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Series Editor's Preface

It is my pleasure to introduce this new volume on Advanced Techniques in Gas Chromatography-Mass Spectrometry (GC–MS–MS and GC–TOF–MS) for Environmental Chemistry, edited by Imma Ferrer and E. Mike Thurman. The reason for having such a book in the Comprehensive Analytical Chemistry series is quite obvious: I was able to convince both editors to do it. My plans to have it in the CAC series date back to my early days as Series Editor, some 15 years ago. I should add that it was not so difficult to convince Imma and Mike since both they are old friends of mine. I met Mike in person for the first time on the occasion of the 209th ACS National Meeting held in Anaheim, CA, in April 1995. Imma had just started to work in my group at that time and successfully defended her PhD thesis in December 1999.

This book can be considered complementary to other two related titles in the CAC series: Two Dimensional GC (Vol. 55) and TOF-MS in Food and Environmental Analysis (Vol. 58). It contains 20 chapters that cover a comprehensive variety of applications mainly in the environmental field but also in related matrices like food and biological samples. The various chapters contain details on the residue analysis of several groups of chemicals including pesticides, odour compounds, hormones, dioxins, PCBs, flame retardants and industrial applications using GC–MS–MS and/or GC–TOF–MS.

The book can be used as an academic text for postgraduate students and technicians, and as a reference for those working in chemical analytical laboratories who want to learn more about the applications of GC-tandem MS systems to environmental issues. Overall this book covers an important, routinely used and continuously improved technique in the field of Analytical Chemistry that has proven its usefulness for solving everyday problems in the analysis of organic chemical contaminants.

Finally I would like to thank both editors again and all the authors who have contributed to this book for their time and effort in preparing this excellent and useful book on GC-tandem MS techniques.

Prof. D. Barceló
Barcelona, Spain, July 18th 2013

Preface

Gas chromatography mass spectrometry (GC-MS) is not a forgotten field! Yes, it is true that LC-MS has been the preferred technique in environmental analyses over the past decade. Nonetheless, there have been major advances in the analysis of volatile and semi-volatile compounds by GC-MS. For example, there have been significant innovations in GC-MS instrumentation, such as the development of time-of-flight mass detectors (TOF) and two-dimensional gas chromatography (GC×GC) coupled to gas chromatographs. Furthermore, tandem mass spectrometry techniques (GC–MS–MS) are still commonly and successfully used for the achievement of high sensitivities.

Mainly, GC-MS techniques are used for the analysis of (i) volatile organic compounds that are analyzed by purge-and-trap techniques, (ii) semi-volatile compounds that don't ionize by LC-MS sources, and (iii) classic compounds (such as dioxins and chlorinated pesticides) where methodologies have been already well established and developed in the past. Today, even after developments for LC-MS ionization, these are still the main three areas for GC-MS applications. Furthermore, most environmental laboratories have people trained and qualified for GC-MS method development. Also, the inexpensive cost of this technique has made it easily available to all government, university, private, and industry labs. Standard methods by the Environmental Protection Agency (US EPA) have been the classic methods of choice by many scientists in the environmental field of research. Another fundamental aspect of GC-MS applications is the development of new sample preparation procedures, including extraction techniques and derivatization methods. This has opened a whole new area of remarkable applications in the field of environmental chemistry research.

Advances in high-resolution and accurate mass analysis by LC-MS have been recently carried over into the GC-MS instrumentation with time-of-flight mass spectrometry. This has only happened in the past 3–5 years, an important factor to take into account. These developments have resulted in innovative applications to environmental problems, as the reader will discover in this book.

The book consists of 20 chapters divided in two parts. The first one deals with both classic and advanced GC-MS and GC–MS–MS applications in the environmental field. The second part describes several applications using high-resolution and accurate mass GC-MS. The first part emphasizes basic features such as sample preparation for GC-MS analysis (Chapters 2, 5, and 8), development of multiresidue methods in food and water (Chapters 1, 3, and 7), analysis

of biological samples (Chapters 6 and 9), air samples (Chapter 4), and water samples (Chapters 9 and 10). The second part focuses on the use of high-resolution and time-of-flight GC-MS, describing and reviewing a whole variety of methodologies for the detection of environmental compounds. The basic principles for time-of-flight (TOF) analysis are described in detail in Chapters 11 and 12. New innovations such as the development of a miniaturized GC–TOF–MS system are illustrated in Chapter 13. Likewise, new developments in soft ionization sources are shown in Chapter 14. Several applications using high-resolution GC-MS techniques are described in Chapters 15 through 19. These applications include the analysis of stable isotopes in fracking waters, halogenated flame retardants, industrial compounds, persistent organic pollutants, and dioxins. Finally, Chapter 20 introduces a new idea called "exposomics" which discusses how GC–TOF–MS can be used to measure changes in specific individuals due to the exposure to a wide range of environmental contaminants. All classes of environmentally relevant compounds are represented in this book such as pesticides, odor-causing compounds, pharmaceuticals and personal care products, polychlorinated dibenzo-*p*-dioxins and furans, polychlorinated biphenyls, polyaromatic hydrocarbons, hormones, phytoestrogens, pyrethroids, hydrocarbon gases and their stable isotopes, halogenated flame retardants, and persistent organic pollutants.

As this is our third book together, we have the tradition of predicting future trends in environmental mass spectrometry: (i) GC-MS will continue to be a popular and highly used analytical technique for the identification of volatile and semi-volatile compounds, (ii) high-resolution and time-of-flight mass spectrometry will become more and more important for nontarget and unknown analyses, gaining terrain in many environmental labs, and (iii) development of new soft ionization techniques will be the key to more effective MS-MS analyses.

Finally, we would like to acknowledge our friend, colleague, and Series Editor, Damià Barceló, for giving us the opportunity to put this book together. As always, it has been an enjoyable endeavor to be part of. And we want to thank each of the individual authors for their outstanding contributions that made possible this state-of-the-art book on GC-MS applications for environmental chemistry.

Imma Ferrer and Mike Thurman
July 2013, Boulder, Colorado

Chapter 1

Gas Chromatography–Mass Spectrometry Techniques for Multiresidue Pesticide Analysis in Agricultural Commodities

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1 GAS CHROMATOGRAPHY

Gas chromatography was developed in the 1950s when James and Martin [1] reported the separation of organic and fatty acids from mixtures using nitrogen as the carrier gas and 10% steric acid in silicone/diatomaceous earth as the stationary phase [2]. Since its development, tremendous advancements and improvements in the hardware, software, and consumables have established GC as an important analytical tool in the isolation of chemical constituents from complex matrices prevalent in the food, flavor and fragrances, petroleum and chemicals, environmental, and biological and medical disciplines [3]. Fused silica capillary columns of diverse stationary phases and column dimensions have since replaced packed columns due to their superior separation efficiency

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and resolution. The pneumatics and microfluidic devices, built into current GCs, allow for precise control of gas flows that provides not only reliable chromatographic retention times [4] but also introduce a variety of sample injection and maintenance techniques such as large volume or programmed temperature vaporization (PVT) injection [5], column backflushing [6], and advanced GC procedures such as multidimensional (GC × GC), low pressure, and fast capillary gas chromatography [7–10] techniques. The integration and use of the computer in today's GC aids in the optimization of instrumental conditions, enables for efficient acquisition and storage of chromatographic (and mass spectra) data, and provides the speed to quantitatively and qualitatively process the data using various software algorithms and programs. The combination of these technological advances has made it possible to couple sampling devices such as headspace, thermal desorption, solid-phase microextraction, stir bar sorptive extraction as well as automated sample preparation workstations, to allow for increased sample throughput and further diversity of GC and gas chromatography–mass spectrometry (GC–MS) applications.

2 GAS CHROMATOGRAPHY WITH ELEMENT SELECTIVE DETECTION FOR MULTIRESIDUE PESTICIDE ANALYSIS

The first significant work of multiresidue pesticide procedures by Mills [11] applied paper chromatography using a chromogenic reagent (silver nitrate/hydrogen peroxide in 2-phenoxyethanol and acetone) to stain the paper as a means to detect organochlorine pesticides and reported "it is possible to rapidly identify and approximately measure residual of commonly used pesticides in a variety of foods and feeds." Identification with paper chromatography depended on the number and size of the developed spots and difficulties arose due to streaking and resolving spots of pesticides with similar migration times. In a follow up on his work, Mills *et al.* [12] replaced paper chromatography with a GC equipped with a Coulson coulometric detector for the separation and detection of 21 organochlorine pesticides, demonstrating one of the first published application of detectors used for GC analysis for pesticides.

Many GC detectors prior to mass spectrometry were of the element selective types, namely, so because detection was dependent on the presence and detection of element heteroatoms in the molecular makeup of the analyte that formed specific ions or emissions when combusted and became ideally suited for pesticide analysis. Commonly used universal GC detectors were the thermal conductivity (TCD) and flame ionization (FID) detectors [13–15] because of their abilities to produce signal responses from the carbon–hydrogen content of the analyte. The TCD signal response is a result of changes in the temperature and electrical resistance when the analyte from the GC effluent comes in contact with a conductivity cell. The FID signal results when the C–H-containing analyte is combusted in a flame jet creating ions which are

collected at an electrode to produce the response. The detectors generally used for the GC analysis of pesticides are electron-capture (ECD) [16,17], electrolytic conductivity (ELCD) [18], nitrogen–phosphorus (NPD) [19], and flame photometric [20], which were effective due to their selectivity and sensitivity for halogens (especially chlorine), nitrogen, phosphorus, sulfur, or a conjugated moiety such as an aromatic ring that were present in organochlorine and organophosphorus pesticides that were commonly used at the time [12,21–23]. Some of these detectors have been modified such as the micro-electron-capture (μECD) and pulsed flame photometric [24] and are of still in use today because of their improved selectivity and sensitivity [25,26]. The ELCD and the closely related halogen-specific detector (XSD) [27–29] are currently used to analyze organochlorine pesticides and have been useful to detect phthalimide fungicides such as captan, captafol, and folpet [30,31], notoriously difficult pesticides to analyze by MS because of their thermal instability under GC conditions and the inability to produce stable fragments with MS. As a result of the availability of these different detector types, many multiresidue pesticide procedures based on gas chromatography coupled with element selective detection for the analysis of foods were developed at several regulatory and private laboratories [21,32,33]. Despite the increasing use of GC–MS, the addition of an element selective detector to the GC–MS or a stand alone GC-element selective detector can be used to compliment GC–MS or enhance the GC–MS performance in the detection or confirmation of difficult pesticides in complex food and agriculture-based matrices [24,30,34–38].

3 CAPILLARY GC–MS

Pesticide confirmation with GC-element selective detection is determined by cross-referencing the retention indices or comparing the variations in the retention times of each pesticide using two different columns of stationary phases of varying polarities [26,39,40]. This practice has since been replaced because MS has become the primary detector of use in most laboratories and is more reliable for identification or confirmation. Today, criteria for pesticide presence from a sample are based on MS technology [41–45]. Due to the separation and resolution capabilities of GC, an analyte is isolated as it undergoes fragmentation typically by electron ionization (EI) in the mass spectrometer to generate a spectrum based on mass-to-charge (m/z) ratios. The analyte can be unambiguously identified based on the chromatographic retention time and the unique spectral fragmentation pattern of the mass spectrum in full-scan mode or the selection of three or four unique ions or precursor-to-product ion transitions in selective ion monitoring (SIM), tandem MS (MS/MS), or selective (or multiple) reaction monitoring (SRM or MRM) MS/MS using nominal or accurate masses. The selection of a variety of mass spectrometric procedures provides the capabilities and means to further elucidate the identity of the analyte based on the presence of the mass of the molecular and

fragmented ions (m/z) and calculations of their ion ratios based on mass fragmentation and distribution [44,45].

The use of a mass spectrometer as a detector for gas chromatography-multiresidue pesticide analysis came about in the 1990s as a result of the commercialization, availability, and most importantly, the affordability of the technology. MS is the standard detector because of its capability to detect, quantitate, and identify chemical analytes in complex mixtures because of its specificity and unambiguous certainty based on the m/z properties of the precursor and fragment ions. Currently, quadrupole ion traps, quadrupole mass filters, time-of-flight (TOF), and double-focusing mass spectrometers have been used for GC–MS pesticide analysis and other applications [13]. Both quadrupole-based technologies were initially studied because of their availability and cost, but much of the current multiresidue pesticide procedures are now based on quadrupole mass filters using single stage or triple quadrupole platforms. Few conventional pesticide laboratories have utilized TOF mass and double-focusing analyzers for multiresidue analysis primarily due to cost, lack of computer speed to acquire data, and popularity of the quadrupole instruments. Although double-focusing technologies have been used for persistent and legacy chlorinated pesticides, its primary role has been focused for high-resolution studies of halogenated persistent organic pollutants and their isomers such as polychlorinated dibenzo-*p*-dioxins, polychlorinated biphenyls, and brominated diphenyl ethers and the reader is referred to more detailed reviews and references elsewhere [46–51], including some of the chapters in this book.

4 GAS CHROMATOGRAPHY–ION TRAP-MASS SPECTROMETRY

The invention of quadrupole mass analyzers such as the quadrupole mass filter and quadrupole ion trap were developed by Paul in the early 1950s [52]. The quadrupole ion trap mass analyzer can exist in either a three-dimensional or linear configuration. The (three-dimensional) quadrupole ion trap is a mass analyzer consisting of two hyperbolic electrodes and a hyperbolic ring electrode situated at the center between the two electrodes. Ions are trapped and manipulated in three-dimensional space between these three electrodes by an oscillating electric potential or a fundamental radiofrequency electric field [52,53]. The linear ion trap mass analyzer consists of quadrupole rods that are used to confine the ions radially and static electric potential on-end electrodes to confine the ions axially. This design allows the analyzer to act either as a quadrupole mass filter or as a trap by creating a potential well for the ions along the axis of the electrodes. The advantage of this design allows for an increased ion storage capacity, faster scan times, and simplicity of construction. Ion trap mass spectrometers became commercially available in the mid-1980s, but it took a few years to develop procedures for multiresidue pesticide analysis. Some problems of the early ion trap instruments were due to

internal ionization sources that overfilled the trap with ions causing space-charge effects that limited the dynamic range and led to poor quantitation. Ion traps are now available with external ionization sources and automated gain control or scanning, which minimize the interferences caused by ion reactions with neutrals and space-charge effects by adjusting the gate time for admitting the ions to enter the trap [54].

Cairns *et al*. [55] demonstrated the use of gas chromatography-ion trap-mass spectrometry (GC-IT-MS) in full-scan mode to determine the quantitative and confirmatory ions for 245 pesticides. They analyzed incurred residues in fresh produce using the FDA acetone-based extraction method developed by Luke *et al*. [21] and compared the MS results with element selective detectors. Zhang *et al*. [56] demonstrated the basic use of the GC–IT-MS by optimizing the number of scans and scan rates in full-scan mode and in combination with the deconvolution software tool, Automated Mass Spectral Deconvolution and Identification System (AMDIS), developed by the National Institute of Science and Technology (NIST), to remove the interfering and overlapping peaks from the background or sample matrix. The resulting mass spectra of a pesticide from the sample and a reference standard generated from the ion trap were effective to qualitatively screen and identify pesticide residues at concentration levels >50 ng/g in cabbage and rice samples. Tahboub *et al*. [57] reported a simultaneous identification and quantitation method to screen organochlorine pesticides in honey using GC–IT-MS in full-scan mode. Makabe *et al*. [58] determine 292 pesticides in processed meat and seafood products by extracting the samples with 1:1 ethyl acetate:cyclohexane, removing lipids with acetonitrile:*n*-hexane partitioning, and solid-phase extraction (SPE) cleanup with C18 and tandem graphitized carbon black/primary-secondary amine (GCB/PSA) mini-cartridge columns before injection into a GC–IT-MS primarily in full-scan mode.

GC–IT-MS can be used in MS/MS mode via its unique ion storage functions. After the sample is introduced (i.e., gas chromatography effluent) and ions generated (i.e., an external ion source), all ions are stored in the ion trapping device, and the selected analytes of interest are defined by their corresponding m/z ratios and are kept as the precursor ions, while all other ions are expelled from the ion trap via resonant expulsion. Using their secular frequencies, excited precursor ions are then fragmented with collision gas (e.g., helium) generating product ions and the resulting product ion spectra. These product ions can also undergo further collisions to generate MS/MS or MS^3 products and spectra to provide additional structural information of the precursor molecule. Schachterle and Feigel [59] were one of the first to demonstrate the use of GC–MS/MS with an ion trap for the analysis of 19 pesticide residues in fresh produce samples using a simple extraction procedure with methylene chloride. Lehotay [60] prepared apple, bean, and green extracts using supercritical fluid extraction followed by analysis by direct sample injection/gas chromatography/tandem mass spectrometry (DSI/GC/MS–MS) in an attempt to develop an effective, efficient, and inexpensive

approach to quantitate and identify targeted pesticides. Gamon *et al*. [61] analyzed 80 pesticides from samples prepared from acetone extraction and dichloromethane/petroleum ether partitioning using GC–IT-MS/MS in both EI and chemical ionization modes. Studies from Hernando *et al*. [62] followed by Aguera *et al*. [63] reported using positive and negative chemical ionization of pesticides followed by MS/MS detection for determining organophosphorus, organochlorine, and pyrethroid pesticides in crops for routine analysis at pesticide regulatory levels. Inoue *et al*. [64] reported that GC–IT-MS/MS was effective for both identification and quantitation analysis of low level pesticide residues in agricultural matrices and found the results comparable to GC–MS/SIM using a single quadrupole mass filter. In a follow up to this work, Hirahara *et al*. [38] reported a GC–IT-MS/MS to determine and achieve quantitation limits of 10 μg/kg or better for 199 pesticides from apple, cabbage, orange, potato, rice, soybean, and spinach matrices.

5 GAS CHROMATOGRAPHY–SINGLE QUADRUPOLE-MASS SPECTROMETRY

The quadrupole mass filter or analyzer consists of four cylindrical and hyperbolic-shaped rods which separate mass ions based on a direct current (DC) voltage superimposed upon an oscillating electric field caused by a radiofrequency voltage applied between each rod pair. An alternating current voltage is responsible for ions to spiral through the quadrupole, while the DC voltage directs the ions axially toward the other end of the electrodes. Ions of interest are selected as the mass-to-charge (m/z) ratios of the ions are matched with the voltages and voltage ratios so that the trajectories of these ions are stable to travel through the quadrupole and are detected by an electron multiplier, while nondesirable ions will collide with the quadrupole due to their unstable trajectories. The quadrupole mass analyzer is widely used in today's GC–MS and liquid chromatography–mass spectrometry instruments because of its relatively simple design of the space requirements between the ion source and the detector, for its durability and reliability for nominal mass measurements, and wide linear dynamic range [65]. The single quadrupole mass filter can operate in full-scan or SIM modes. In full-scan mode, mass fragments over a wide range (typically, m/z 50–600) are monitored, whereas in the SIM mode, ion fragments characteristic of the analyte are selected for detection.

The quadrupole filter in full-scan mode can provide the complete mass spectra of all analytes present in the sample provided that the analytes can be effectively ionized. Kakimoto *et al*. [66,67] evaluated pesticides in fresh produce samples by using full-scan GC–MS and attempted to quantitate at the 0.1 and 0.5 μg/kg levels using one extracted target ion of each pesticide analyzed. Norli and Christiansen [68] performed studies by analyzing produce extracts with GC–MS using the quadrupole in full-scan mode in combination

with the AMDIS deconvolution software algorithms and was able to screen and identify target compounds by purifying the mass spectra from the real sample and then matching the spectra against reference libraries. Results showed better match factors for samples fortified (0.02 and 0.1 μg/kg) or incurred at higher pesticide concentrations.

Due to the lack of sensitivity in full-scan MS mode, most applications of the single quadrupole mass filter are operated in SIM mode. Gas chromatography-mass spectrometry/selective ion monitoring mode (GC-MS/SIM) is effectively used for quantitation because lower detection limits can be achieved due to the lower numbers of scans that reduce the number of mass fragments from targeted compounds. Typically, three or four ions characteristic and unique to the analyte of interest are selected for identification, one ion being the target is used for quantitation and the remaining three ions are used for qualification with respect to the target ion. Fillion *et al*. [69] described the application of GC–MS/SIM for the multiresidue analysis of 189 pesticides for fresh produce samples using acetonitrile salt-out extraction followed by celite–charcoal cleanup. Later, Fillion *et al*. [70] modified their procedure by replacing the celite–charcoal cleanup with a combination of octadodecyl-linked (C18), graphitized carbon, and aminopropyl-linked SPE cartridges for the analysis of about 200 pesticides by GC–MS/SIM. The same technique was used to analyze the pesticides by identification of the target compounds using the GC retention times, the presence of the target and qualifier ions (2–3 ions), and the ion ratios of the qualifier to the target ions. Quantitation was based on the signal of the target ion, which usually provides the highest signal intensity of all of the ions used to characterize the pesticide and calibration standards. Due to the effectiveness of acetonitrile-based extraction, cleanup by C18 and GCB/PSA columns, and subsequent analysis by GC–MS/SIM, this procedure has been adopted as an accepted or official method by many laboratories in Canada [71], Korea [72], Japan [73,74], China [75], and the United States [76]. Nemoto *et al*. [77] and Ueno *et al*. [78] developed acetonitrile extraction procedures utilizing gel permeation chromatography followed by PSA or GCB cleanup steps to analyze pesticides in fresh produce matrices. In the past few years, Pang *et al*. have optimized the most of GC–MS/SIM capabilities for multiresidue pesticide procedures by developing procedures to analyze from >400 to approximately 900 pesticides in different agricultural commodities using a variety of sample preparation techniques such as acetonitrile salt-out extraction/SPE column cleanup for fresh produce, honey, fruit juice, wine, and teas [78–81], ethyl acetate extraction/gel permeation chromatography cleanup for animal tissue [82,83], and pressurized liquid extraction for grain products [84].

GC–MS/SIM analysis of pesticides utilizing acetone-based extraction techniques has its basis from FDA, that is, Pesticide Analytical Manual (PAM) procedures [21,85] which utilized acetone extraction, petroleum ether partitioning, and SPE cleanup procedures using octadodecyl-linked silica (C18) and strong anion exchange/primary-secondary amine sorbents

(SAX/PSA) tandem cartridges [86,87]. Stan [88] modified the well-established DFG Method S19 developed by Specht *et al.* [32,33] by changing the GC-element selective detectors (NPD, ECD), previously used in the method, to capillary GC–MS equipped with a single quadrupole mass filter for full-scan and SIM modes. Štajnbaher and Zupančič-Kralj [89] developed a method based on acetone extraction followed by SPE cleanup to analyze and quantitate several pesticide classes using GC–MS/SIM. After evaluation of several SPE sorbents, they found that a SPE consisting of poly(styrene-divinylbenzene) sorbent was most effective in removing matrix coextractives from fresh fruit and vegetable samples for the analysis of 90 pesticides. Mercer [86,87] developed procedures for the analysis of halogenated as well as nitrogen-, sulfur-, and oxygen-containing pesticides in fruits and vegetables due to difficulties or lack of identifying these classes of pesticides by element selective detectors (ELCD, XSD, NPD, and ECD). Samples were prepared by acetone extraction, petroleum ether partitioning, and SPE cleanup procedures using octadodecyl-linked silica (C18) and SAX/PSA tandem cartridges before analysis by GC–MS/SIM. The results of these studies revealed as much as 10 times more improvement in sensitivity in the pesticide detection and identification of pesticides that could not be detected by previous detectors. Ogawa *et al.* [90] developed a procedure utilizing acetone–ethyl acetate–*n*-hexane as the extracting solvent, followed by gel permeation chromatography cleanup and analysis of 95 pesticides by GC–MS/SIM in cabbage, onion, orange, rice, and spinach matrices.

6 GC–QQQ-MS/MS

One disadvantage of the GC–MS/SIM procedures using the single quadrupole mass filter is the nonspecificity or the abundance of contributing ions from the sample matrix that can interfere with analyte identification. Since chemical components in a complex matrix can often contribute to the abundances of the chemical analyte of interest, this can affect the ion ratios of the fragments used for identification and create false positives or negatives. This problem can be addressed by additional selectivity of ions pertinent to the analyte and filtering of nonspecific ions from the sample matrix using gas chromatography-triple quadrupole mass spectrometry, developed by Yost and Enke [91]. Three quadrupoles are aligned in series, with the first and third quadrupoles used as mass selective filters, while the second is used as a collision chamber. The first quadrupole is used to isolate and accelerate only the target or precursor ion of the analyte of interest toward the collision chamber, whereas all other analyte ions are removed. The collision chamber allows the precursor ion to fragment in the presence of an energetic inert gas such as Ar or N_2 via collision-induced dissociation and the fragment ions are accelerated toward the third quadrupole. The selective product ions are sorted in the third quadrupole and allowed to pass and enter the detector. A tandem instrument in selective or multiple reaction monitoring (SRM or MRM) mode in a

triple quadrupole instrument is more specific and sensitive than SIM because the precursor and product ions have been specified and selected and there is a higher signal-to-noise ratio of the specific ion (target-to-product) transitions. The difference between MS/MS in a triple quadrupole and an ion trap mass analyzer is that the quadrupole MS/MS experiments are performed in space, while MS/MS in the ion trap is performed in the time domain [92].

One of the first to demonstrate the capability of gas chromatography-triple quadrupole mass spectrometry in tandem mass mode (GC-QQQ-MS/MS) for multiresidue pesticide analysis was Garrido-Frenich *et al.* [93], who demonstrated the identification and quantitation of 130 pesticides in fresh vegetable samples. They showed that a simple ethyl acetate extraction without any cleanup was all that was required for the identification or confirmation of pesticides at trace levels, reducing the analysis time and increasing sample throughput. The ethyl acetate extraction procedure combined with GC–QQQ-MS/MS analysis was also performed by Pihlström *et al.* [94] and has replaced the GC pesticide procedures used by the Swedish National Food Administration [95], since 1989 that involved the use of gel permeation chromatography cleanup, GC-element selective detectors, and GC–IT-MS. Belmonte-Valles *et al.* [96] validated the procedure for 53 pesticides in tomato, orange, and apple matrices using GC-QQQ-MS/MS with negative chemical ionization and diluting the sample 10 times, while still achieving acceptable analytical performance (LOD < 2.5 ng/g, 70–120% mean recoveries, and RSD $< 20\%$). The quick, easy, cheap, effective, rugged, and safe (QuEChERS) method based on acetonitrile salt-out extraction followed by solid-phase dispersive cleanup [97] has been used as a sample preparation procedure by many laboratories for multiresidue GC–QQQ-MS/MS analysis of pesticides in a variety of food matrices [31,98–103]. Other extraction solvents such as ethyl acetate have been used as an alternative to acetonitrile for the QuEChERS extraction step [104]. Banerjee *et al.* [105] developed a GC-QQQ-MS/MS using QuEChERS ethyl acetate extraction for the determination of 349 pesticides, 11 polychlorinated biphenyls, and 15 polycyclic aromatic hydrocarbons in fruits and vegetables. QuEChERS and dilute-and-shoot procedures have been implemented and current procedures have been simplified by the elimination of preparation steps that were used to remove interfering matrix co-extractives for standard GC–MS procedures, due to the enhanced selectivity and sensitivity of GC–QQQ-MS/MS.

Okihashi *et al.* [106] and Wong *et al.* [107] adapted the acetonitrile cleanup procedure by Fillion *et al.* [70] using GCB/PSA sorbent SPE for better cleanup of samples to analyze 260 pesticides in fresh produce and 168 pesticides dried ginseng root using GC–MS/MS, respectively. Wong *et al.* [103,107] optimized the use of toluene as both as an eluting solvent to elute planar and aromatic pesticide residues from GCB and as the final GC solvent extract via solvent exchange from acetonitrile due to its preferable properties for GC analysis [108]. Hayward *et al.* [109] expanded this work and were able to validate the procedure to 310 pesticides and 24 botanical matrices. Their work indicated some of the limitations of GC–MS/MS since matrix

interferences from some of the botanical matrices contributed to shifting retention times and changes in the chromatographic peak shapes that affected the quantitation and identification of the pesticides due to changes in the precursor-to-product ion transition ratios.

7 CAPILLARY GC–TOF-MS

GC–SIM/MS and GC–MS/MS generated by quadrupole mass filters or ion traps are techniques that can be used to identify compounds from the matrix background from characteristic ions associated with these targeted compounds. Although target-oriented procedures are extremely sensitive and quantitative, they do not provide complete information of the screening because pesticides or additional chemical contaminants of concern not targeted in SIM or MS/MS are not screened or identified during the process.

A time-of-flight (TOF) mass spectrometer determines the mass-dependent time it takes when ions of different masses migrate from the ionization source to the detector [13]. A TOF instrument consists of a reflectron, which is an optical device that acts as an ion mirror by reversing the direction of an ion, thus enhancing the resolving power of the instrument by increasing the flight path and time and exploiting the detector arrival times of ions of varying kinetic energies [110]. TOF mass analyzers can operate in full-scan mode and are commercially available in two forms. One type can operate high scan rates up to 500 spectra/s at unit mass resolution and linearity of four orders of magnitude, which makes it ideal for fast GC and multidimensional (GC × GC) GC applications. The second is a high-resolution TOF (HRTOF) that can evaluate mass spectra data at 5000–15,000 full width at half maximum (FWHM) mass resolution, achieve mass accuracy of 5–10 ppm, extend the mass range up to 1500 amu, and provide linearity of three orders of magnitude. The analytical capabilities of a HRTOF can provide reliable detection and identification of pesticides by providing accurate mass data.

Koesukwiwat *et al.* [111] and Lehotay *et al.* [112] applied fast, low pressure gas chromatography by installing a short microbore capillary column to the inlet to maintain normal GC pressures and operation and connecting it to a megabore analytical column (10 m × 0.53 mm ID × 1 μm thickness) that leads to a high scan rate TOF detector. By employing QuEChERS and fast chromatographic run times (<10 min per run), the authors' goals were to increase sample throughput by completely analyzing 150 pesticides in 32 samples in an 8-h workday. Validation of the quantitative aspects of the QuEChERS procedure were consistent as in previous studies, but the investigators also evaluated the qualitative performance between software tools (developed for automated detection) and the analyst evaluation (using conventional instrument software) by randomly spiking different extracts with different pesticides and concentration levels. Although the analyst evaluation was slightly better in recognizing false positives (0.8% vs. 1.2%) and false negatives (17% vs. 23%), the analyst approach was time-consuming and would not be practical in routine, high-throughput analytical applications.

Multidimensional gas chromatography coupled to a full-scan TOF-MS (GC × GC/TOF-MS) provides additional separation because coeluting peaks resulting from a one-dimensional separation can be separated by a second column possessing a different stationary phase. The resulting GC × GC chromatograms form narrow peaks and require a mass spectrometer such as TOF to be fast enough to scan enough data points for each individual peak. Dallüge *et al*. [113] demonstrated the use of GC × GC/TOF-MS equipped with a PVT injector for large volume injection on an ethyl acetate extract from food samples for multiresidue pesticide screening. Their work showed that the effects of interfering matrix compounds could be minimized resulting in better quality TOF-MS spectra due to the enhanced and increased separation from GC × GC and the reporting of two retention times from the two columns could also provide additional identification of the pesticide. van der Lee *et al*. [114] developed a targeted method for the analysis of 106 pesticides and contaminants in animal feed using ethyl acetate extraction, gel permeation chromatography and PSA dispersive SPE for cleanup, and GC × GC–TOF-MS analysis in full-scan mode. The quantitative method was validated at 10 and 100 μg/kg levels, with recoveries between 70% and 110%, and RSD below 20% for most of the pesticides evaluated. The system software was able to identify pesticides at levels greater than 50 μg/kg and quantitation was made possible since correlation coefficients for 90% of the pesticides were $r^2 > 0.995$. Mol *et al*. [115] validated the qualitative aspect of the method by detecting and reporting pesticides in plant-based materials using library-based software tools and determining the occurrence of false detections and false negatives of 235 pesticides in various leaf crops fortified at concentrations levels ranging from 10 to 200 μg/kg. Eighty-three compounds were detected at 10 μg/kg with 95% confidence and increased to 185 pesticides at 200 μg/kg. The qualitative determination of the 235 pesticides in plant leaves was shown to be compound and concentration dependent, but they proposed that rather than routinely validate the procedure quantitatively (recovery studies, linearity, calibration curves), the qualitative approach of counting the number of fortified pesticides found and the number of false detects in unfortified samples is more efficient and effective. This concept was further evaluated by Lommen *et al*. [116], who developed their own metabolomics-based screening software tools for processing raw GC × GC–TOF-MS data for automated library-based identification in residue and chemical contaminant analysis. The features of their software tool (metAlignID) have been tested in its ability for automated library-based identification and concentration quantitation. The software has been adapted for standard PC configurations and can process large GC × GC–TOF-MS files that are routinely generated and required to be screened for large numbers of chemicals. The software was able to find more correct hits than the conventional software when processing the same data but for the most part, both software tools are comparable since both found approximately the same number of false positives and negatives when samples were tested at the 10 μg/kg level.

Čajka and Hajšlová [110] reported the advantages and limitations of GC–HRTOF-MS for pesticide analysis applications by analyzing peach extracts and optimizing and evaluating the chromatographic conditions and detector settings. They found the procedure provided unbiased identification and reliable quantitation of the target ion due to the narrow mass window for extracting the ions, the availability of the full spectra at low levels, and acceptable sensitivity since most of the pesticides studied were detected at levels below 10 μg/kg. Leandro *et al*. [117] evaluated baby food using GC–HRTOF-MS and reported the use of dynamic range enhancement that improved the working dynamic range for exact mass measurements and quantitation, problems reported by Čajka and Hajšlová [110]. Hayward and Wong [118] evaluated dried ginseng powders using extensive extraction and cleanup procedures and comparing the validation data of 170 pesticides evaluated on both GC–MS/SIM and GC–HRTOF-MS and found few significant differences between the performances of the two instruments. HRTOF-MS data provided additional accurate mass information on incurred pesticide residues to aid in the identification, which was not present from the MS/SIM acquisition. Cervera *et al*. [119] evaluated the QuEChERS multiresidue method with GC–HRTOF-MS for nontargeted and targeted analysis of 55 pesticides in apple, carrot, olive, orange, and tomato samples. For evaluation of the targeted analysis, validation was based on the linearity, accuracy, precision, selectivity, and limit of quantitation. The procedure was shown to be reliable in all samples, with the exception of olives. Using the system's software tools, retrospective analysis of the acquired chromatograms was carried out to discover the presence of imazalil and two polycyclic aromatic hydrocarbons in one of the carrot samples analyzed.

Zhang *et al*. [120] recently evaluated a simultaneous screening and identification procedure for pesticide analysis in vegetables using capillary gas chromatography coupled to a hybrid quadrupole time-of-flight mass spectrometer (GC–QTOF-MS) and a database based on chemical formula match of the precursor ion and structural confirmation of product ions by accurate mass measurement for 187 pesticides. Vegetable samples were extracted by salt-out acetonitrile extraction and cleaned up by carbon/aminopropyl SPE and toluene and analyzed by TOF-MS in full scan at 5 spectra/s over the mass range m/z 50–600 at approximately 13,500 FWHM resolution. The MS^2 conditions were fixed for isolation of the precursor ion at medium MS resolution and using nitrogen as the collision gas. The limit of identification of the database was determined to be at 5 ppb and accurate mass errors were less than 2.5 mDa. The method was tested on fresh produce samples purchased from a local market and 13 pesticides were found in celery, rape, scallion, and spinach samples. The results of this work reveal the increased selectivity of the hybrid Q-TOF mass analyzer for the identification of the pesticides and its product ions based on their accurate masses.

8 LIMITATIONS AND FUTURE OUTLOOK OF GC–MS

Despite the advances in GC–MS technologies, the role of GC–MS as the major analytical procedure for pesticides has been surpassed by LC–MS, primarily because many of the pesticides that were favorable to GC analysis are no longer being used or replaced with pesticides that are thermally labile and not amenable to GC [121]. GC is still a major component for multiresidue pesticide analysis but the number of pesticides screened by LC–MS is now far greater than the ones requiring GC–MS.

GC samples typically require additional sample preparation due to the limited capacities of the inlet and the capillary columns, which especially becomes important with dirty sample matrices. Due to the popularity of simplified sample preparation procedures such as dilute-and-shoot and QuEChERS and the tendency to avoid cleanup procedures such as gel permeation chromatography and SPE, either GC systems need to become more rugged or the mass detectors need to become more sensitive to handle these dirty or dilute food extracts. High water content fruits and vegetables are a majority of samples analyzed for pesticides and there are comprehensive and straightforward procedures for their analysis based on acetonitrile, acetone, ethyl acetate extraction, and cleanup by SPE or dispersion procedures. In contrast, food samples such as vegetable and fish oils, spices and dried botanical products, seeds, plant, and animal tissue, and animal feed-containing oils, lipids, fats, and sterols are notoriously difficult to analyze by GC–MS. Components in fatty extracts can saturate and overwhelm the column causing for retention times to shift and greatly affect the chromatographic peak shapes and the fragmentation patterns in the mass spectra. These effects can lead to problems in quantitation and identification. In addition, many nonpolar pesticides tend to partition and concentrate in the fat portion of the food. Dilution can be used to dilute the influence of the fat provided that the sensitivity of the method or the instrument is enough to meet the concentration levels of the analyte. This has been demonstrated by Chamkasem *et al*. [122], who analyzed avocado extracts using QuEChERS and applied sufficient cleanup and dilution to analyze the extract by GC–QQQ-MS/MS. A second option is partitioning the acetonitrile extract with acetonitrile-saturated hexane where the lipids are partitioned in the nonpolar hexane phase and the pesticides partition in the more polar acetonitrile phase. This has been demonstrated by Hirahara *et al*. [37], who partitioned acetonitrile extracts from cereal and seed samples with acetonitrile-saturated hexane before SPE cleanup. Low temperature fat precipitation and solid-phase sorbents have also been described as a successive procedure to remove fats from the samples [123,124]. Future procedures need to include new or improved sample procedures that can eliminate the need for matrix-matched standards, analyte protectants and standard additions to compensate for matrix effects.

Most GC–MS systems utilize EI to ionize the analyte of interest. EI spectra at 70 eV are robust and reproducible, which is the basis of the NIST and commercially available mass spectral databases. On the contrary, the excessive energy of 70 eV can cause extensive fragmentation of some compounds that prevents the formation of the molecular ion and make identification difficult. Spectra based on the molecular ion and less fragmentation can make structural elucidation easier. Chemical ionization techniques, such as positive and negative chemical ionization, are available but are sometimes very difficult to reproduce. Other soft ionization techniques such as atmospheric pressure chemical ionization (APCI) could rectify excessive EI fragmentation and provide an intact molecular ion. Research performed by Portolés *et al.* [125–127] have coupled APCI to a triple quadrupole or high-resolution Q-TOF mass analyzers and demonstrated excellent performance based on the formation of the molecular ion and fragmentation patterns of the pesticides studied using QuEChERS extracts of fresh produce samples. Additional APCI-MS/MS experiments led to more useful structural determination than compared to those subjected to EI–MS/MS.

Another ionization procedure, supersonic molecular beam mass spectrometry developed by Amirav [128–131] also shows promise in improving EI. Helium gas enters through a small nozzle into a vacuum chamber such that the carrier gas combined with the helium makeup gas and the heavier analyte molecules form a supersonic molecular beam, resulting in the supercooling of the analyte molecules. The result of this supercooling allows for improved analyte identification and sensitivity by providing the enhancement of the molecular ions, extension of the range of low volatile and thermally labile compounds to become more amenable to GC analysis, and versatility in injection techniques and column choices [132].

Finally, Li *et al.* [133] developed a new GC–MS technique by utilizing resonance enhanced multiphoton ionization as a third ionization alternative. The investigators used capillary gas chromatography coupled with a multiphoton ionization/time-of-flight mass analyzer (GC/MPI/TOF-MS) to analyze 49 pesticides in extracted vegetable and fruit samples and compared the results obtained from GC/EI/MS. An *n*-alkane standard was used for the calibration of the retention indices for the pesticides. Pesticides were efficiently ionized and the limits of detection were in the subpicogram to pictogram range, better than those obtained by GC/EI/MS. Three pesticides, 3-(2-naphthyl)acetamide, thiabendazole, and tricyclazole present in real samples (cucumber and lemon) were detected by the new procedure, whereas none of these pesticides were detected by GC/EI/MS–MS. This new approach shows promise for trace analysis of pesticides in foods and in the environment.

High-resolution mass spectrometry using an orbital trap mass analyzer has attracted much attention due to its capability to achieve high mass resolution of $>100{,}000$ and mass accuracy <5 ppm [134]. Current and commercial instrumentation includes the coupling of liquid chromatography with single

stage orbital trap and to hybrid instruments consisting of a quadrupole or an ion trap to an orbital trap mass analyzer. Therefore, it is conceivable that a commercially available capillary GC system coupled to a single stage orbital trap or to a hybrid quadrupole-orbital trap MS system for analyzing pesticides in complex food matrices may materialize in the future if there is a demand for this technology. The development of this new high mass resolution MS technology can be evaluated along with GC–HRTOF-MS procedures to address analytical challenges in multiresidue pesticide procedures.

9 CONCLUSIONS

GC and GC–MS have played significant roles in multiresidue pesticide procedures over the past 50 years. The major GC detector has become the mass spectrometer in the form of the ion trap, quadrupole mass filter, and recently, triple quadrupole and TOF mass analyzers. The technological advances in chromatography and mass spectrometry have affected the way the samples are being prepared for GC–MS analysis. Extensive clean-up procedures were once required to remove interfering matrix components but selective and sensitive GC–MS systems have allowed procedures to become simplified. The development of QuEChERS and simplified organic solvent extraction procedures could not have existed if it were not for the development and availability of these advance MS instrument technologies. However, challenges do exist for GC–MS systems. Although procedures for high water content produce samples are comprehensive and straightforward, new cleanup procedures are required and need to be implemented to address more difficult matrices. Better ionization procedures are needed to replace or compliment EI so that the sensitivity and identification of the system can be improved. LC–MS procedures may have become more prominent in multiresidue pesticide procedures but the role of GC–MS has not been eliminated. Both procedures are required in the laboratory to perform comprehensive and routine multiresidue pesticide analysis but both procedures combined may not be able to address every pesticide or chemical contaminant that may be present in food. The universal instrument for the multiresidue analysis of pesticides and other chemical contaminants in foods is still yet to come.

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Chapter 2

Microextraction Techniques Coupled to Advanced GC–MS Techniques for Analysis of Environmental Samples

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1 INTRODUCTION

Currently, gas chromatography (GC) coupled to mass spectrometry (MS) is still the method of choice in environmental analysis for a wide variety of volatile, semivolatile, and nonpolar compounds (e.g., certain pesticides, persistent organic pollutants (POPs), etc.) [1–4]. Among the developed methods, those based on chromatographic separation with time-of-flight (TOF) [5] or tandem MS (MS/MS) using triple quadrupole (QqQ) or ion trap (IT) mass analyzers [2,6] have opened new perspectives in terms of identification and are acknowledged as the major useful and authoritative methods for determination of trace environmental contaminants. Moreover, they allow substantially reduced analysis time and improved sensitivity and specificity, especially when dealing with complex matrixes, thus facilitating the detection of a larger number of compounds within one run and enabling low detection limits (LODs). However, despite significant technological advances in the development of highly efficient GC analytical instruments, most of them

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cannot directly handle the complex environmental matrices, yet. So, one or more pretreatment steps before instrumental analysis named as "sample preparation" are still mandatory in many applications, for enrichment, cleanup, and signal enhancement, especially for analysis of trace components [3,7,8].

In environmental analysis, traditional methods for sample preparation are laborious, time consuming, and usually involve large amounts of solvents, which are expensive, generate considerable waste, and contaminate the sample. In addition, usually more than one clean-up stage prior to detection is required [7,8]. As a result, modern sample enrichment techniques run toward the great simplification, miniaturization, and easy manipulation of the analytical devices; strong reduction or absence of organic toxic solvents; as well as low sample volume requirements in agreement with the green analytical chemistry principles. Hence, conventional extraction methods have gradually been replaced by SPE, and more recently by several novel microextraction techniques, which remove only a portion of the analyte of interest and are based on equilibrium between the analyte and the extraction phase [3]. Over the past 15 years, a number of sorbents, solvents, and membrane-based microextraction techniques are being developed, and they offer new possibilities in sample treatment. Advantages such as a drastic reduction of the extraction time, improved sensitivity, and high sample throughput can be highlighted [6,9–18]. All these, almost solvent-free, techniques, which are continually being developed, optimized, and often modified, are available for the researchers and are readily combined with GC, offline, at-line, or sometimes even online.

In this context, a vast amount of research has been conducted in this field and a number of novel and elegant microextraction methods have been proposed in recent years for trace analysis of organic contaminants in environmental samples by using GC–MS. Most applications deal with the use of single quadrupole (Q) MS, and analysis was performed by acquiring data in full scan and/or selected ion monitoring (SIM) modes for identification and quantitation purposes, respectively. A number of excellent reviews [6,9–18] giving a comprehensive theoretical background for these techniques and focusing on these GC–MS topics are available in the literature, so such information does not fall within the scope of this chapter. Instead, the scope is limited to the new trend observed toward microextraction techniques coupled to advanced GC–MS instrumentation (MS/MS or TOF), with a stress on some promising methods appearing and/or developing rapidly during the past decade, in order to achieve the outstanding advantages over traditional extraction methods. Interested readers are encouraged to read earlier dedicated reviews [6,9–18] as well as the references cited in this chapter for additional, more comprehensive information. The results show that sorbent- and solvent-based microextraction techniques can be a promising tool to improve nowadays the performance of GC methods used in contaminant environmental control.

2 MICROEXTRACTION TECHNIQUES

Up to now, a variety of microextraction techniques for the simultaneous measurement of a cocktail of organic contaminants have been set up (Figure 1). Meanwhile, the choice of the most appropriate technique over the numerous existing ones, or the development or the innovation on a new approach is generally based on physicochemical properties of analytes, features of the techniques, and instrumentation to be used as well as on a compromise between cost, selectivity, and sensitivity [19]. As previously mentioned, the number of these techniques which deal with coupling of advanced GC–MS techniques (i.e., tandem MS and TOF) is relative small compared with that coupled to specific GC detectors or single quadrupole and IT instruments. Overall, only one sorptive and five solvent microextraction (SME) configurations have appeared during the reviewed period, based on solid-phase microextraction (SPME) as well as on single-drop microextraction (SDME), ultrasound-assisted emulsification microextraction (USAME), dispersive solid-phase extraction (DSPE), dispersive liquid–liquid microextraction (DLLME), and solidified floating organic drop microextraction (SFO–DME) concepts.

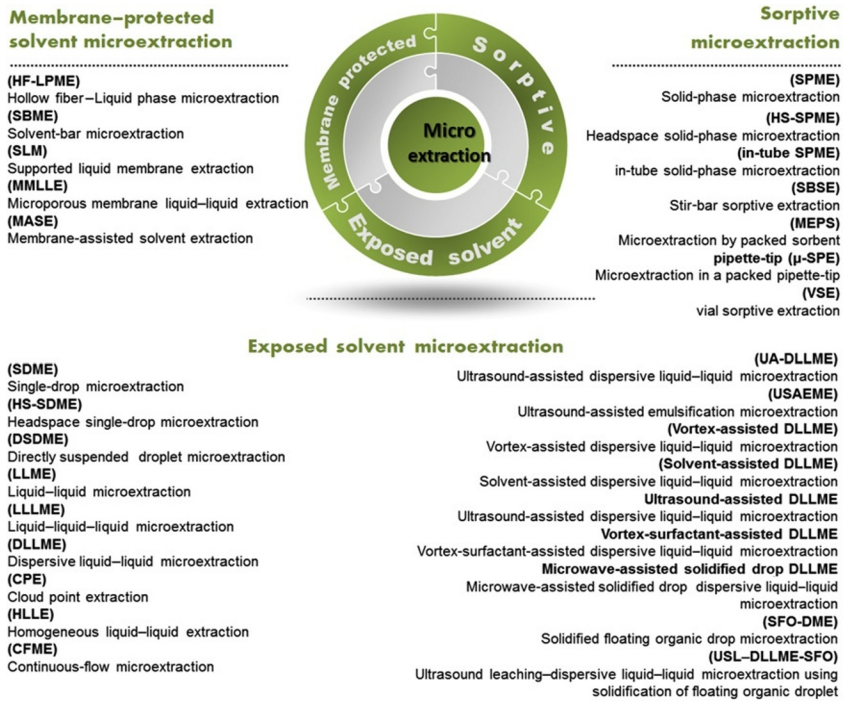

FIGURE 1 Terminology and acronyms used for the most popular microextraction techniques.

These microextraction methods will be next reviewed paying special attention to the key aspects of method development and to their applications in environmental analysis by GC–MS.

2.1 Sorptive Microextraction Techniques

2.1.1 Liquid Environmental Samples

Obviously, SPME [20] in environmental analysis has reached a level of maturity that makes it an attractive alternative to SPE in terms of liquid samples. As expected, it is the microextraction technique that is most frequently coupled to advanced GC–MS instruments due to its distinct advantages, the most important of which are as follows: (i) solvent free; (ii) easy interfacing with GC systems; (iii) simplicity of operation; (iv) integration of sampling, extraction, concentration, and sample introduction into a single step; (v) time efficient; (vi) lack of artifacts; (vii) no need of expensive or complex equipment; (viii) possibility of full automation [3,10,20]. Unlike SPE, SPME relies on quantitative but nonexhaustive transfer of analytes on the basis of equilibration between the analyte and a fused silica fiber coated with a stationary phase, which can be a liquid polymer, a solid sorbent, or a combination of both. It can combine sampling, isolation, and enrichment in one step, by two conformations: fiber SPME and in-tube SPME. Fiber SPME is the initially developed and most widely used design.

Three basic types of extraction can be performed using fiber SPME: direct extraction in which the coated fiber is immersed in the aqueous sample, headspace (HS) configuration for sampling air or the volatiles from the HS above an aqueous sample in a vial, and a membrane protection approach which protects the fiber coating for the analysis of analytes in polluted samples. The choice of sampling mode has a very significant impact on extraction kinetics and the nature of both the sample matrix and the analytes [10]. The other SPME design, in-tube SPME, is suitable for automation and online coupling with analytical instruments, which not only shortens the analysis time but often provides accuracy and precision relative to manual techniques [21–24]. After the sample is repeatedly drawn and ejected through the capillary for extraction, the extracted analytes can be desorbed by introducing a stream of mobile phase and then analyzing it by an online instrumental system. It has been demonstrated as a very efficient extraction method for the analysis of polar and thermally labile analytes and therefore is commonly used in combination with HPLC and LC–MS [21–24].

The sorptive extraction is primarily controlled by the partition coefficient of the solute between the sample and sorbent phases. Therefore, coating type is one of the most critical aspects in the SPME techniques. Different commercially available coating types (i.e., poly(dimethylsiloxane) (PDMS), poly (acrylate) (PA), carboxen/poly(dimethylsiloxane) (CAR/PDMS), carbowax/

templated resin (CW/TPR), divinylbenzene/carboxen/poly(dimethylsiloxane) (DVB/CAR/PDMS), carbowax-polyethylene glycol (PEG)) have been introduced to accomplish the extraction capability of a wide range of analytes. Also, resistances to the organic solvents along with the thermal, mechanical, and chemical stability of the SPME coatings specify their scope of applications and commercial capabilities. Despite the commercial fibers, home-made coatings [25–28] have been recently introduced to overcome some problems of commercial fibers, such as solvent instability and swelling, low operating temperature, and stripping of coating.

Besides sorbents, several other key factors that affect SPME performance, including the extraction mode, the temperature, extraction and desorption time, and concentration of NaCl, are usually properly optimized in order to achieve good accuracy and precision together with LODs and high throughput [3].

Determination of pollutants in environmental matrices by coupling SPME with advanced MS techniques has attracted considerable interest in the past few years (Table 1). With respect to mass spectrometers that allow MS/MS experiments, most of the research reviewed here reported the use of IT tandem in-time mass spectrometry detection (GC–IT-MS/MS), probably because of its ability to perform simultaneous quantitative analysis and characterization of trace-level compounds. As regards the SPME modes, HS-SPME is the most frequently used, especially in cases when modification of the matrix (i.e., change of the pH, derivatization, etc.) is necessary, since with this mode the fiber is primarily protected, expanding its lifetime. For example, POPs such as organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), and brominated flame retardants are organic compounds that exhibit very good thermostability and hence HS-SPME preconcentration can be successfully implemented at temperatures higher than 80 °C, achieving good sensitivity and selectivity.

Derouiche *et al.* [29] have developed an HS-SPME–GC–IT-MS–MS method for the simultaneous determination of 15 OCPs and 20 PCBs in aqueous samples. It was shown that the phase of fiber coating as well as parameters like extraction time, temperature, agitation, and ionic strength greatly affect the extraction process, the efficiency of which is correlated with the volatility, distribution constant, and the structures of the chlorinated congeners. Apart from extraction variables, instrumental parameters such as collision-induced dissociation (CID), resonant excitation amplitude, and RF storage level were studied and optimized for each OCP and PCB to increase specificity and sensibility for IT-MS/MS analysis. The parent ion isolated from the primary ionization was selected by the authors with the aim of achieving a compromise between both selectivity (the highest m/z ion) and sensitivity (the most abundant ion). For CID spectra of PCBs, the base peaks were always M-Cl or M-2Cl, with the latter ones being always the predominant ions for non- and mono-ortho-chlorinated congener, improving the specificity and precision of

TABLE 1 Sorptive and Solvent Microextraction Methods for the Determination of Organic Compounds in Environmental Samples

Sample Type	Class of Compounds	Analytes	Optimized Extraction Conditions	Instrumental Analysis	Rec. (%)	RSD (%)	LODs/LOQ (ng/l)	Ref.
Sorptive Microextraction								
Liquid Samples								
Deionized, river	OCPs and PCBs	HCB, PCB 8, Lindane, PCB 18, PCB 28, Heptachlor, PCB 52, Aldrin, PCB 44, Heptachlor epoxide, PCB 66, 2,4′-DDE PCB 101, *cis*-chlordane, *trans*-nonachlor, 4,4′-DDE, Dieldrin, 2,4′-DDD, PCB 77, PCB 118, PCB 153, PCB 105, 4,4′-DDT, PCB 138, PCB 126, PCB 187, PCB 128, PCB 180, PCB 170, Mirex, PCB 195, PCB 206, PCB 209	HS-SPME: PDMS 100 µm fiber; DP, 2 ml; ET, 60 min at 80 °C; stirring, desorption: 5 min, 260 °C	GC–IT-MS/MS	75–105	5–21	0.4–26	[29]
Ultrapure, river, wastewater	Chlorinated toluenes	2-CT, 3-CT, 4-CT, 2,4-DCT, 2,5-DCT, 2,6-DCT, 3,4-DCT, 2,4,5-TCT, 2,3,6-TCT	HS-SPME: PDMS/DVB fiber; DP, 10 ml; ET, 30 min at 25 °C; stirring, desorption: 2 min, 270 °C	GC–IT-MS/MS	84–112	2–12	0.030–0.330	[30]

Ultrapure, surface, drinking, groundwater, wastewater	Fragrances and earthy–musty compounds	IB, MIB, TCA, GSM, GAL, MX, TON, MK	HS-SPME: PDMS/DVB fiber; DP, 10 ml; ET, 40 min at 70 °C; 200 g/l NaCl; stirring, 250 rpm; desorption, 8 min, 250 °C	GC–IT-MS/MS	67–119	6–20	0.02–20	[31]
Milli-Q, river, wastewater	Estrogens	DES, E1, E2, MES, EE2	SPME: PA fiber; DP, 100 ml; ET, 60 min; 300 mg/ml NaCl; pH 6; stirring, 400 rpm; derivatization HS-SPME: 1.5 vial, 50 µl MSTFA, 60 °C, 30 min; desorption, 5 min, 280 °C	GC–IT-MS/MS	1–12	80–120	0.2–3.0	[32]
Ultrapure, river, swimming pool water, wastewater	Parabens, triclosan, and related chlorophenols	2,4-DCP; 2,4,6-TCP; MP; EP; PP; BP; TCS	HS-SPME: DVB/CAR/PDMS fiber, DP, 10 ml, 0.1 g sodium hydrogen phosphate heptahydrate, 100 µl AAA (derivatization reagent), 35% NaCl, 100 °C	GC–IT-MS/MS	82–102	2–12	4–21	[33]

Continued

TABLE 1 Sorptive and Solvent Microextraction Methods for the Determination of Organic Compounds in Environmental Samples—Cont'd

Sample Type	Class of Compounds	Analytes	Optimized Extraction Conditions	Instrumental Analysis	Rec. (%)	RSD (%)	LODs/LOQ (ng/l)	Ref.
Ultrapure, wastewater	Primary amines	MA, EA, IPA, IBA, BA, IAA, AA, CA, HA, PEA	HS-SPME: PA fiber; DP, 10 ml; ET, 45 min at 40 °C; 360 mg/ml NaCl; pH 12; stirring, 750 rpm; derivatization: 1.5 vial, 200 µl PFBAY, 60 °C, 30 min; desorption, 8 min, 250 °C	GC–IT-MS/MS	–	2–11	10–2500/25–7500	[34]
Tap, potable, wastewaters	*N*-nitrosamines	NDBA, NDEA, NDMA, NDPA, NDPhA, NMEA, NMOR, NPIP, NPYR	HS-SPME: DVB/CAR/PDMS fiber; DP, 10 ml; ET, 60 min; 360 g NaCl; pH 7; *T*, 45 °C; desorption: 18 min, 250 °C	GC–IT-MS/MS	–	3–14	1–5/10–15	[35]
Tap, drinking, wastewaters	*N*-nitrosamines	NDMA, NDEA, NDPA, NMOR	HS-SPME: CAR/PDMS fiber; DP, 4.5 ml; 1.54 g NaCl; ET, 60 min; *T*, 85 °C; desorption: 4 min, 250 °C	GC–IT-MS/MS	92–122	3.3–15.9	3.2–15.2	[36]
Ultrapure, mineral, tap water	Pesticides	Propoxur, carbofuran, pirimicarb, carbaryl, methiocarb	SPME: PDMS/DVB fiber; DP, 10 ml; ET, 45 min; 1 g NaCl; desorption: 6.5 min, 270 °C	GC–QqQ-MS	71–125	1–9	0.04–1.7/0.64–2.9	[37]

Disinfected water, untreated water	Disinfection by-products (DBPs)	Trichloromonofluoromethane, benzene, dichloromethane, 1,1-dichloroethene, 1,2-dichloroethene (E), carbon tetrachloride, 1,1-dichloroethane, trichloromethane, 1,1,1-trichloroethane, trichloroethylene, 1,2-dichloroethane, 1,2-dichloropropane, chlorobenzene, bromodichloromethane, 1,3-dichloro-1-propene (E), 1,3-dichloro-1-propene (Z), toluene, 1,1,2-trichloroethane, 2-chloroethoxyethene, ethylbenzene dibromochloromethane, 1,2-dichlorobenzene tribromomethane, tetrachloroethylene, 1,1,2,2-tetrachloroethane, 1,4-dichlorobenzene, 1,3-dichlorobenzene	HS-SPME: DVB/CAR/PDMS fiber; DP, 5 ml; NaCl 1% (v/v); ET, 2 min; *T*, 35 °C; desorption 270 °C, 45 s	GC–TOF-MS	–	1.4–14.4	15–477	[38]
Solid Samples								
Sediments	PBDEs	BDE-47, BDE-100, BDE-99, BDE-85, BDE-153, BDE-154	HS-SPME: PA fiber, 0.5 g sediment, 40 mg $KMnO_4$; 0.5 ml H_2SO_4, 5 ml water, ET, 40 min, *T*, 100 °C; desorption, 300 °C, 2 min	GC–IT-MS/MS	76–111	1–14	<0.15 ng/g	[39]

Continued

TABLE 1 Sorptive and Solvent Microextraction Methods for the Determination of Organic Compounds in Environmental Samples—Cont'd

Sample Type	Class of Compounds	Analytes	Optimized Extraction Conditions	Instrumental Analysis	Rec. (%)	RSD (%)	LODs/LOQ (ng/l)	Ref.
Sewage sludge	Primary amines	Methylamine, ethylamine, isopropylamine, isobutylamine, butylamine, isoamylamine, amylamine, cyclohexylamine, heptylamine, 2-phenylethylamine	PHWE-HS-SPME: PHWE: 1 g sediment, 1 g diatomaceous earth, *T*, 100 °C; EPR: 1500 psi; 2 cycles, FV: 60%; ET,15 min, pH 4; HS-SPME: PA fiber; PHWE extract to 25 ml water, 10 ml sample, pH 12; 3.6 g NaCl; derivatization: PFBAY (200 µl); *T*, 40 °C; ET, 15 min; stirring, 750 rpm; desorption, 250 °C, 8 min	GC–IT-MS/MS	–	5–24	9–135 ng/g/ 50–450 ng/g	[40]

Sewage sludge	*N*-nitrosamines	NDBA, NDEA, NPYR, NDPhA	PHWE-HS-SPME: PHWE: 5 g sludge, 1 g diatomaceous earth, *T*, 125 °C; EPR: 1500 psi; 2 cycles, FV: 60%; ET, 5 min, pH 7.5; HS-SPME: PHWE extract, 10 ml sample; DVB/CAR/PDMS fiber; DP, 10 ml; ET, 60 min; 360 g NaCl; pH 7; *T*, 45 °C; desorption, 18 min, 250 °C	GC–IT-MS/MS	–	5–19	30–150 ng/g 100–500 ng/g	[41]
Soil	Organochlorine pesticides	Mirex, α- and γ-chlordane, p,p′-DDT, heptachlor, heptachlor, epoxide isomer A, γ-hexachlorocyclohexane, dieldrin, endrin, aldrine, and hexachlorobenzene	MAE-HS-SPME: 20 ml hex:acet (1:1); pressure 200 psi; *T*, 115 °C; ET, 10 min; HS-SPME: MAE extract, 15 ml was evaporated to dryness, redissolved to 40 ml water sample; PDMS fiber; DP, 40 ml; ET, 60 min; *T*, 65 °C; desorption, 15 min, 290 °C	GC–IT-MS/MS	8–51	14–36	0.02–3.6 ng/g	[42]
Solvent Microextraction								
Liquid Samples								
Ultrapure, wastewater, potable	Musk fragrances	DPMI, ADBI, AHMI, ATII, HHCB, AHTN, MX, MM, MK, HHCB-lactone	IL–HS-SDME: DP, 10 ml; AP, [OMIM][PF_6] (1 µl); *T*, 60 °C; ET, 45 min; stirring, 750 rpm, 300 g/l NaCl	GC–IT-MS/MS	–	3–11	10–30	[43]

Continued

TABLE 1 Sorptive and Solvent Microextraction Methods for the Determination of Organic Compounds in Environmental Samples—Cont'd

Sample Type	Class of Compounds	Analytes	Optimized Extraction Conditions	Instrumental Analysis	Rec. (%)	RSD (%)	LODs/LOQ (ng/l)	Ref.
Ultrapure, river, wastewater	Personal care products	TCS, MTCS	DLLME, DP, 10 ml; disperser, MeOH (1 ml); AP, CH_3CCl_3 (40 µl); derivatization: MTBSTFA (40 µl); centrifugation, 3 min (3500 rpm); sedimented phase (39 µl)	GC–IT-MS/MS	82–105	3–9	2–5	[45]
Ultrapure, river, swimming pool water, wastewater	Parabens, triclosan, and related phenols	2,4-DCP, 2,4,6-TCP, MP, EP, PP, BP, TCS	USAME, DP, 10 ml; AP, 1,1,1-trichloroethane (100 µl); 1 g Na_2HPO_4, ET, 5 min; derivatization: acetic anhydride (200 µl)	GC–IT-MS/MS	85–100	7–13	3.9–12.5	[46]

Solid Samples

Sediments	PBDEs	BDE-47, BDE-100, BDE-99, BDE-153	USAL–DSPE–DLLME: USAL: 0.25 g, 1.5-ml acet; 0.2% detergent; 35 °C; 5 cycles (5 min); centrifugation (3500 rpm, 5 min); DSPE: 1.2 ml USAL extract, 100 mg of activated silica gel, vortex (30 s), centrifugation (3500 rpm, 3 min); DLLME: 1 ml DSPE extract, 5 ml water, CCl_4 (60 µl), shaking (10 s), thermostatic bath (35 °C, 5 min); centrifugation (3500 rpm, 2 min)	GC–IT-MS/MS	80–111	5–10	0.25 and 1 ng/g	[47]
Sediments	PBDEs	BDE-47, BDE-100, BDE-99, BDE-153	USL–DLLME–SFO: USAL: 1 g, 1.5 ml MeOH, 40 °C, 2 cycles (9.2 min), centrifugation (200 rpm, 10 min); DLLME: 0.4 ml USAL extract; disperser: MeOH (100 µl); ES, 1-dodecanol (26.5 µl, 20 °C), 1 ml NaCl 6.15 M; SFO: 4.4 ml water, 40 °C; ice bath: 10 min; 3 µl isooctane	GC–IT-MS/MS	71–104	5–9	500–1800 ng/g/ 170–600 ng/g	[48]

Continued

TABLE 1 Sorptive and Solvent Microextraction Methods for the Determination of Organic Compounds in Environmental Samples—Cont'd

Sample Type	Class of Compounds	Analytes	Optimized Extraction Conditions	Instrumental Analysis	Rec. (%)	RSD (%)	LODs/LOQ (ng/l)	Ref.
Sewage sludge	Musk fragrances	DPMI, ADBI, AHMI, ATII, HHCB, AHTN, MX, MM, MK, HHCB-lactone	PLE–HS-SPME: PLE: 1 g sludge, H_2O/methanol (1:1); 1 g florisil, *T*, 80 °C; EPR: 1500 psi; 2 cycles, FV: 60%; ET, 5 min; IL–HS-SDME: DP, 10 ml; AP, [OMIM][PF_6] (1 µl); *T*, 60 °C; ET, 45 min; stirring, 750 rpm, 300 g/l NaCl	GC–IT-MS/MS	–	3–10	0.5–1.5 ng/g/ 2.5–5 ng/g	[44]

1,1,3,3,5-pentamethyl-4,6-dinitroindane (MM, musk moskene); 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-(g)-2-benzopyran (HHCB, galaxolide); 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-[g]-2-benzopyran-1-one (HHCB-lactone, galaxolidone); 17β-estradiol (E2); 17β-ethinylestradiol (EE2); 1-pentanethiol ($C_5H_{12}S$); 2,2′-dihydroxy-4-methoxybenzophenone (BP-8); 2,3,4,5,6-pentafluorobenzaldehyde (PFBAY); 2,3,6-trichlorotoluene (2,3,6-TCT); 2,4,5-trichlorotoluene (2,4,5-TCT); 2,4,6-trinitro-1,3-dimethyl-5-tert-butylbenzene (MX, musk xylene); 2,4-dichlorotoluene (2,4-DCT); 2,4-dihydroxybenzophenone (BP-1); 2,5-dichlorotoluene (2,5-DCT); 2,6-dichlorotoluene (2,6-DCT); 2-chlorotoluene (2-CT); 2-hydroxy-4-methoxybenzophenone (BP-3); 2-phenylethylamine (PEA); 3,3,5-trimethylcyclohexyl salicylate (homosalate, HMS); 3,4-dichlorotoluene (3,4-DCT); 3,4-methylenedioxyamphetamine (MDA); 3-chlorotoluene (3-CT); 4-acetyl-1,1dimethyl-6-tert-butyllindane (ADBI, celestolide); 4-chlorotoluene (4-CT); 4-methylbenzylidene camphor (4-MBC); 5-acetyl-1,1,2,6-tet-ramethyl-3-isopropylindane (ATII, traseolide); 6,7-dihydro-1,1,2,3,3-pentamethyl-4(5H)-indanone (DPMI, cashmeran); 6-acetyl-1,1,2,3,3,5-hexamethylindane (AHMI, phantolide); 7-acetyl-1,1,3,4,4,6-hexamethyl-1,2,3,4-tetrahydro-naphthalene (AHTN, tonalide); acetic acid (AA); acetic acid anhydride (AAA); amphetamine (AMP); amylamine (AA); acceptor phase (AP); butyl mercaptan ($C_4H_{10}S$); butyl methoxydibenzoylmethane (BM-DBM); carbon disulfide (CS_2); cyclohexylamine (CA); dibutyltin (DBT); diethylstilbestrol (DES); dimethyl disulfide ($(CH_3)_2S_2$); dimethyl sulfide ($(CH_3)_2S$); donor phase (DP); estrone (E1); ethyl mercaptan (CH_3CH_2SH); ethylamine (EA); ethylhexyl methoxycinnamate (EHMC); ethylhexyl salicylate (EHS); ethylhexyl salicylate (EHS); extraction solvent (ES); extraction time (ET); formic acid (FA); homosalate (HMS); isoamyl methoxycinnamate (IAMC); isoamylamine (IAA); isobutylamine (IBA); isopropylamine (IPA); liquid desorption (LD); mestranol (MES); methamphetamine (MAMP); methylamine (MA); methylmercury ($MMHg+$); methyltriclosan (MTCS); monobutyltin (MBT); *n*-butylamine (BA); *N*-ethyl-3,4-methylenedioxyamphetamine (MDEA); *n*-heptylamine (HA); *N*-methyl-3,4-methylenedioxyamphetamine (MDMA); *N*-methyl-*N*-(tert-butyldimethylsilyl) trifluoroacetamide (MTBSTFA); *N*-nitrosodiethylamine (NDEA); *N*-nitrosodimethylamine (NDMA); *N*-nitroso-di-*n*-butylamine (NDBA); *N*-nitroso-di-*n*-phenylamine (NDPhA); *N*-nitroso-di-*n*-propylamine (NDPA); *N*-nitrosomethylethylamine (NMEA); *N*-nitrosomorpholine (NMOR); *N*-nitrosopiperidine (NPIP); *N*-nitrosopyrrolidine (NPYR); octocrylene (OC); octyldimethyl-*p*-aminobenzoic acid (ODPABA); pentachlorotoluene (PCT); propyl mercaptan (C_3H_8S); temperature (*T*); thermal desorption–gas chromatography–mass spectrometry (TD–GC–MS); tributyltin (TBT); triclosan (TCS); volatile organic sulfur compounds (VOCs).

the detection method. For almost all the studied OCPs, several predominant ions were produced corresponding to losses of one and/or two chlorines, and/or HCl and/or two HCl neutrals, except for heptachlor epoxide, for which losses of H_2O and CO were also observed. The authors stressed the weakness to identify and quantify the 4,4′-DDD and 2,4′-DDT congeners because of its similarity in both the retention time and the CID spectra. It was concluded that the proposed method combined sensitivity, specificity, precision, and reliability and certainly relieves the analyst of some of the pitfalls of present GC–ECD-based methods, without sacrificing performance. It was proved, however, crucial to optimize parameters that affect the efficiency of the combined methodology.

Like POPs, ring-chlorinated derivatives of toluene constitute a group of thermostable chemicals with relatively high vapor pressures and therefore would be easily subjected to HS-SPME determination. In this context, a simple and fast methodology has been developed by Regueiro *et al*. [30] for the analysis of chlorotoluenes in water samples using SPME coupled to GC–MS/MS. A multifactorial experimental design strategy was used for studying the influence on extraction yield of factors such as fiber coating, extraction mode, temperature, and addition of sodium chloride. Based on the evaluation of the results, it can be concluded that fiber and extraction mode were the most significant factors for all studied compounds, achieving higher extraction yields using PDMS/DVB fiber in the HS mode. The concentration of NaCl was also significant for the polychlorinated toluenes, whereas temperature only presented statistical significance for pentachlorotoluene. Magnetic stirring is also essential for the HS-SPME mode improving the sensitivity of the method in a shorter time. Similar to stirring, extraction time heavily affects the response of the target analytes. The extraction profiles for some representative compounds showed that the time required for reaching equilibrium is related to the number of chlorine atoms in the toluene ring. For example, monochlorotoluenes reach the equilibrium conditions after 30 min, whereas dichlorotoluenes and trichlorotoluenes do not reach equilibrium till up to 60 min, although they seem to be close to this condition in 30 min of fiber exposure. At optimal conditions, the method was sensitive enough to ensure reliable determination at low pg/l levels. It is worth pointing out that the scarcity of information and methodologies for the analysis of these compounds emphasize the importance of this developed method.

Besides POPs and chlorinated toluenes, other potent contaminant categories, such as personal care products and earthy–musty compounds, have also been analyzed by the HS-SPME method. Alpendurada's group has employed HS sampling in order to demonstrate the applicability of this SPME extraction mode for the combined determination of earthy–musty compounds and fragrances in waters [31]. The extraction performance of six fibers with different coatings was evaluated with the PDMS/DVB being the most suitable for most of the studied compounds (best for five out of eight analytes). Analyzing each

group individually, the earthy–musty odors are better extracted with the DVB/CAR/PDMS fiber, except for 2,4,6-trichloroanisole (TCA), while the fragrances can be analyzed either with the PDMS/DVB or the PDMS fibers. After the selection of the best SPME fiber for the simultaneous analysis of fragrances and earthy–musty compounds, a central composite design (CCD) was implemented and analyzed with desirability functions to determine the optimal values of three variables: extraction time, temperature, and ionic strength. Considering all the compounds under study, the Pareto chart shows that all the tested factors have an important impact in the SPME extraction efficiency. Individually, the earthy–musty odors require lower temperatures (50 °C), while for fragrances, a higher temperature should be used (90 °C). The fitness for purpose of the developed method for the analysis of fragrances and earthy–musty compounds, under both full scan and MS/MS mode, has been assessed by taking into consideration the following figures of merit: detection (LODs) and quantitation limits (LOQs), repeatability, intermediate precision, calibration parameters, and matrix effect. In almost all cases, MS/MS mode has been found superior compared to full scan mode revealing its distinct advantages. For example, in MS/MS mode, the relative standard deviation (RSD) for earthy–musty odors is noticeably better than for fragrances. In addition, LODs for geosmin and TCA in MS/MS mode are in the range of sub-ppt, namely, about 20 and 100 times lower than in full scan mode. As regards the matrix effect, it is apparent that it influences the extraction efficiency, especially in the case of the most loaded samples like wastewaters and surface water receiving wastewater treatment plant (WWTP) effluents. Due to the different type of matrix effect, correction of results by recovery experiments or use of deuterated internal standards (ISs) for the most ubiquitous compounds is recommended by the authors. The method was effectively applied to the analysis of several water samples showing the occurrence of some of the target compounds. For instance, galaxolide, despite its retraction, was found in significant concentration levels (up to 250 ng/l) in the four terminal sampling stations of river Leca (Portugal), which are downstream of WWTPs and polluted tributaries. Geosmin was ubiquitously distributed in natural waters similarly in rivers Leca and Douro (Portugal), at levels not causing concern (<7 ng/l).

All the previous HS-SPME approaches are generally limited to volatile and thermally stable compounds. However, many polar analytes need to be derivatized before GC separation, either to increase their volatility and thermal stability or to decrease their adsorptivity. Typically, SPME derivatization is performed previously in the sample, or simultaneously with the HS extraction step (*in situ*), but it is also possible to include on-fiber derivatization in the HS analysis. The second approach, however, is more time consuming than simply adding the derivatization agent to the sample because, in fact, a two-step procedure is mandatory. Therefore, *in situ* derivatization may be preferred in HS-SPME [10].

Both *in situ* and on-fiber derivatization approaches have been performed in HS-SPME analysis for the determination of environmental pollutants by using different kinds of derivatization reagents. As an example, *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA) has been used by Carpinteiro *et al*. [32] for on-fiber derivatization of five estrogenic hormones. The analytical procedure involved direct SPME enrichment of estrogens on a coated fiber followed by an on-fiber derivatization step on the HS of a vial containing 50 μl of MSTFA. Derivatization conditions (derivatization time (from 10 to 60 min), temperature (from 40 to 80 °C), and volume of MSTFA (from 20 to 100 μl)) were studied and optimized. The results showed that the volume of MSTFA did not affect the yield of the derivatization, whereas for time and temperature, two different patterns in the behavior of the tested analytes were observed. The silyl derivatives of diethylstilbestrol (DES), estrone (E1), and 17β-estradiol (E2) were scarcely affected by the operating conditions, while an opposite effect has been observed for 17α-ethinylestradiol (EE2) and mestranol (MES). The influence of various factors (pH, NaCl, stirring) on the yield of the microextraction was evaluated using a factorial experimental design at two levels (2^3) with two central points. Stirring was the most important factor with a significant and positive effect on the yield of the microextraction, especially for the samples spiked with NaCl. The study of matrix effects demonstrated that the method is applicable for the determination of all the compounds in surface and sewage water, with the exception of DES and MES in nontreated sewage water. To overcome the matrix limitations, isotopic dilution analysis was proposed by the authors by using E2 as a model compound. The proposed method was proved to be an attractive alternative to the standard addition technique, improving the precision of the results and reducing the experimental effort. However, according to the authors, this approach should be tested for other estrogens using their corresponding isotopic labeled species in order to reveal its applicability for determining trace amounts of estrogens in sewage water samples containing a high degree of interference.

On the other hand, as an alternative, *in situ* derivatization has been proposed by Llompart's group for the HS-SPME determination of parabens, triclosan (TCS), and related chlorophenols in water by using acetic anhydride as acetylation reagent [33]. Acetylated derivatives are selectively determined using GC–MS/MS. Parameters affecting both derivatization and SPME procedures, such as fiber coating, extraction mode, temperature, volume of derivatizating reagent, and ionic strength, are studied and optimized through a multifactorial experimental design. Extraction mode was the most important factor, showing statistical significance for all studied parabens and also for 2,4,6-TCP, whereas fiber coating and volume of derivatizating reagent were not significant for any of the target compounds. Concentration of NaCl and temperature presented statistical significance only for parabens. The latter parameter was also significant for triclosan. Magnetic stirring and extraction

time were also important factors. Based on the extraction time profiles for some representative compounds, different equilibrium time can be achieved depending on the chemical family. Chlorophenols reach the equilibrium conditions in only 15 min, whereas parabens need 30 min of fiber exposure to achieve this condition. The proposed method was demonstrated to be suitable for the intended purpose, in terms of sensitivity, precision, and accuracy. The absence of matrix effects, even for most complex samples, suggests the use of external calibration with ultrapure water standards as a reliable quantification approach.

Given the advantages of automation, a fully automated method based on *in situ* HS-SPME derivatization with pentafluorobenzaldehyde (PFBAY) followed by GC–IT-MS/MS has been recently proposed for determining 10 primary amines in wastewater with different origins and matrix complexities [34]. The influence of main factors on the efficiency of derivatization and of HS-SPME is described in detail and optimized by a CCD. For all species, the highest enrichment factors were achieved using a 85-μm PA fiber exposed in the HS of stirred water samples (750 rpm) at pH 12, containing 360 g/l of NaCl, at 40 °C for 15 min. The mass spectra of the PFBAY-imine derivates showed typical fragment ions at *m/z* 181 and 208, corresponding to the fragment $[CH—C_6F_5]^+$ and the typical α-cleavage product ion $[CH_2—NCH—C_6F_5]^+$, respectively. The latter ion was observed in all cases except for methyl-d_3-amine hydrochloride (dMA), isopropylamine (IPA), and cyclohexylamine (CA) for which base peak ions having three deuterated protons (*m/z* 221) and corresponding to the losses of a methyl group, $[M—CH_3]^+$, and ethyl group, $[M—C_2H_5]^+$, respectively, were observed. For amylamine (AA) and *n*-heptylamine (HA), a base peak ion at *m/z* 250, which points to the fragment $[M—C_3H_7]^+$, formed by the loss of a propyl group, was observed. The entire analytical process, including sample preparation and determination, is fully automated and offers many improvements over the existing extraction methods, enabling satisfactory precision (RSD $\leq$ 11%) and high sample throughput. Moreover, the use of MS/MS rather than single MS detection provides high selectivity for the determination of primary amines in complex matrices, such as industrial wastewater samples. The last feature of the proposed method is of great importance taking into account that both GC and LC analysis of aliphatic amines in aqueous samples have traditionally been difficult due to their particular physicochemical properties, such as high volatility and polarity, basic character, and high solubility in water. In the case of GC analysis, many shortcomings have been reported including poor chromatographic performance, tailing peaks, and memory effects. Furthermore, problematic identification or quantification through SIM mode is usually observed because of the α-cleavage, which usually results in a base peak at *m/z* 30 $[CH_2{=}NH_2]^+$ that provides little possibility for confirmation. These limitations are overcome with the combination of a simple SPME technique and a GC–MS/MS system, and it

is clear that the MS/MS proved to be much more powerful for both identification and quantitation compared to Q MS systems. Finally, the applicability of the method was tested for several real wastewater samples, including industrial wastewater, municipal wastewater, and potable water, confirming the presence of the target analytes at ppb levels. Due to matrix effects in the case of industrial wastewater samples, the use of standard addition method is proposed by the authors for quantification instead of an IS method.

Another automated method based on the HS-SPME followed by GC–IT-MS/MS using chemical ionization has been also proposed by the same group for determining *N*-nitrosamines in different water samples [35]. The divinylbenzene/carboxen/PDMS fiber was the most suitable for all the target compounds among the three different tested fibers (carboxen/PDMS, divinylbenzene/carboxen/PDMS, and PEG). The fully automated, simple, and environment-friendly method provided low LODs (between 1 and 10 ng/l) and satisfactory precision (RSD 15%). A similar GC–IT-MS/MS using chemical ionization has been reported by Hung *et al.* also for the *N*-nitrosamines [36]. The work showed that the use of NDMA-d_6 as IS for the determination of the analytes is a reliable quantification strategy. The authors noted that the proposed method, although slightly less sensitive for some compounds compared to the current methods commonly used for the analysis of *N*-nitrosamines, requires less sample volume and is solventless and more rapid.

As previously mentioned, all of the above published methods are mostly focused on the use of GC–IT-MS/MS systems. Examples of tandem MS using a QqQ mass spectrometer are scarce. To the author's knowledge, only one study has been reported in the literature, which came very recently from Cavaliere *et al.* [37]. In this study, SPME was combined with GC–QqQ-MS for the determination of seven carbamate pesticides in water samples. The PDMS/DVB 65-μm fiber was the most suitable among the five tested coatings (carboxen/polydimethylsiloxane 85 μm (CAR/PDMS), polyacrylate 85 μm (PA), divinylbenzene/carboxen/polydimethylsiloxane 50/30 μm (DVB/CAR/PDMS), polydimethylsiloxane/divinylbenzene 65 μm (PDMS/DVB), and polydimethylsiloxane 100 μm (PDMS)) for preconcentrating the analytes. The efficiency of SPME analysis was further investigated and optimized by using a multivariate approach of design of experiment and, in particular, a CCD. Pareto charts were evaluated to know the weight of contribution of each factor to response and possible cross-effect among these variables. The results obtained showed that the percentage of NaCl, followed by the extraction time, had the greater contribution to the response of almost all studied carbamates except for pirimicarb which was not significantly affected by any of these variables and interaction terms. The analytical utility of multivariate chemometric techniques was also used to explore the chromatographic behavior of the carbamates and to evaluate the importance of each variable investigated and possible cross-effect among these factors on signal of the studied

carbamates. Based on the overall appraisement of the results, it can be concluded that injection temperature was the most important variable which significantly affected the response of the analytes, while GC oven parameters and age of column showed a negligible effect. The authors stressed the importance of quantification by IS calibration in order to avoid the matrix effects and interferences with GC signals and to achieve good reproducibility and robustness for the whole procedure. Three compounds (2,3,5-trimethacarb, 4-bromo-3,5-dimethylphenyln-methylcarbamate (BDMC), and carbaryl-d_7) with a thermal behavior similar to those analytes were evaluated as ISs. Among them, 2,3,5-trimethacarb and carbaryl-d_7 were found suitable for quantitative analysis of propoxur, carbofuran, and pirimicarb, and carbaryl and methiocarb, respectively, whereas BDMC was immediately discharged due to significant memory effect and insufficient desorption of analyte from fiber. It was concluded that the proposed method was a convenient new approach to the analysis of carbamates in water samples due to its distinct advantages such as simplicity, speed, ease, and automatable operation without any derivatization step. Furthermore, it offered much better sensitivity (0.04–1.7 ng/l) compared to EPA Method 531.1 (2.0 and 4.0 μg/l for carbaryl and methiocarb, respectively) and it was superior to previously published methods based both on LC and GC approaches.

As in the case of QqQ spectrometer, only one specialized work has been published until now on the GC–TOF-MS system. In this study, an HS-SPME method was coupled to GC–TOF-MS with a split/splitless injector for extracting and analyzing volatile organic compounds, including disinfection by-products [38]. The DVB/CAR/PDMS fiber was the most suitable among those tested (PDMS (7 and 100 μm), CAR/PDMS, PDMS/DVB, and DVB/CAR/PDMS), although the CAR/PDMS fiber was also good for some analytes. Additional parameters that affect the extraction process such as extraction temperature, and extraction, incubation, and desorption times were also tested and optimized. The procedure has been applied to a number of water samples where several VOCs were detected. The results from these quantitative measurements were compared with results obtained by liquid–liquid extraction (LLE). Overall, these results were comparable and helped to validate the method of analysis developed in this interlaboratory study. The proposed method offers many advantages for analyzing VOCs in water samples and can be used as an alternative to the purge and trap EPA Method 624.

2.1.2 Solid Environmental Samples

The SPME assay for the determination of organic pollutants in solid matrices, such as sewage sludge, sediments, and soils, is extremely attractive because of the well-established advantages of the HS mode. Four publications dealt with this issue. In the first one, Montes *et al.* [39] have developed a method by combining an oxidative sample treatment (based on the use of $KMnO_4$ under

acidic conditions) with the HS extraction mode to determine polybrominated diphenyl ethers (PBDEs) from sediment samples. The quantification was performed using GC–MS/MS. Certain factors that affected the performance of the microextraction process were thoroughly evaluated and related to the total organic carbon of the sample. The amount of $KMnO_4$ had a major effect on the efficiency of the HS extraction, with the optimum mass being correlated to the content of organic carbon in the sample. The method described was very simple, low cost, and fast, and could constitute an alternative method to monitor the levels of tetra- to hexabrominated PBDEs in sediments that contain up to 6% of organic carbon.

In the second publication, integrating the advantages of pressurized hot water extraction (PHWE) and HS-SPME, Llop *et al*. [40] proposed a method for the determination of 10 primary amines in sewage sludge. Derivatization of the aqueous sludge extract was performed with PFBAY based on their previous HS-SPME method [34]. The influence of most critical factors (i.e., pH of water as the solvent extraction, the extraction time, and the extraction temperature) affecting the extraction efficiency in PHWE was optimized by a CCD. In most cases, the extraction temperature was the most important parameter in the PHWE process, improving the efficiency of the process at higher values (100 °C). The pH of water as the extraction solvent was the second most important factor, followed by the extraction time, which was the least influential factor for most analytes. Higher responses were observed at the lowest pH value (pH 4) and highest extraction time (15 min). Main attention during method development was given to the negative effects of the sludge matrix on the SPME efficiency and on the chromatographic resolution. The authors concluded that the matrix of the samples affects the quantification of target compounds and they suggested the use of standard addition calibration in order to avoid this problem. Despite the matrix effect, validation data were satisfactory, indicating a useful method. The method was tested for its applicability to the analysis of the target compounds in several types of sewage wastewater samples, confirming the presence of most of the target compounds at mg/kg (dry weight (d.w.)) levels, increasing from the sewage sludge of the potable water plant to the sewage sludge of industrial WWTPs.

The same integration approach has been proposed also by the same research group for the quantitative determination of the nine *N*-nitrosamines in sewage sludge [42]. Extracts of PHWE which were optimized using a CCD with regard to operational parameters were subjected to previous optimized HS-SPME process [41]. The determination of the analytes was performed by GC tandem MS by using the chemical ionization mode (GC–CI–IT-MS/MS). The integration approach provided LODs between 0.03 and 0.15 μg/kg, and the use of CI–MS/MS instead of single CI–MS detection was found superior in terms of selectivity and sensitivity for the determination of target *N*-nitrosamines in such highly complex sewage sludge samples from WWTPs.

In the fourth report, Herbert and coworkers [42] presented a powerful approach to directly coupling microwave-assisted extraction (MAE) with HS-SPME process for the determination of 11 OCs in soil samples by using a PDMS fiber. Comparing the precision obtained in the direct injection with that of the whole MAE–HS-SPME–GC–MS/MS process, the authors noted that the variability came mainly from the chromatographic step, which could possibly be reduced using deuterated pesticide surrogates. Overall, the proposed method integrates the advantages of MAE and enrichment and sample-clean-up capability of the HS-SPME into a single step, making it a promising sample preparation method for complex samples.

2.2 Solvent-Based Microextraction

2.2.1 Liquid Environmental Samples

Since its inception in 1995, SME techniques for sample preparation have grown increasingly popular due to their simplicity, low cost, and adaptability to a wide variety of sample types and analytes [9,12,14,49]. SME configuration combines the classic extraction by liquid and microextraction in a stationary phase, and it has generally been considered as an alternative SPME concept, in which a microvolume (<100 μl) or a single drop of an extractant phase is utilized as the collection phase replacing the coated fiber. Over the past years, a number of versatile acronyms (Figure 1) based on principle, configuration, scale or size, operation procedure, physical state of samples and/or solvent, and the physical or chemical nature of sampling process have appeared in the literature to describe the process, making it sometimes difficult or confusing to differentiate between the developed SME techniques. For example, SME is also frequently called liquid-phase microextraction (LPME), but this descriptive term is associated by many with the membrane-protective modes of SME, rather than as a general term. Taking into consideration the SME terminology, which was given in a recent review article [49], SME techniques can fall into two broad categories: exposed solvent and membrane-protected solvent (Figure 1).

SDME was the first SME method developed and has some major advantages, as well as some significant disadvantages. According to this technique, a drop of the selected water-insoluble solvent is suspended at the tip of a GC syringe and immersed in an aqueous sample or exposed to the HS of a sample (contained in a vial) for a preselected time. The SDME technique, in any of its modes, is simple, cheap, and rapid; requires very small amounts of solvent; and does not require specialized apparatus. Despite this, however, it inherits some drawbacks from LLE such as the formation of emulsion and dissolution of the liquid droplet in dealing with some dirty samples. The droplet may be lost from the needle tip of the syringe during the extraction, especially when samples are stirred vigorously. If the samples are not perfectly clean, an

emulsion may form [14,15]. This limits the usable volume of the extracting medium and directly affects the precision and also the sensitivity of the determination. This limitation is more marked when HS-SDME is performed at high temperature because of the evaporation of the organic solvent during the extraction [3,9,10,49,50]. Improving the stability of the droplet is thus a challenging topic to be overcome. In this regard, ionic liquids (ILs) have been recently proposed as alternatives to organic solvents due to their unique physical and chemical properties [51,52]. ILs are organic salts which consist largely of organic cations paired with organic or inorganic anions. These compounds are often defined to possess melting points less than or equal to 100 °C. The structure of ILs can be designed to produce desired properties including negligible vapor pressure, elevated thermal stability, tunable viscosity and miscibility with other solvents, as well as the capability of undergoing numerous solvation interactions. An interesting example of coupling ILs with HS-SDME was recently reported by Vallecillos *et al.* [43]. In this study, a fully automated IL–HS-SDME procedure has been developed for the first time to preconcentrate trace amounts of 10 musk fragrances extensively used in personal care products (six polycyclic musks, three nitromusks, and one polycyclic musk degradation product) from wastewater samples prior to analysis by GC–IT-MS/MS. To adapt the IL (viscous solvent) to the GC, a large internal diameter (3.4 mm) liner was used in order to improve the IL evaporation. A piece of glass wool was introduced to avoid the entrance of the ILs in the GC column. Furthermore, a guard column was used to prevent analytical column damages. Several parameters that affect the performance of HS-SDME such as extraction solvent, drop volume, stirring rate, extraction temperature, salt concentration, sample volume, and extraction time were tested and optimized. As previously outlined, the drop instability is the major drawback of the SDME technique. Therefore, special attention was given by the authors to the first four variables which are strongly correlated and affect drop stability. At optimal conditions, the method was demonstrated to be feasible for qualitative and quantitative analysis of the target compounds in environmental water samples. Matrix effects upon extraction were also evaluated by investigating the applicability of the proposed method to determine fragrance contamination in influent and effluent samples from WWTPs. The results showed that the matrix considerably influenced the sensitivity of the proposed method, especially in the case of influent samples. To overcome this effect, an IS 2,4,6-trinitro-1, 3-dimethyl-5-tert-butylbenzene (d_{15}-MX, d_{15}-musk xylene) was used but without good results, as no differences were observed between the external calibration curve and that obtained with IS. Alternatively, the authors proposed the dilution of the samples with ultrapure water (1:2) with the addition of IS to IL, in order to improve the precision of the method. Under these conditions, the matrix had little effect on the extraction performance and the proposed method proved to be a rapid, simple, and sensitive technique for the analysis of fragrances in wastewater samples,

representing a nice alternative to traditional and other recently introduced methods. The applicability of the proposed method was tested for the monitoring of musk fragrances in wastewaters over a period of 6 months, confirming that 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-(g)-2-benzopyran (HHCB, galaxolide) and 7-acetyl-1,1,3,4,4,6-hexamethyl-1,2,3,4-tetrahydro-naphthalene (AHTN, tonalide) were the most abundant compounds in the samples.

Despite the fact that ILs–HS-SDME looks to be especially promising for the selective simultaneous extraction of nonpolar and polar compounds from relatively complex aqueous samples, many challenges still remain to be addressed. In an effort to overcome the issues associated with drop stability, a new SME concept called DLLME has been developed [12,49]. It is based on a ternary component solvent system like homogeneous liquid–liquid extraction and cloud point extraction in which the acceptor to donor phase ratio is greatly reduced. In this method, a cloudy state consisting of fine droplets is formed when an appropriate mixture of extraction and disperser solvents is injected into an aqueous sample containing the analytes of interest. The fast injection of the mixture of organic solvents into the water causes the water-immiscible solvent to be dispersed in the aqueous mass as small microdrops, into which the target analytes are rapidly extracted. The enriched organic phase is then separated from the aqueous sample by centrifugation (or frozen). After centrifuging the cloudy solution, the sedimented phase at the bottom of a conical tube is recovered and directly subjected to instrumental analysis, typically by GC. The extracting solvent must be a high-density water-immiscible solvent, such as chlorobenzene, carbon tetrachloride, or tetrachloroethylene, whereas the disperser solvent must be a water-miscible, polar solvent, such as acetone, methanol, or acetonitrile. Operational simplicity, rapidity, low cost, high recovery and enrichment factors, and the extremely small consumption of the extraction solvent are some of the features of DLLME [3,12,49]. At present, it is competing with other microextraction techniques such as SPME and SDME and has been successfully applied to the concentration of different organic compounds in water samples [3,12,49]. Montes *et al*. [45] reported the use of a DLLME coupled to tandem GC–MS for determination of TCS and methyltriclosan (MTCS) in different water samples. The method was based on the use of a ternary mixture consisting of a disperser, an extractant, and *N*-methyl-*N*-(tert-butyldimethylsilyl) trifluoroacetamide (MTBSTFA) as derivatization reagent. The influence of several experimental factors on the yield of the sample preparation process is thoroughly discussed. The evaluation of the results showed that the proposed method is mainly controlled by the nature and volume of disperser, extractant, and silylation reagent, as well as the ionic strength of the sample. The efficiency of the DLLME process was affected in a minor extension by the nature of water samples, suggesting that external calibration would be an alternative quantification technique, instead of the time-consuming

standard addition protocol. It was demonstrated that the selected compounds could be efficiently determined under the optimized experimental conditions, while a specific case application (depending either on the matrix or on the target species) can further enhance the analytical utility of the proposed approach. For example, in case of MTCS, a further improvement in sensitivity is advisable to measure concentrations of this pollutant below the 5 ng/l level. Overall, performance of the proposed method is comparable, or even better, to those reported for other approaches applied to the determination of the same compounds, with the advantage of a shorter sample preparation step. This report constitutes the first application of DLLME, which incorporates a silylation reaction in the sample preparation scheme, and it exhibits clear advantages in comparison to current methodology for this type of matrix and analytes. According to the authors, this approach could be of interest for the determination of other phenolic and acidic PPCPs, whose ability to react with MTBSTFA, in organic media, has been previously demonstrated. Figure 2 depicts GC–MS/MS chromatograms of the target compounds.

Apart from the aforementioned SME configurations, ultrasound-assisted solvent extraction (USAE) is also considered a good option for organic-compound extraction from different matrices, as it provides more efficient contact between sample and solvent due to an increase of pressure (which

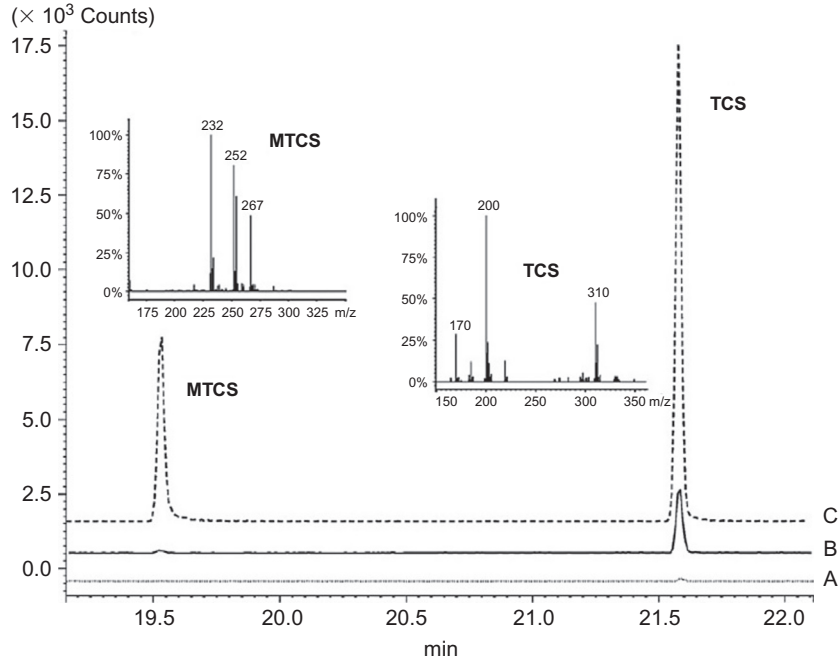

FIGURE 2 GC–MS/MS chromatograms for a procedural blank (A) and sample, before (B) and after (C) addition of TCS and MTCS at 400 ng/l [45].

favors penetration and transport) and temperature (which improves solubility and diffusivity) [53,54]. Combining the benefit of microextraction and ultrasound radiation, a novel microextraction technique, named ultrasound-assisted emulsification microextraction (USAEME or UAEME) or ultrasound-assisted DLLME, was recently introduced by Regueiro *et al*. [46]. This approach is based on emulsification of a microvolume of water-immiscible extraction solvent in the aqueous sample solution by ultrasound radiation, which can increase the contact surface between the two immiscible phases, favoring the mass transfer of analytes into the organic phase and improving the extraction efficiency. More importantly, UAEME does not need the disperser solvent used in conventional DLLME, which avoids losses of the target analytes. The main effects of ultrasound are the fragmentation of one of the phases to form an emulsion of submicron droplet size that extends the contact surface between both liquids. The fundamentals of USAE, including the different available formats, aspects of method development, and applicability to organic and inorganic analytical problems have been recently reviewed in the literature [53,54].

The concept of USAEME was proposed by Regueiro *et al*. [46] as an alternative preconcentration method for parabens, TCS, and related phenols in water samples. The method was based on the formation of acetylated derivatives and its further extraction by USAEME and detection by GC–MS/MS. A multifactorial experimental design was employed to study and optimize the main variables potentially affecting the microextraction and derivatization processes (extraction solvent, phase ratio, sodium chloride concentration, extraction time, and acetic anhydride volume). *In situ* derivatization with acetic anhydride was demonstrated to be successful under optimized conditions. The proposed method possessed high sensitivity and was uninfluenced by the sample matrix, suggesting that the use of external calibration with acetylated standards would be a reliable quantification approach. The method offers the advantages of increased sample throughput and procedural simplicity if compared with nonexhaustive extraction techniques such as SPME.

2.2.2 Solid Environmental Samples

A field where USAE seems to be particularly advantageous, due to the complexity of the matrixes, is the determination of pollutants in solid environmental samples such as sediments, sludge, and soils. Two nice examples of coupling USAE with other SME configurations for extracting, cleaning up, and preconcentrating of PBDEs in sediment samples prior to GC tandem MS analysis are reported in the literature over the review period of this chapter. In the first one [47], PBDEs were first leached (ultrasound-assisted leaching, USAL) from sediment samples by using acetone. This extract was cleaned up by DSPE using activated silica gel as sorbent material. After cleanup, PBDEs were preconcentrated by using DLLME technique. The combination of USAL

and DSPE led to an increment of methodology selectivity and sensitivity, and it explains the DLLME preconcentration capabilities to complex sediment matrixes. USAL–DSPE–DLLME–GC–MS/MS showed comparable or lower MDLs to Soxhlet–GC–MS/MS, SPME–GC–MS/MS, and MAEGC–MS/MS methodologies. However, the proposed methodology offers a large time-saving and requires lower volumes of solvents. USAL–DSPE–DLLME employs simple and inexpensive equipment, and it is applicable for most of the analytical laboratories. Furthermore, the developed USAL–DSPE–DLLME provides good linearity, precision, and quantitative recoveries.

In the second one [48], a modified USAL–DLLME method by adding a new solid floating organic droplet (SFO) step [55] is proposed for the analysis of the same groups of analytes (PBDEs) in sediment samples. Namely, the ultrasound-assisted leaching–dispersive liquid–liquid microextraction using solidification of floating organic droplet (USAL–DLLME–SFO) technique is proposed for extraction and isolation of PBDEs from sediment and further determination by GC–MS/MS. Variables showing significant effects on the analytical responses were considered within a further CCD. The performance of the method was evaluated and compared to that of other extraction methods taking data from the open literature. The results showed that the proposed method was not only very simple, fast, and inexpensive, but, in comparison to conventional procedures, such as Soxhlet-GC–MS/MS methodology, significantly improved the sensitivity achieving lower LODs. By comparing against LC analytical methodologies (i.e., UPLC–APCI/MS/MS) reported for the determination of PBDEs in sediment and sludge samples, in addition to the aforementioned advantages, other technical aspects would be also recorded as additional benefits, including the limited use of toxic solvents and the fewer requirements on advanced equipment or materials. Finally, compared with USAL–DSPE–DLLME, the proposed method showed comparable advantages, such as minimum sample manipulation, and lower amount of organic solvents and time consumption, and therefore could be a viable alternative for the determination of PBDEs in sediment samples by GC–MS/MS. The applicability of the method was also tested in real sediment samples. The PBDE concentration in the samples was at the pg/g level (n.d. to 29 pg/g d.w.), with BDE-47 being the most abundant congener followed by the BDE-153 congener. BDE-99 was detected in 28% of the analyzed samples, while BDE-100 was below LOQ in all samples.

Finally, the benefits of integration of pressurized liquid extraction (PLE) with IL–HS-SDME have been recently explored by Vallecillos *et al.* [44]. After the optimization of the PLE method, half of the extracted volume was evaporated, diluted to 10 ml with ultrapure water (dilution 1:2), and finally subjected to previous validated IL–HS-SDME protocol [43]. The method showed clear advantages compared to methods including standard SPE clean-up step in terms of simplicity, rapidity, and solvent consumption.

3 CONCLUDING REMARKS, TRENDS, AND PERSPECTIVES IN MICROEXTRACTION

The variety of application fields that have been addressed in this chapter reveal that, due to its feasibility, flexibility, versatility, low costs, and rapidity, microextraction techniques coupled to advanced GC–MS techniques such as tandem MS and TOF have proved themselves successful in virtually any analytical challenge that makes them effectively applicable alternatives in contaminant analysis. Hence, over the past decade, a large number of contaminants belonging to different chemical groups found their way into the field of GC–MS/MS and GC–TOF-MS analysis.

In summary, the works reviewed here indicated that:

- Despite the clear advantages and the potential of advanced GC–MS instrumentation, current application of such systems in environmental analysis is substantially less than those with specific detectors or Q and IT instruments. This is principally due to their heavier operating performance, their higher requirements on advanced equipment or materials, and their high supply and maintenance cost, when compared to simple instruments. However, commercial competition and continuous development in instrumentation systems are anticipated to turn over this trend in the years ahead, broadening and expanding their application.
- It is obvious that there is no universal sample microextraction technique suitable for all types of samples, and the sample preparation required is dependent on the nature of the analytes, the matrix, and the final separation method.
- Among the tested microextraction techniques, SPME, especially in the HS mode, is the method of choice for many analytes and matrices. However, given the matrix effect observed in many applications, special attention should be given to calibration methods used for quantification.
- Due to their inherent drawbacks, the use of first SME design concepts like SDME or hollow fiber liquid-phase microextraction (HF-LPME) either are thoroughly modified introducing new extraction phases (i.e., ILs) and formats or they are gradually replaced with other more robust and flexible ones, such as DLLME, USAME, Vortex DLLME, etc.
- For semisolid or solid samples, SME is usually performed as pretreatment method prior to more sophisticated extraction of the samples. Often, a conjugation of two or more SME techniques is applied for cleaner extracts and better sensitivity.
- Given the fact that numerous variables affect the microextraction process, the optimization process continues to be the most laborious and difficult task in the development and validation of the analytical protocols. To eliminate this obstruction as well as to reduce the experimental cost and obtain the maximum information from the most economical set of experiments, many of the published works made use of multivariate statistical approaches (experimental design).

- Derivatization plus microextraction seems a reliable strategy which gives the green light for its direct coupling with GC–MS systems and expanding its range of application to weakly volatile and thermally unstable compounds, improving in parallel its performance in terms of sensitivity.
- Considering the advances of the instrumental techniques, MS/MS-based approaches necessitate high sensitivities achievable only via rigorous optimization as well of all instrumental parameters.
- In recent years, increasing effort has also been placed on innovating sorptive and SME to allow for online and automated procedures. Hence, microextraction has a promising future in analytical methodology.

Despite the available data and the current state of knowledge, there are a number of gaps that need to be addressed and several innovations that are still challenging. Some examples focusing on the most important areas are illustrated below:

- Considering the research potential of sorptive microextraction techniques, future research will definitely be directed toward the design of improved extraction phases capable of overcoming drawbacks and challenges associated with the commercially available sorbents and supporting materials. In this promising new area of research, ILs and polymeric ionic liquids, highly selective (highly selective molecularly imprinted polymers) or nano-shaped materials (i.e., multiwalled carbon nanotubes (MWCNTs), graphenes, and magnetic nanoparticles), due to their unique nanostructure and extraordinary properties, are expected to play a vital role, holding great promise for potential applications in environmental analysis.
- Similarly, in SME, significant efforts should be devoted to the use of more selective (ILs) and greener extraction solvents and low-density solvents for DLLME. ILs exhibit valuable characteristics which can significantly broaden the applicability of SME.
- Despite the improvements and innovations that have been achieved in the application of SME, the technique is still far from being accepted for routine analysis. Further development of the sample-collection devices is required in order to facilitate achieving reliable, reproducible results. Furthermore, commercial devices need to be developed to enable the simple, robust, and accurate analysis of organic pollutants in environmental samples.
- Finally, as regards the online in-tube SPME system, although it can be easily automated in combination with GC, it is not yet available for routine analyses. Hence, further development is also needed to make the system applicable to large-scale analysis in routine laboratories.

Obviously, principal benefits and advantages of microextraction are nowadays widely acknowledged. However, there is a huge area of research in front of us that can contribute to the development of new methodologies for higher throughput, cleaner extracts, and cost-effective results.

ABBREVIATIONS

CAR	carboxen
CCD	central composite design
CFME	continuous-flow microextraction
CPE	cloud point extraction
CW/TPR	carbowax/templated resin
DHF-LPME	dynamic hollow fiber liquid-phase microextraction
DI-SDME	direct single-drop microextraction
DI-SPME	direct solid-phase microextraction
DLLME	dispersive liquid–liquid microextraction
DVB	divinylbenzene
GC	gas chromatography
HF	hollow fiber
HF-LPME	hollow fiber liquid-phase microextraction
HLLE	homogeneous liquid–liquid extraction
HS	headspace
HS-LPME	headspace liquid-phase microextraction
HS-SDME	headspace single-drop microextraction
HS-SPME	headspace solid-phase microextraction
IT	ion trap
LC	liquid chromatography
LLE	liquid–liquid extraction
LLLME	liquid–liquid–liquid microextraction
LPME	liquid-phase microextraction
MASE	membrane-assisted solvent extraction
MIPs	molecularly imprinted polymers
MMLLE	microporous membrane liquid–liquid extraction
MS	mass spectrometry
PA	poly(acrylate)
PDMS	poly(dimethylsiloxane)
PHWE	pressurized hot water extraction
POPs	persistent organic pollutants
QqQ	triple quadrupole
SBSE	stir-bar sorptive extraction
SDME	single-drop microextraction
SFO	solid floating organic droplet
SLM	supported liquid membrane extraction
SME	solvent microextraction
SPE	solid-phase extraction
SPME	solid-phase microextraction
USAE	ultrasound-assisted extraction
USAL	ultrasound-assisted leaching
VOCs	volatile organic contaminants

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Chapter 3

Determination of Pesticide Residues in Environmental and Food Samples Using Gas Chromatography–Triple Quadrupole Mass Spectrometry

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1 INTRODUCTION

To ensure the compliance with the regulations and guidelines of maximum residue limits (MRLs) of pesticides in the environment and foods established by government agencies (e.g., *U.S.* 40 *CFR Part* 180; *Ontario Regulation* 169/03; 2005/396/*EC*; 2008/299/*EC*) [1–4], various analytical technologies have been evaluated and analytical methods developed so that pesticide residues could be identified and quantitated with high confidence for timely enforcement of tolerances. Over the past 30 years, the applications of mass spectrometry (MS) have been promoted by the advances in ionization sources and commercial availability of MS instruments. MS has become an

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indispensable tool in the routine analysis of chemical contaminants in the environment and foods [5–9].

Among various MS instruments, triple quadrupole mass spectrometry (QqQ-MS), first reported by Yost and Enke [10,11], has its unique designs and applications and the first commercial QqQ-MS was introduced by Finnigan and Sciex in the early 1980s [12,13]. The QqQ-MS is different from single quadrupole (Q) MS systems, which can be operated only in two data acquisition modes, that is, single ion monitoring (SIM) or full scan. A typical QqQ-MS consists of two quadrupole MS analyzers (Q1 and Q3) and a collision cell (q) sandwiched between the two MS analyzers. Depending on the purposes of applications (structure elucidation or quantitation), the instrument can be operated in several modes such as full scan, precursor ion scan, neutral loss scan, product ion scan, or selected reaction monitoring (SRM) mode (also referred as multiple reaction monitoring, MRM). When operating in SRM mode, QqQ-MS can be used as a quantitative tool for target analysis of small molecules in environmental and food samples.

When operated in the SRM mode, the first and second mass analyzers (Q1 and Q3) focus on a set of predefined ions, precursor ions at Q1 and the corresponding product ion at Q3. A collision gas (typically N_2 or Ar) is introduced into the collision cell (q) at a pressure to ensure that ions entering the collision cell will undergo collision-induced dissociation (CID) with collision gas molecules. Only ions with the same m/z as those predefined precursor ions will be selected by Q1 and sent into the collision cell (q). These ions will be subjected to CID in the collision cell (q), generating fragments. Those fragments with the same m/z as the predefined product ions will be selected by Q3 and measured by the detection system. The use of a molecular-specific precursor/product ion pair in the SRM mode for each molecule analyzed also implies a MS-based separation of molecules and, as it will be discussed in this review, an ideal approach in multiresidue pesticide analysis.

The intensity of detected product ions is usually less than that of their precursor ions because of fragmentation and imperfect ion transmission efficiency. The loss of signal is offset by noise eliminated in the product ion m/z. Signal-to-noise ratio (SNR) is enhanced by the absence of any signal arising from the precursor and product ion monitored, except when the specified pesticide is present. Additionally, U shaped collision cells are often used to minimize interference of neutral species. Therefore, the overall SNR is increased, resulting in better sensitivity of GC–QqQ-MS as compared to GC–MS full scan or GC–MS-SIM. Meanwhile, higher selectivity is achieved by choosing specific precursor and product ions informed by molecular structures associated with mass spectra ions. Theoretically, it is possible to perform higher order MS^n experiments by combining N ($N > 2$) mass analyzers together. This approach, however, will increase the complexity and cost of the system and makes it a less practical instrument for routine analysis.

Pesticide residue analysis using GC–QqQ-MS takes advantage of both chromatographic separation and mass spectrometric determination and has been

applied in many multiresidue pesticide methods [14]. A large number of pesticides can be included in one GC–QqQ-MS method, improving the screening efficiency of pesticide laboratories. Unlike SIM methods where four ions are generally needed for confirmation of each pesticide, QqQ methods only require two transitions and unlike full scan methods, QqQ does not sacrifice sensitivity in a comprehensive method. In a GC–QqQ-MS analysis, target pesticides are first separated from matrix components on a GC column and sequentially introduced into a QqQ-MS analyzer. Using pesticide-specific SRM mode, pesticides undergo ionization in the ion source; precursor ions are selected and separated in the Q1, and fragmented in the collision cell; and those selected product ions are detected in the Q3. The SNR of a detected pesticide can be further improved by setting a specific detection time window on Q1 and Q3 based on each pesticide's GC retention time (RT; time-segment-based SRM data acquisition). This approach reduces the number of MRM transitions monitored by the QqQ at a specific time, allows Q1 and Q3 to be operated in a more efficient manner, and distributes more QqQ measurement time (dwell time) for each pesticide to improve the SNR of the target pesticides [15,16].

2 METHOD DEVELOPMENT

To develop a GC–QqQ-MS method, one needs to first generate MRM transitions for each pesticide. According to EU identification criteria [17], it is enough to achieve identification of a pesticide using two MRM transitions and their relative ion abundance ratio, provided the RT matches. The transition with higher abundance could be used for quantitation and the other transition can be served as a confirmatory ion, but the selection should be dependent on the conditions of the analysis.

An optimized MRM transition should include a precursor ion, a product ion, and an optimized collision energy; optimal choices can be generated based on the structure and full scan mass spectra. This is done by first selecting a precursor ion with the highest *m/z* (molecular ion) and abundance because background interferences decrease with an increasing *m/z* [18] and enhances the SNR further. The 70 eV energy used by electron ionization (EI) source of GC–QqQ-MS often cannot generate molecular ions with enough abundance. Under this circumstance, a compromise has to be made between selectivity and sensitivity by choosing EI-induced fragments with enough abundance in full scan spectra as precursor ions [19]. Then each selected precursor ion will undergo CID to generate products ions at different collision energies. The optimal SRM transitions carry specific structural information of the target pesticides for identification (selectivity and separation) and have enough abundance to be detectable (sensitivity and SNR). To ensure selectivity of GC–QqQ-MS analysis, unspecific MRM transitions such as $[M]^+ \rightarrow [M]^+$ (such a transition does not provide additional specificity than would be obtained through SIM) as well as $[M]^+ \rightarrow [M-1]^+$ or $[M]^+ \rightarrow [M-2]^+$ (due to deprotonation in the course of ionization) ought not be used to avoid interferences.

Once all transitions are ready, one needs to arrange those transitions in different time segments based on each pesticide's GC RT. When performing multiresidue pesticide analysis, a QqQ-MS has to monitor many coeluting MRM transitions in one GC chromatographic run. A QqQ-MS needs a minimum of a few milliseconds to monitor a transition. In a given cycle time, too many concurrent transitions will overwhelm the QqQ-MS. However, if the cycle time is increased to accommodate those transitions, insufficient data points might be collected to actually define a peak. There are no official documents that define the minimum data points required for peak definition, but six to eight points per peak would be sufficient [20]. When building MRM data collection segments, one needs to factor in the cycle time, number of transitions in a segment, dwell time of each transition, minimum data points per peak required, and RT shifts due to matrix effects or column performance. It is challenging to have a large number of overlapping segments based on RTs because of the large number of targeted analytes included in the method. In recent years, scheduled multiple reaction monitoring (sMRM) or similar software algorithms have been introduced. The main advantage of these algorithms is to automatically optimize dwell time, data points, and response for each MRM transition within a user-defined time window. Compared to the conventional time segment approach, sMRM decreases the number of concurrent MRM transitions monitored at any time, thus increasing SNR and reproducibility, and simplifying the method development [15,16].

3 APPLICATIONS OF GC–QqQ-MS

The determination of pesticides in environmental and food samples involves sample preparation (including extraction, cleanup, solvent exchange, concentration, etc.) and instrumental analysis. The purpose of sample preparation is to selectively extract the targeted pesticides based on their physicochemical properties and further separate from the matrix components to make the sample more suitable for instrumental analysis. If the instruments do not have sufficient sensitivity, selectivity, and ruggedness, the emphasis will rely on sample preparation, which could be time consuming and laborious. With the availability of sensitive and selective instruments, the sample preparation task could be significantly simplified, enabling sample dilution to minimize matrix interferences and eliminating or reducing additional concentration and cleanup efforts.

3.1 Determination of Pesticides in Environmental Samples

One of the main areas of interest to environmental chemists is the identification and quantification of pesticides in water, air, soil, and sediments. Pesticides are an important class of hazardous organic chemicals in the environment due to their potential persistence and bioaccumulation. The applications of QqQ-MS in environmental analysis can be traced back to the early 1980s [21], but the high cost and technological limitations such as data acquisition speed and

operating software of QqQ-MS slowed down the use of this technology until 2000s.

A recent environmental application of GC–QqQ-MS involves the potential adsorption of pesticides onto aerosol particles (<2.5 μ), which can pose a threat to human health, so it is important to monitor pesticides in particulate matters. Coscollà *et al*. [22] developed a GC–QqQ-MS method that can monitor 40 pesticides in airborne particulate matter (PM 10). PM 10 samples were collected using quartz filters (150 mm, diameter). Pesticides were extracted using microwave-assisted extraction, cleaned up using gel permeation chromatography (GPC), and determined using two MRM transitions for each pesticide. To ensure the selectivity of the QqQ-MS system, Coscollà *et al*. also proposed to use accurate masses, using a 10 mDa mass accuracy for each transition. Unlike time-of-flight (TOF) or magnetic-based sector MS systems, QqQ-MS is designed to carry out unit MS resolution experiments. The proposed approach can be difficult for GC–QqQ-MS, as it requires using a narrow mass tolerance window on the QqQ-MS and could lead to a decrease in sensitivity or missing target ions.

Nonpolar pesticides (e.g., organochlorines) with low solubility tend to be strongly adsorbed by sediment, requiring aggressive extraction technologies such as pressurized liquid extraction (PLE), Soxhlet, or ultrasound/microwave-assisted extraction to extract pesticide residues from soil or sediment matrices prior to GC–QqQ-MS analysis. Luo *et al*. [23] employed PLE and GC–QqQ-MS to determine 12 pyrethroids in soils. Camino-Sánchez [24] developed a method for the simultaneous determination of PCBs, PAHs, PBDEs, and pesticides in marine sediments using PLE extraction, stirring bar concentration, and GC–QqQ-MS analysis. Sánchez-Avila [25] used ultrasound-assisted extraction and GC–QqQ-MS to analyze PAHs, PCBs, PBDEs, APS, and pesticides in sediments. These results reveal GC–QqQ-MS can perform target analysis for a wide range of chemical contaminants with excellent sensitivity and selectivity.

Pitarch *et al*. conducted a series of studies to evaluate the potential of GC–QqQ-MS for monitoring the occurrence of PAHs, PCBs, PBDEs, octyl/nonylphenols, and pesticides in wastewaters [26,27]. Water samples were prepared using simple solid-phase extraction (SPE) cleanup and concentration procedures. More than 50 chemical contaminants could be detected below 25 ng/mL using GC–QqQ-MS. The analytical strategy proposed using GC–QqQ-MS and LC–QqQ-MS for targeted pesticides and GC-TOF and LC-TOF for nontargeted analysis of chemical contaminants in wastewater [27]. Results of the study provide valuable information for wastewater treatment and emphasize the challenging aspects of determining a wide range of organic pollutants with varying physicochemical properties. A summary of GC–QqQ-MS methods for different environmental samples are listed in Table 1 (#1–10), including instrument parameters, sample preparation procedures, target pesticides, and sample matrices.

TABLE 1 Applications of GC–QqQ-MS for Pesticide Analysis

	Pesticide	Matrix	Sample Preparation	GC–QqQ-MS	References
1	Pesticides, PCBs, PAHs, PBDEs, octyl/ nonylphenols	Wastewater	1. 100 mL water sample + 1 mL of surrogate mixture (5 IS) 2. SPE cartridge (C18) cleanup 3. Cartridge was dried by passing air and pesticides eluted using 5 mL ethyl acetate:DCM (50:50) 4. Collected extract evaporated to dryness and redissolved in 1 mL of hexane	An Agilent 6890N GC coupled with a Quattro Micro QqQ-MS A fused silica HP-5MS capillary column (30 m × 0.25 mm i.d. × 0.25 μm) EI, positive; CI, negative; 1 μL injection, splitless injection mode, ~42 min run time Carrier gas: helium; collision gas: nitrogen	[26]
2	PAHs, octyl/ nonylphenols, PCBs, pesticides, PBDEs	Wastewater	1. 100 mL water sample + 1 mL of surrogate mixture (5 IS) 2. SPE cartridge (C18) cleanup 3. Cartridge was dried by passing air and pesticides eluted using 5 mL ethyl acetate:DCM (50:50) 4. Collected extract evaporated to dryness and redissolved in 1 mL of hexane	An Agilent 6890N GC coupled with a Quattro Micro QqQ-MS A fused silica HP-5MS capillary column (30 m × 0.25 mm i.d. × 0.25 μm) EI, positive, 1 μL injection, splitless injection mode, ~42 min run time Carrier gas: helium; collision gas: nitrogen	[27]
3	20 pesticides	Soil	1. 5 g soil sample + 10 mL water or 5 g soil sample + 10 mL 1.0 M aqueous Na_2-EDTA solution for 30 min 2. 10 mL acetonitrile + acetic acid mixture (99:1, v/v) added to the centrifuge tube containing the hydrated sample	A Varian CP-3800 GC coupled with a Varian 1200 QqQ-MS A Zebron ZB-50 capillary column (30 m × 0.25 mm × 0.25 μm) EI, positive, 3 μL injection, splitless injection mode, 25 min run time Carrier gas: helium; collision gas: argon	[28]

			3. Salting out with addition of 4 g anhydrous $MgSO_4$ (4.0 g) and $NaAc{\cdot}3H_2O$ (1.7 g) 4. Centrifugation and acetonitrile layer used for cleanup 5. Concentrated acetonitrile extract to 1 mL and then mixed with water (1 mL) and *n*-hexane (5 mL) 6. Concentrated hexane extract to dryness + IS 7. Redissolved in 1 m hexane for GC–QqQ-MS analysis		
4	32 endocrine disrupting compounds and pesticides	Water	1. Conditioned SPE cartridge using 7 mL of ethyl acetate, 7 mL of methanol, 7 mL of Milli-Q water at 1 mL/min 2. 500 mL sample passed through the cartridge at 5 mL/min 3. Rinsed the cartridge with 5 mL water and dried by vacuum pressure for 60 min 4. Eluted pesticides with $2\times$ 2.5 mL of methanol and 2×2.5 mL acetonitrile at 1 mL/min 5. Extracts were evaporated to dryness and resuspended until a final volume of 500 µL in methanol	A Shimadzu GC-QP2010 coupled with a Shimadzu QP 2010 QqQ-MS A Varian VF-5 fused silica capillary column (30 m × 0.25 mm × 0.25 µm) EI, positive, 1 µL injection, 34.5 min run time Carrier gas: helium; collision gas: argon	[29]

Continued

TABLE 1 Applications of GC–QqQ-MS for Pesticide Analysis—Cont'd

	Pesticide	Matrix	Sample Preparation	GC–QqQ-MS	References
5	Pyrethroids	Soils	1. 10 g soil sample extracted using PLE. DCM, acetone, 50% DCM in acetone (selected), or 50% DCM in ethyl acetate was evaluated as extraction solvent 2. Extracts concentrated to near dryness and redissolved in *n*-hexane (1 mL) 3. The PLE extracts were cleaned up using SPE. Graphitized-carbon black (GCB) cartridge (selected), Florisil cartridge, silica cartridge, or C18 cartridge was evaluated	An Agilent 6890N GC coupled with a Waters Micromass Quattro Micro QqQ-MS A DB-5 capillary column (30 m × 0.25 mm × 0.25 μm) EI, positive, 25 min run time; carrier gas: helium; collision gas: argon	[23]
6	PCBs, PAHs, PBDEs, pesticides	Marine sediment	1. 10 g dried sample was extracted using PLE (methanol, 80 °C, 1000 psi, 10 min) 2. After extraction, volume was adjusted to 50 mL using methanol 3. 10 mL extracts from step 2 + 200 mL deionized water extracted using stir-bar sorptive extraction (Twisters) for 12 h 4. Addition of 60 g NaCl and extracted for another 12 h 5. Twisters were analyzed using the thermal desorption unit and GC–QqQ-MS	An Agilent 7890 GC coupled with an Agilent 7000B QqQ-MS A fused silica HP-5MS capillary column (30 m × 0.25 mm i.d. × 0.25 μm) EI, positive, ~42 min run time Carrier gas: helium; collision gas: nitrogen	[24]

7	40 pesticides	Airborne	1. The PM10 filters extracted MAE (50 °C for 20 min, using a power of 1200 W, and 30 mL of ethyl acetate) 2. 20 L of nonane (keeper) added to the extract and concentrated with Turbo Vap 500 3. Extracts were redissolved with 750 µL of dichloromethane prior to GPC cleanup 4. GPC cleanup 5. Collection fractions evaporated to dryness and redissolved with 1 mL of hexane prior to GC–QqQ-MS analysis	A Trace GC Ultra coupled with a Thermo-Finnigan TSQ Quantum A TR-5MS capillary column (30 m × 0.25 mm i.d. × 0.25 µm) EI, positive, 1 µL injection, splitless injection mode, 36.6 min run time Carrier gas: helium; collision gas: nitrogen	[22]
8	16 PAHs, 5 PEs, 7 PCBs, 6 PBDEs, 6 APs, 3 OCs, BPA	Seawater, river water, wastewater treatment plant (WWTP) effluents, sediments, mussels	Water samples SPE using Oasis HLB cartridges and eluting with dichloromethane/hexane (1:1, v/v) and dichloromethane/acetone (1:1, v/v) Mussels and sediments Ultrasound-assisted extraction using DCM as solvent	An Agilent 7890A GC coupled with an Agilent 70000 A QqQ-MS A 15 m HP-5MS capillary column used to detect BDE 209 A HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm) used to detect PAHs, PCBs EI, positive, 1 µL injection, ~60 min run time Carrier gas: helium; collision gas: nitrogen	[25]
9	12 pyrethroids	Water, sediment, milk	Water 1. 20 mL unfiltered water fortified with surrogates 2. Extracted using sonication and 1 mL DCM for 5 min	An Agilent 7890A GC coupled with 7000A QqQ-M A DB-5MS capillary column (15 m × 0.25 mm × 0.1 µm) containing 5% phenyl methyl siloxane	[30]

Continued

TABLE 1 Applications of GC–QqQ-MS for Pesticide Analysis—Cont'd

	Pesticide	Matrix	Sample Preparation	GC–QqQ-MS	References
			3. Organic phase evaporated and redissolved with 100 µL ethyl acetate Sediment 1. 1 g sediment fortified with surrogate standards 2. Extracted twice with 20 mL of hexane/dichloromethane (2:1) 3. Cleaned up using a Florisil cartridge 4. Eluted with 25 mL of ethyl acetate, evaporated and redissolved with 100 µL ethyl acetate	EI, positive; CI, negative; 3 µL injection, splitless injection mode; 20 min run time Carrier gas: helium; collision gas nitrogen; NCI:ammonium	
10	77 pesticides	Natural spring water, tap, and commercial mineral waters	Solid phase microextraction (SPME) 1. 14 mL sample (+IS) extracted by immersion of a StableFlex 65 µm PDMS-DVB fiber (60 min, 70 °C, sample agitation at 500 rpm, addition of NaCl, pH adjusted to 6) 2. Desorption of the pesticides at 250 °C for 5 min in the split–splitless injector and held for 4 min at the split ratio at 100:1 3. The fiber baked out for 10 min at 250 °C	A Varian CP-3800 GC coupled with a 1200L QqQ-MS A Varian VF-5 fused silica capillary column (30 m × 0.25 mm × 0.25 µm) coupled with a deactivated guard column (2 m × 0.25 mm i.d.) EI, positive, SPME fibers or 10 µL injection (HF-LPME), split injection mode, 27 min run time Carrier gas: helium; collision gas: argon	[31]

			Hollow fiber liquid phase microextraction (HF-LPME) 1. A syringe plunger inserted into the hollow fiber soaked with 1-octanol:dihexyl ether (75:25, v/v; 1 min), and then placed into a 15 mL screw top vial containing 14 mL of sample 2. Addition of NaCl to adjust the ionic strength and adjusted 3. Shook the vial for 90 min at 90 rpm 4. The fiber transferred on a 2 mL vial containing 1.5 mL of cyclohexane and agitated for 5 min at 30 rmp 5. The extract passed through $MgSO_4$ and evaporated to dryness 6. The residue redissolved with 250 µL of cyclohexane and 5 µL of a solution of parathion ethyl d-10 (IS) for GC–QqQ-MS/MS analysis		
11	12 pesticides, metabolites	Baby foods of fruit and rice, fish and pasta, and potato and pork	1. 10 g sample + 10 mL acetonitrile, anhydrous magnesium sulfate (4 g), sodium chloride (1 g) 2. Addition of volumetric standard (delta-hexachlorocyclohexane, -HCH) 3. Centrifugation and transferred 1 mL supernatant for dispersive cleanup with PSA sorbent (50 mg), anhydrous $MgSO_4$ (150 mg), C18 sorbent (100–300 mg) 4. Centrifugation and supernatant used for GC–QqQ-MS analysis	A Varian CP-3800 GC coupled with a Varian 1200 QqQ-MS A Zebron ZB-50 capillary column (30 m × 0.25 mm × 0.25 µm) EI, positive, 8 µL injection, LVI, 21.5 min run time Carrier gas: helium; collision gas: argon	[32]

Continued

TABLE 1 Applications of GC–QqQ-MS for Pesticide Analysis—Cont'd

	Pesticide	Matrix	Sample Preparation	GC–QqQ-MS	References
12	130 pesticides	Vegetables	1. 5 g sample + 10 mL ethyl acetate 2. Extract filtered with a cartridge containing glass wool and 3 g anhydrous sodium sulfate 3. Filtered extract was evaporated to dryness and redissolved in 1 mL cyclohexane 4. Screen 1 MRM; reanalyzed nonnegative samples using 2–3 MRMs	A Varian CP-3800 GC coupled with a Varian 1200L QqQ-MS A Varian VF-5 fused silica capillary column (30 m × 0.25 mm × 0.25 μm) coupled with a deactivated guard column (2 m × 0.25 mm i.d.) EI, positive, 10 μL injection, splitless injection mode, 11.6 min run time Carrier gas: helium; collision gas: argon	[14]
13	130 pesticides	Cucumbers	1. 5 g sample + 10 mL of ethyl acetate 2. Filtration with a home-made cartridge containing a glass wool plug and 3 g of anhydrous sodium sulfate 3. Evaporation of the solvent and redissolved residue with 1000 mL of cyclohexane	A Varian 3800 GC coupled with Varian 1200L QqQ-MS A Varian VF-5 fused silica capillary column (30 m × 0.25 mm × 0.25 μm) coupled with a deactivated guard column (2 m × 0.25 mm i.d.) EI, positive, 10 μL injection, split injection mode, 11.6 min run time Carrier gas: helium; collision gas: argon	[33]
14	78 pesticides	Tomato, onion	1. 20 g sample + 40 mL ethyl acetate 2. Salt out with 20 g of anhydrous sodium sulfate 3. Dispersive cleanup of 10 mL extract from step 2 + 0.25 g NH_2 adsorbent and 1.5 g anhydrous magnesium sulfate 4. 1 mL aliquot + IS for GC–MS/MS analysis	A Varian CP-3800 GC coupled with a Varian 1200L QqQ-MS A DB-5 MS column (30 m × 0.25 mm × 0.5 μm) coupled with a guard column (2 m × 0.53 mm i.d.) EI, positive, 5 μL injection, splitless injection mode, 30.3 min run time Carrier gas: helium; collision gas: argon	[34]

15	151 pesticides	Strawberry	1. 10 g sample + 10 mL acetonitrile 2. Salt out with anhydrous NaCl (1 g) and anhydrous $MgSO_4$ (4 g) 3. Centrifuged and 1 mL upper layer used for dispersive cleanup with 25 mg of PSA and 150 mg of $MgSO_4$	A Varian CP-3800 GC coupled with a 1200L QqQ-MS A Varian VF-5 fused silica capillary column (30 m × 0.25 mm × 0.25 μm) coupled with a deactivated guard column (2 m × 0.25 mm i.d.) EI, positive, 10 μL injection, splitless injection mode, 20.5 min run time Carrier gas: helium; collision gas: argon	[35]
16	129 pesticides	Lettuce and other green leafy vegetables	1. 10 g sample + 10 mL acetonitrile + IS 2. Salt out 0.5 g disodium hydrogen citrate sesquihydrate, 1 g trisodium citrate dihydrate, 4 g anhydrous magnesium sulfate, and 1 g sodium chloride 3. Dispersive cleanup of an aliquot of the extract using 150 mg anhydrous magnesium sulfate, 25 mg PSA, and 12.5 mg GCB per mL acetonitrile extract 4. Acidification of an aliquot of the supernatant (1.5 mL) with 50 μL of 5% formic acid in acetonitrile (v/v) 5. The extract was evaporated and redissolved in toluene prior to GC–MS/MS analysis. The sample concentration in the final extract was 1 g/mL	A Varian CP-3800 GC coupled with a Varian 1200L QqQ-MS A DB-5 MS column (30 m × 0.25 mm × 0.5 μm) coupled with a guard column (2 m × 0.53 mm i.d.) EI, positive, 5 μL injection, splitless injection mode, 30.3 min run time Carrier gas: helium; collision gas: argon	[36]

Continued

TABLE 1 Applications of GC–QqQ-MS for Pesticide Analysis—Cont'd

	Pesticide	Matrix	Sample Preparation	GC–QqQ-MS	References
17	140 pesticides	Cucumber, orange	1. 10 g sample + 10 mL acetonitrile 2. Salt out with anhydrous NaCl (1 g) and anhydrous $MgSO_4$ (4 g) 3. Centrifuged and 1 mL upper layer used for dispersive cleanup with 25 mg of PSA and 150 mg of $MgSO_4$	A Varian CP-3800 GC coupled with a 1200L QqQ-MS A Varian VF-5 fused silica capillary column (30 m × 0.25 mm × 0.25 μm) coupled with a deactivated guard column (2 m × 0.25 mm i.d.) EI, positive, 10 μL injection, splitless injection mode, 13.3 min run time Carrier gas: helium; collision gas: argon	[37]
18	12 pesticides	Orange, cucumber	1. 15 g sample + 50 mL ethyl acetate and 10 g of anhydrous sodium sulfate 2. Extract was filtered through a porous plate funnel to a spherical flask 3. Evaporation of the extract to nearly dryness and residue was redissolved in 5 mL of a cyclohexane + IS 4. 1 mL extract from step 3 diluted to 2 mL	A Varian 3800 GC coupled with 1200L QqQ-MS A Varian VF-5 fused silica capillary column (30 m × 0.25 mm × 0.25 μm) coupled with a deactivated guard column (2 m × 0.25 mm i.d.) EI positive, 10 μL injection, splitless injection mode, 18.5 min run time Carrier gas: helium; collision gas: argon	[38]
19	167 OHs, OPs, pyrethroids	Fruit and vegetables	1. 15 g sample + 15 mL ACN (salt-out extraction) + IS 2. Solid-phase dispersive cleanup with octadecyl-bonded silica (C18) 3. Graphitized carbon black/primary–secondary amine (GCB/PSA) sorbents and toluene	A Varian CP-3800 GC coupled with a Varian 1200L QqQ-MS A Varian VF-5 fused silica capillary column (30 m × 0.25 mm × 0.25 μm) coupled with a deactivated guard column (5 m × 0.25 mm i.d.)	[39]

			4. Concentrated 6 mL extracts from step 3 to ~100 µL 5. Reconstitution to 1.0 mL using toluene for GC–MS/MS analysis	EI, positive, 1 µL injection, splitless injection mode, 45 min run time Carrier gas: helium; collision gas: argon	
20	130 pesticides	Fruits and vegetables (orange, nectarine, and spinach)	Accelerated solvent extraction (ASE) 1. 7 g diatomaceous earth + 10 g of triturated sample and then homogenized in a mortar 2. The content was transferred to a 33-mL extraction cell + IS 3. Ethyl acetate was selected as extraction solvent and the extraction temperature, pressure 70 °C, and 1500 psi. The preheating and static times, 2 and 3 min; the contact solvent time was 5 min, with a flush volume of 60% and executing two cycles 4. Extracts (~50 mL) evaporated to ~35 mL and salt out with 2 g anhydrous sodium sulfate, volume adjusted to 50 mL 5. 10 mL of ASE extracts evaporated to dryness and redissolved with 0.5 mL ethyl acetate GPC (only for spinach samples) 1. ASE extracted concentrated to 2 mL with ethyl acetate 2. 1 mL aliquot injected into the GPC system and eluted with cyclohexane–ethyl acetate (1:1, v/v) 3. Collected fraction time (14.5–21.0 min) evaporated to dryness and residue redissolved with 1 mL of ethyl acetate	An Agilent 6890N GC coupled to a Quattro Micro QqQ-MS A HP-5MS fused silica capillary column (30 m × 0.25 mm × 0.25 µm) EI, positive, 1 µL injection, splitless injection mode, 38 min run time Carrier gas: helium; collision gas: argon	[40]

Continued

TABLE 1 Applications of GC–QqQ-MS for Pesticide Analysis—Cont'd

	Pesticide	Matrix	Sample Preparation	GC–QqQ-MS	References
21	160 pesticides	Wines	1. 10 g of wine + 10 mL ACN + IS 2. Salt out with 0.5 g disodium hydrogen citrate sesquihydrate, 1 g trisodium citrate dihydrate, 4 g anhydrous magnesium sulfate, and 1 g sodium chloride 3. A 5 mL aliquot of the acetonitrile extract dispersive cleanup with 0.75 g anhydrous magnesium sulfate, 0.125 g PSA and 0.250 g C18 4. A 1.5 mL aliquot of the acetonitrile extract + 50 µL of 5% formic acid in acetonitrile (v/v) 5. Extract was evaporated to dryness and reconstituted in 1.5 mL toluene prior to GC–QqQ–MS/MS analysis	A Varian CP-3800 GC coupled with a Varian 1200L QqQ-MS A DB-5 MS column (30 m × 0.25 mm × 0.5 µm) coupled with a guard column (2 m × 0.53 mm i.d.) EI, positive, 5 µL injection, splitless injection mode, 30.3 min run time Carrier gas: helium; collision gas: argon	[41]
22	140 pesticides	Carrots, tomatoes, and strawberries	Citrated buffered QuEChERS 1. 10 g sample + 10 mL acetonitrile salt-out with 4 g anhydrous magnesium sulfate, 1 g sodium chloride, 1 g trisodium citrate dihydrate, and 0.5 g disodium hydrogen citrate sesquihydrate	A Varian CP-3800 GC coupled with a Varian 1200L QqQ-MS A DB-5 MS column (30 m × 0.25 mm × 0.5 µm) coupled with a guard column (2 m × 0.53 mm i.d.) EI, positive, 5 µL injection, splitless injection mode, 30.3 min run time	[42]

			2. Dispersive cleanup: 5 mL aliquot + 125 mg PSA and 750 mg anhydrous magnesium sulfate 3. 1.5 mL aliquot of the upper layer + 50 µL 5% formic acid in acetonitrile 4. For the analysis by GC/MS/MS, the extract was evaporated and reconstituted in 1.5 mL toluene	Carrier gas: helium; collision gas: argon	
23	150 pesticides	Fruits and vegetables	1. 15 g sample + 15 mL of MeCN with 1% HOAc + IS 2. Salt out with 6 g of anhydrous $MgSO_4$ and 1.5 g of anhydrous NaOAc 3. 1 mL of MeCN extract for d-SPE cleanup with 150 mg anhydrous $MgSO_4$, 50 mg PSA, 50 mg C18, 7.5 mg GCB 4. 0.5 mL of the extract + add 50 µL QC and analyte protectants mixture and 50 µL MeCN	An Agilent 7890A GC coupled with 7000A QqQ-MS An Rti-5 ms analytical column (10 m × 0.53 mm × 1 µm) coupled to a 3 m × 0.15 mm i.d. HydroGuard noncoated restriction capillary (3 m × 0.15 mm i.d.). A virtual column length entered into the GC configuration (3.13 m × 0.15 mm i.d.) EI positive, 5 µL injection, splitless injection mode; 9.5 min run time Carrier gas: helium; collision gas: nitrogen	[43]
24	25 pesticides	Apple, orange, tomato, carrot	1. 15 g sample + 15 mL acetonitrile 2. Salt out with anhydrous NaCl (1.5 g) and anhydrous $MgSO_4$ (6 g) 3. Centrifuged and 1 mL upper layer used for dispersive cleanup with 50 mg of PSA, 150 mg of $MgSO_4$ and (50 mg C18 for orange) 4. 500 µL extract + 1 mL toluene 5. Evaporated to ~300 µL and adjusted to 500 µL with toluene	An Agilent 7890 GC system coupled to a Quattro Micro QqQ-MS, EI An Agilent 7890A GC system coupled to a Xevo TQ-S QqQ-MS, CI A DB-5 MS column (30 m × 0.25 mm × 0.5 µm) 1 µL injection, splitless injection mode, 22.2 min run time Carrier gas: helium; collision gas: nitrogen	[44]

Continued

TABLE 1 Applications of GC–QqQ-MS for Pesticide Analysis—Cont'd

	Pesticide	Matrix	Sample Preparation	GC–QqQ-MS	References
25	42 pesticides	Tobacco	1. 7.5 g ground tobacco + IS 2. Extracted using PLE (100 °C; 100 atm; heating time: 5 min; static extraction time: 3 min; flush volume: 60% of extraction cell volume; purge: N_2, 60 s; number of cycles: 3) 3. Concentrated PLE extracts to 1 mL 4. SPE cleanup Low polarity pesticides: 0.1 mL concentrated extract loaded onto a Florisil cartridge; elutes loaded on silicagel cartridge Intermediate- and/or high-polarity pesticides: 0.1 mL concentrated extract loaded onto a silicagel cartridge *N*-methylcarbamate: 0.1 mL concentrated extract loaded onto an aminopropyl cartridge	A HP-6890 GC coupled with a A TSQ-7000 QqQ-MS A HP capillary column (30 m × 0.25 mm × 0.25 μm) EI, positive; CI, negative, splitless injection mode, ~38 min run time Carrier gas: helium; collision gas: argon; CI reagent gas: methane (one MRM transition for each pesticide)	[45]
26	122 pesticides	Dry cereal and animal feeds	1. 5 g sample + 10 mL water + 15 mL ACN + IS 2. Salt out with 0.5 g disodium hydrogen citrate sesquihydrate, 1 g trisodium citrate dihydrate, 4 g anhydrous magnesium sulfate, and 1 g sodium chloride	A Varian CP-3800 GC coupled with a 1200L QqQ-MS A DB-5 MS column (30 m × 0.25 mm × 0.5 μm) coupled with a guard column (2 m × 0.53 mm i.d.)	[46]

			3. A 7.5 mL aliquot of the supernatant dispersive cleanup with 0.75 g anhydrous magnesium sulfate, 0.5 g C18, and 0.125 g PSA 4. A 3 mL aliquot of the supernatant + 50 µL of 5% formic acid in acetonitrile (v/v) 5. Evaporated to dryness and redissolved in 1.5 mL toluene prior to GC–MS/MS analysis	EI, positive, 5 µL injection, splitless mode, 30.3 min run time Carrier gas: helium; collision gas: argon	
27	140 pesticides	Dry cereal and animal feeds	1. 5 g sample + 10 mL water + 15 mL acetonitrile + IS 2. Salt out with 0.5 g disodium hydrogen citrate sesquihydrate, 1 g trisodium citrate dihydrate, 4 g anhydrous magnesium sulfate, and 1 g sodium chloride 3. Freeze out for 2 h or overnight) at −26 °C 4. Dispersive cleanup 100 mg anhydrous magnesium sulfate, 75 mg C18, 20 mg PSA per mL acetonitrile extract 5. Acidification with 15 µL of 5% formic acid in acetonitrile per mL of extract 6. The extract was evaporated under a stream of nitrogen and the residue was redissolved in toluene + second IS 7. The sample concentration in the final extracts was 0.66 g/mL	A Varian CP-3800 GC coupled with a 1200L QqQ-MS A DB-5 MS column (30 m × 0.25 mm × 0.5 µm) coupled with a guard column (2 m × 0.53 mm i.d.) EI, positive, 5 µL injection, splitless injection mode, 30.3 min run time Carrier gas: helium; collision gas: argon	[47]

Continued

TABLE 1 Applications of GC–QqQ-MS for Pesticide Analysis—Cont'd

	Pesticide	Matrix	Sample Preparation	GC–QqQ-MS	References
28	168 OHs, OPs, pyrethroids	Dried powdered ginseng	1. 2 g sample + 10 H_2O + IS 2. Addition 20 mL ACN or 2:1:1 acetone/cyclohexane/ethyl acetate 3. Solid-phase dispersive cleanup with octadecyl-bonded silica (C18) 4. Concentration 15 mL of extracts from step 3 to 1 mL 5. SPE graphitized carbon black/ primary–secondary amine sorbents and eluted using acetone:toluene (3:1) 6. Concentration elutes from step 5 to ~100 μL 7. Reconstitution to 1.0 mL using toluene for GC–MS/MS analysis	A Varian CP-3800 GC coupled with a Varian 1200L QqQ-MS A Varian VF-5 fused silica capillary column (30 m × 0.25 mm × 0.25 μm) coupled with a deactivated guard column (2 m × 0.25 mm i.d.) EI, positive, 1 μL injection, splitless injection mode; 45 min run time Carrier gas: helium; collision gas: argon	[48]
29	140 pesticides	Cereal samples (wheat, rye, barley, oats, maize, buckwheat, etc.) and various animal feeds	Procedure A 1. 5 g sample + 10 mL water were added + IS 2. Extracted with 15 mL acetonitrile (salt out with 0.5 g disodium hydrogen citrate sesquihydrate,1 g trisodium citrate dihydrate, 4 g anhydrous magnesium sulfate, and 1 g sodium chloride) 3. Hand shaken and centrifuged and an aliquot of acetonitrile supernatant (7.5 mL) was transferred to a 15 mL disposable screw-capped	A Varian CP-3800 GC coupled with a Varian 1200L QqQ-MS A DB-5 MS column (30 m × 0.25 mm × 0.5 μm) coupled with a guard column (2 m × 0.53 mm i.d.) EI, positive, 5 μL injection; splitless injection mode, 33.33 min run time Carrier gas: helium; collision: argon	[49]

4. Dispersive-SPE agents (125 mg PSA, 500 mg C18, and 750 mg $MgSO_4$
5. 3 mL extract evaporated to dryness and residue dissolved using 1.5 mL toluene

Procedure B

1. 5 g sample + 10 mL water were added + IS
2. Extracted using 15 mL acetonitrile
3. Salt out 0.5 g disodium hydrogen citrate sesquihydrate, 1 g trisodium citrate dihydrate, 4 g anhydrous magnesium sulfate, and 1 g sodium chloride were added
4. An aliquot of acetonitrile supernatant was transferred into a glass test tube and stored for at least 2 h (or overnight) in a freezer (−26 °C)
5. Dispersive-SPE agents (150 mg PSA, 550 mg C18, and 750 mg $MgSO_4$) with an aliquot of the extract (7.5 mL)
6. An aliquot of sample extract (3 mL) was evaporated and reconstituted in 1.5 mL toluene for GC–MS/MS analysis

Continued

TABLE 1 Applications of GC–QqQ-MS for Pesticide Analysis—Cont'd

Pesticide	Matrix	Sample Preparation	GC–QqQ-MS	References
		Procedure C 1. 5 g sample + 25 mL water/acetonitrile mixture (2:3, v/v) + IS 2. Salt out with 0.5 g disodium hydrogen citrate sesquihydrate, 1 g trisodium citrate dihydrate, 4 g anhydrous magnesium sulfate, and 1 g sodium chloride were added 3. An aliquot of acetonitrile supernatant (7.5 mL) cleanup with dispersive-SPE agents (150 mg PSA, 550 mg C18, 75 mg GCB, and 750 mg $MgSO_4$) 4. An aliquot of sample extract (3 mL) was transferred to a glass tube, evaporated and reconstituted in 1.5 mL toluene for GC–MS/MS analysis Procedure D 1. 5 g sample + 25 mL water/acetonitrile mixture (2:3, v/v) + IS 2. Salt out with 0.5 g disodium hydrogen citrate sesquihydrate, 1 g trisodium citrate dihydrate, 4 g anhydrous magnesium sulfate, and 1 g sodium chloride were added 3. An aliquot of acetonitrile supernatant (7.5 mL) cleaned up		

			with dispersive-SPE agents (550 mg PSA, 375 mg C18, and 750 mg $MgSO_4$) 4. An aliquot of sample extract (3 mL) was transferred to a glass tube, evaporated, and reconstituted in 1.5 mL toluene for GC–MS/MS analysis		
30	135 pesticides	Green and black dry tea leaves and stalks	Procedure A 1. 2 g tea sample + 10 mL water 2. Extracted with 10 mL MeCN (salt out 4 g anhydrous $MgSO_4$ and 1 g NaCl) 3. Centrifuged and used 1 mL extract for partitioning with 1 mL hexane + 5 mL 20% NaCl (w/w) solution 4. A part of hexane layer used for GC–MS/MS analysis Procedure B 1. 2 g tea sample + 10 mL water 2. Extracted with 10 mL MeCN 1% HOAc (salt out 4 g anhydrous $MgSO_4$ and 1.7 g $NaOAc.3H_2O$) 3. Centrifuged and used 1 mL extract for partitioning with 1 mL hexane + 5 mL 20% NaCl (w/w) solution 4. A part of hexane layer used for GC–MS/MS analysis Procedure C 1. 2 g tea sample + 10 mL water	An Agilent 7890A GC coupled with an Agilent 7000B QqQ-MS A HP-5 ms Ultra Inert column (15 m × 0.25 mm × 0.25 µm) coupled with a DB-5 ms Ultra Inert column (0.50 m × 0.15 mm × 0.15 µm) EI, positive, 2 µL injection, splitless injection mode, 20 min run time Carrier gas: helium; collision gas: nitrogen	[50]

Continued

TABLE 1 Applications of GC–QqQ-MS for Pesticide Analysis—Cont'd

Pesticide	Matrix	Sample Preparation	GC–QqQ-MS	References
		2. Extracted with 10 mL MeCN 1% HOAc (salt out 4 g anhydrous $MgSO_4$, 1 g NaCl, 1 g $Na_3Citr{\cdot}2H_2O$, and 0.5 g $Na_2HCitr{\cdot}1.5H_2O$) 3. Centrifuged and used 1 mL extract for partitioning with 1 mL hexane + 5 mL 20% NaCl (w/w) solution 4. A part of hexane layer used for GC–MS/MS analysis Procedure D 1. 5 g tea sample + 15 mL MeCN 2. Extracted with an Ultra-turrax macerator at 15,000 rpm for 1 min twice 3. The MeCN extract was purified using LLE 1 mL hexane and 5 mL 20% NaCl (w/w) solution 4. A part of hexane layer used for GC–MS/MS analysis		

32	19 OPs	Fats and oils	1. 25 g sample dissolved with GPC mobile phase (ethyl acetate–cyclohexane, 1:1, v/v) to 10 mL 2. 1 ml was cleaned up by GPC, collecting the fraction eluting between 14.5 and 24.5 min 3. The GPC fraction was evaporated to near dryness 4. Residue was dissolved with 1 mL hexane + IS	A Varian CP-3800 GC coupled with a 1200L QqQ-MS A Varian VF-5 fused silica capillary column (30 m × 0.25 mm × 0.25 µm) coupled with a deactivated guard column (2 m × 0.25 mm i.d.) EI, positive, 4 µL injection, splitless injection mode, 24 min run time Carrier gas: helium; collision gas: argon	[51]
33	Isofenphos, famfur, mirex, p,p-DDT, thionazin, gamma-lindane	Meat	1. 5 g sample extracted with 20 mL ethyl acetate 2. Extract filtered with a cartridge containing glass wool and 3 g anhydrous Na_2SO_4 3. Filtered extract was evaporated to dryness and redissolved in 5 mL cyclohexane–ethyl acetate (1:1, v:v) 4. 2 mL was cleaned up using GPC 5. Collected GPC fraction was evaporated to dryness and dissolved with 1 mL cyclohexane	A Varian CP-3800 GC coupled with a 1200L QqQ-MS A Varian VF-5 fused silica capillary column (30 m × 0.25 mm × 0.25 µm) coupled with a deactivated guard column (2 m × 0.25 mm i.d.) EI, positive, 10 µL injection, split injection mode, 11.6 min run time Carrier gas: helium; collision gas: argon	[52]
34	45 OCs, OPs	Muscle of chicken, pork, and lamb	A. Polytron extraction 1. 5 g sample drenched with 3 × 20 mL ethyl acetate and salted out with 3 g of anhydrous Na_2SO_4 using a Polytron PT2100 2. Filtered the extract and evaporated to almost dryness 3. Redissolved residue with 5 mL of mixed cyclohexane–ethyl acetate (1:1 v/v) and used 2 mL into GPC	A Varian CP-3800 GC coupled with a 1200L QqQ-MS A Varian VF-5 fused silica capillary column (30 m × 0.25 mm × 0.25 µm) coupled with a deactivated guard column (2 m × 0.25 mm i.d.) EI, positive, 10 µL injection, split injection mode, 11.6 min run time Carrier gas: helium; collision gas: argon	[53]

Continued

TABLE 1 Applications of GC–QqQ-MS for Pesticide Analysis—Cont'd

Pesticide	Matrix	Sample Preparation	GC–QqQ-MS	References
		Soxhlet extraction 1. 5 g sample extracted with 150 mL of ethyl acetate or 6 h 2. Evaporated the extract to almost dryness 3. Residue was redissolved with 5 mL of a mixed cyclohexane–ethyl acetate (1:1 v/v) and used 2 mL into GPC ASE 1. 5 g sample freeze-dried at 0.1–0.2 mbar for 8 h and mixed with 7 g Hydromatrix 2. Extracted at 120 °C; 1800 psi; 11 min; ethyl acetate 60 mL 3. Evaporated extract to almost dryness 4. Residue was redissolved with 5 mL of mixed cyclohexane–ethyl acetate (1:1 v/v) and used 2 mL for GPC GPC 1. Cyclohexane–ethyl acetate (1:1 v/v) in isocratic mode, flow rate was 5 mL/min, collected fractions 15–22 min 2. Evaporated the collected fraction to dryness 3. Residue was redissolved with 950 µL cyclohexane and 50 µL of IS (caffeine)		

35	57 pesticides	Hen eggs	1. 0.5-g homogenized egg + 2.0 g of C18 sorbent (previously washed with two volumes each of *n*-hexane, dichloromethane and methanol) and 1 g of anhydrous $MgSO_4$ 2. SPE (2g Florisil) cleanup 3. The SPE cartridge was eluted with 1.5 mL of ACN saturated in *n*-hexane (85:15, v/v) and 8.5 mL of EtAc (3 + 3 + 2.5 mL) 4. The extract was evaporated to near dryness and the residue was redissolved with 950 L of cyclohexane + IS	A Varian CP-3800 GC coupled with a 1200L QqQ-MS A Varian VF-5 fused silica capillary column (30 m × 0.25 mm × 0.25 μm) coupled with a deactivated guard column (2 m × 0.25 mm i.d.) EI, positive, 1 μL injection, splitless injection mode, 17.7 min run time Carrier gas: helium; collision gas: argon	[54]
36	34 OCs and OPs	Animal livers	A. Polytron extraction 1. 5 g sample drenched with 3 × 20 mL ethyl acetate and salted out with 3 g of anhydrous Na_2SO_4 using a Polytron PT2100 2. Filtered the extract and evaporated to almost dryness 3. Redissolved residue with 5 mL of mixed cyclohexane–ethyl acetate (1:1 v/v) and used 2 mL into GPC 4. Collected GPC fraction evaporated to dryness and redissolved using 975 μL cyclohexane and 25 μL IS MSPD 1. 0.5 g liver sample blended with 2.0 g C18 2. SPE extraction (2 g Florisil) and eluted using 10 mL ethyl acetate 3. Evaporated extract and redissolved with 975 μL cyclohexane and 25 μL IS	A Varian CP-3800 GC coupled with a 1200L QqQ-MS A Varian VF-5 fused silica capillary column (30 m × 0.25 mm × 0.25 μm) coupled with a deactivated guard column (2 m × 0.25 mm i.d.) EI, positive, 1 μL injection, splitless injection mode, 16.6 min run time Carrier gas: helium; collision gas: argon	[55]

Continued

TABLE 1 Applications of GC–QqQ-MS for Pesticide Analysis—Cont'd

	Pesticide	Matrix	Sample Preparation	GC–QqQ-MS	References
37	OPs	Fish feeds	1. 10 g fish feed samples extracted by the Soxtec system to generate fat extracts 2. 0.5 g of fat extracts + 3 mL hexane 3. SPE cleanup with an Extrelut cartridge and the pesticides were eluted with 3 × 5 mL portions of acetonitrile/*n*-hexane (80/20, v/v) 4. Extracts + 4 mL methanol evaporated and redissolved 1 mL of *n*-hexane 5. SPE cleanup with a Bond Elut PCB column 6. Elutes from step 5 were evaporated to dryness and the residue was dissolved in 1 mL of *n*-hexane. The dilution factor was two (HCB as the internal standard, which is used to check the cleanup steps. A minimum recovery of 70% for HCB guarantees the absence of procedural errors and/or instrumental failures that can compromise the analysis)	A Varian CP-3800 GC coupled with a 1200L QqQ-MS A Varian VF-5 fused silica capillary column (30 m × 0.25 mm × 0.25 μm) coupled with a deactivated guard column (2 m × 0.25 mm i.d.) EI, positive, 1 μL injection, splitless injection mode, 40 min run time Carrier gas: helium; collision gas: argon	[56]

38	12 pyrethroids	Milk	1. 1 g milk+IS extracted twice with 20 mL hexane/dichloromethane (2:1) 2. SPE cleanup C18 cartridge in tandem with basic alumina cartridge 3. Pyrethroids were eluted with 30 mL of acetonitrile 4. Elutes were evaporated and redissolved with 100 µL ethyl acetate	An Agilent 7890A GC coupled with 7000A QqQ-M A DB-5MS capillary column (15 m × 0.25 mm × 0.1 µm) containing 5% phenyl methyl siloxane EI, positive; CI, negative; 3 µL injection, splitless injection mode; 20 min run time Carrier gas: helium; collision gas nitrogen; NCI:ammonium	[30]
39	100 pesticides	Olive oil	Procedure 1 1. 4 g olive oil dissolved in 20 mL *n*-hexane 2. Extracted using 20 mL ACN saturated with *n*-hexane twice 3. Acetonitrile phase collected and evaporated to almost dryness and dissolved with 5 mL ethyl acetate-cyclohexane (1:1) 4. 2.5 mL was used for GPC cleanup 5. Evaporated collected GPC fraction to dryness 6. Dissolved the residue with 1 mL cyclohexane Procedure 2 1. 1 g olive oil dissolved with 8 mL ethyl acetate–cyclohexane, 1:1, v/v 2. 2.5 mL was used for GPC cleanup 3. Evaporated collected GPC fraction to dryness 4. Dissolved the residue with 1 mL cyclohexane	A Varian CP-3800 GC coupled with a 1200L QqQ-MS A Varian VF-5 fused silica capillary column (30 m × 0.25 mm × 0.25 µm) coupled with a deactivated guard column (2 m × 0.25 mm i.d.) EI, positive, 5 µL injection, splitless injection mode, 19 min run time Carrier gas: helium; collision gas: argon	[57]

Continued

TABLE 1 Applications of GC–QqQ-MS for Pesticide Analysis—Cont'd

	Pesticide	Matrix	Sample Preparation	GC–QqQ-MS	References
40	19 OCs, OPs, pyrethroids	Vegetables, eggs	Vegetables 1. 10 g sample extracted with 10 mL acetonitrile (1% HAc) 2. Salt out with anhydrous NaAc (1 g) and anhydrous $MgSO_4$ (4 g) 3. 1 mL extract dispersive cleanup PSA (25 mg) and anhydrous $MgSO_4$ (150 mg) 4. Evaporated 800 µL extract to dryness and redissolved with cyclohexane 400 µL Eggs 1. 0.5 g egg blended with C18 sorbent (2.0 g) anhydrous $MgSO_4$ (1.0 g) were added 2. SPE (2 g Florisil) and eluted with 1.5 mL acetonitrile saturated with hexane and 8.5 mL ethyl acetate 3. Evaporated the extract to near dryness and redissolved with 975 µL of cyclohexane and 25 µL IS	A Varian 3800 GC coupled with a Varian 1200L QqQ-MS A Varian VF-5 fused silica capillary column (30 m × 0.25 mm × 0.25 µm) coupled with a deactivated guard column (2 m × 0.25 mm i.d.) EI, positive, 10 µL injection, splitless injection mode; 20.5 min run time Carrier gas: helium; collision gas: argon	[58]

3.2 Determination of Pesticides in Food Samples

For simplicity, food sample matrices can be categorized as dry foods (e.g., animal feeds, dry botanicals, grains, cereals, etc.), high water content foods (e.g., fresh produce and vegetables), and fatty foods (meat, oil, animal organs, etc.). Depending on the sample matrices, different sample preparation techniques are required to obtain satisfactory results in the GC–QqQ-MS analysis of pesticides in these food matrices.

3.2.1 High Water Content Food Samples

Typically, pesticide residues in fresh produce have been analyzed with gas chromatography equipped with element selective detectors such as ECD, FPD, PFPD, etc. Since the 1980s, many of these detectors have been replaced by GC–MS/SIM as the main instrument of analysis and the element selective detectors are used complementarily for pesticides not suitable for MS because of poor ionization. However, recently, GC–QqQ-MS is beginning to replace GC–MS-SIM because of the improved selectivity and sensitivity. Table 1 (#11–25) includes different GC–QqQ-MS methods, targeting fresh produce, primarily fruits, and vegetables. In general, these methods start with an organic solvent extraction procedure, followed by SPE cartridge or dispersive SPE (d-SPE) cleanup using different sorbents (e.g., C18, graphitized carbon black, primary–secondary amines, etc.), followed by GC–QqQ-MS analysis.

QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) and its subsequent modifications were first applied to pesticide analysis in fresh produce samples [59–61]. The QuEChERS procedure is an efficient salt-out organic extraction followed by dispersive solid extraction cleanup in a test tube. Compared to conventional salt-out extraction methods [62–64], QuEChERS uses less lab glassware, smaller sample size (10–15 g), and less extraction solvent (10–15 mL). The method provides satisfactory recoveries (70–120%) for a wide range of pesticides, including polar pesticides such as methamidophos and acephate. The success of QuEChERS was partially due to its introduction at the time that QqQ-MS technology was becoming popular in pesticide laboratories.

Wong *et al.* [39] compared the instrument performance of GC–QqQ-MS and GC–MS-SIM using samples prepared by an acetonitrile extraction procedure based on QuEChERS and Fillon's method [65]. Their results demonstrate that GC–QqQ-MS outperforms GC–MS-SIM in terms of sensitivity and selectivity. GC–QqQ-MS detects 90% of the 168 target pesticides fortified into 10 different fresh produce and vegetables at 10 and 25 ng/g. The corresponding recoveries range from 70% to 120% with RSD $<20\%$, while GC–MS-SIM could only detect $<80\%$ of the pesticides at the same fortification concentrations. Koesukwiwat *et al.* [43] developed a low-pressure GC–QqQ-MS method for a fast analysis of 150 pesticides. Using a 10 m, 0.53 mm i.d., 1 μm film analytical column, a 3 m, 0.15 mm i.d. capillary at

the inlet, and a rapid oven temperature ramp rate, the method can separate and detect the 150 pesticides within 6.5 min. Though average peak width is just 2–3 s, the method takes advantage of fast data acquisition capability of the GC–QqQ-MS by setting dwell times to 2.5 ms and arranging the number of transitions in each segment to achieve at least eight data points per peak. Cervera *et al*. [40] developed a method that can detect 130 pesticides in vegetables and fruits using accelerated solvent extraction and GC–QqQ-MS. Only spinach extracts underwent GPC cleanup procedure. No cleanup was applied to orange or nectarine samples. Even though recoveries were between 70% and 120% and RSDs were <20%, matrix enhancement was observed in all tested samples for the majority of pesticides investigated. Pesticides had to be quantitated using matrix-matched calibration standards. The observation of signal enhancement reported in this study underlines the importance of evaluating matrix effects.

3.2.2 Fatty Food Samples

In fatty foods, the major interferences include lipids, sterols, waxes, saturated/unsaturated fatty acids, as well as fatty acid esters and proteins. It requires significant efforts to defat the samples by different extraction and cleanup procedures. With fat content <20% (e.g., milk), samples can be prepared using QuEChERS. For samples with fat content >20%, GPC is a commonly used cleanup technology. Garrido Frenich *et al*. [58] compared the performance of ion trap-MS and QqQ-MS using solvent, high water content, and fatty food samples. In the cucumber (high water content) or solvent samples, the two instruments generated similar intraday precision, linearity, and sensitivity. The QqQ-MS provided better sensitivity in egg matrix, suggesting that, for complex matrices, QqQ-MS can better separate target analytes from matrix inferences. Different from the QqQ-MS, the ion trap uses its storage feature to perform MS/MS in time by sequentially storing precursor ions, fragmenting them, and then scanning out the product ions using the same ion storage compartment. In comparison, the selection of precursor ions, fragmentation, and detection of product ions happen in different devices in QqQ-MS. When operating in SRM mode, QqQ-MS has a faster data acquisition rate so that it can monitor more concurrent ions [35,66]. This has been a very desirable feature for improving pesticide screening capacity.

3.2.3 Dry Food Samples

Pesticides have been monitored in dry foods such as medicinal plants, herbs, dietary supplements, and grains. Water content in these matrices is <1%. Compared to fatty samples or high water content samples, dry food samples have more concentrated matrix per mass unit. When analyzing dry food samples, smaller sample sizes compared to high moisture foods are used. A large sample size will lead to more matrix components, requiring additional cleanup

efforts. In order to improve extraction efficiency, hydration is often employed for sample swelling so that enclosed pesticides could be extracted. Cajka *et al.* [50] evaluated four different sample preparation procedures that could be coupled with GC–QqQ-MS for the detection of pesticides in dry tea samples. Compared to a method using GC–MS-SIM [67], Cajka's method achieved satisfactory recoveries (70–120%) for the majority of 135 target pesticides using much less sample cleanup.

The use of GC–QqQ-MS can help identify pesticides from complex matrices such as dry ginseng powder. Due to matrix interference, two BHC isomers could not be identified using GC–MS-SIM, but using GC–QqQ-MS, the four isomers are clearly separated and identified without any difficulty [48]. The presence of ε-BHC could not be confirmed due to the lack of reference standard.

This is best demonstrated in Figure 1 where the presence of incurred BHC residues in dried ginseng powder (*Panax quinquefolius*) was determined by two extraction methods (acetonitrile, ACN, and 2:1:1 acetone/cyclohexane/ethyl acetate, ACE) and analyzed by either GC–QqQ-MS (A) or GC–MS-SIM (B). From Figure 1A, GC–QqQ-MS shows the presence and separation of α-, β-, γ-, δ-, and ε-BHC isomers by the two transitions, 181 → 146 (primary, quantitation) and 219 → 183 (secondary, qualifier). Transition ratios 181 → 146/219 → 183 of the two extraction procedures showed that the two procedures are similar. GC–MS-SIM (Figure 1B), on the other hand, shows

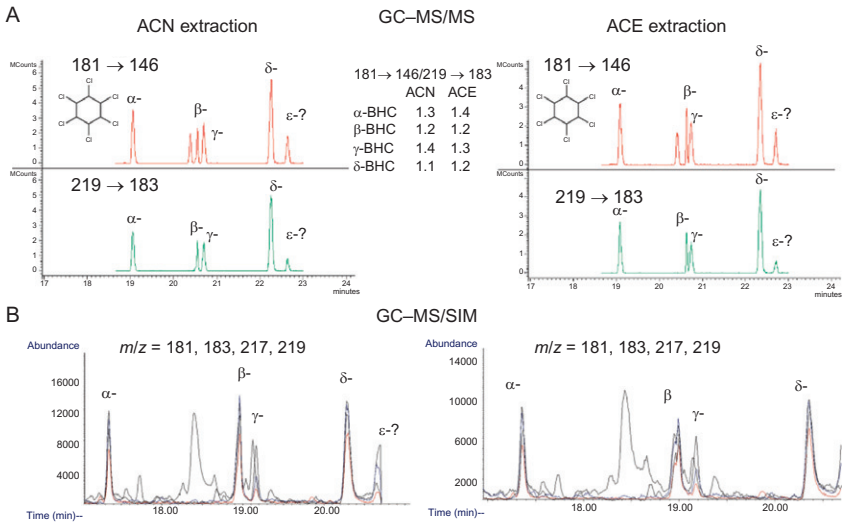

FIGURE 1 BHC residues in dried ginseng powder by (A) GC–QqQ-MS versus (B) GC–MS-SIM.

interferences in the screening and identification of the β- and γ-BHC isomers using *m/z* 181, 183, 217, and 219 (modified from Ref. [48]). Additional studies of dry foods are listed in Table 1 (#25–40).

4 CHALLENGING ISSUES OF GC–QqQ-MS

GC–QqQ-MS only can be applied to semivolatile, nonpolar, and thermally stable pesticides that are amenable to the analysis. When analyzing polar, thermally labile, and low-volatility pesticides, researchers have to use LC–MS for the same selectivity and sensitivity [68]. It is impossible to directly introduce pesticides such as macrocyclic lactones, carbamates, or neonicotinoids into QqQ-MS via GC conditions. Even for pesticides which could be introduced into QqQ-MS, their on-column masses (sensitivity) are limited due to the sample capacity of GC injection system.

The GC–QqQ-MS analysis could also suffer from matrix effects. Two types of matrix effects might occur and have to be addressed. One is signal suppression or enhancement and the other is isobaric interferences (spectral interferences) [69–71]. Overlapping spectral peaks mask the peaks of interest and can provide false-positive or -negative results. Common solutions to signal alteration include matrix-matched calibration standards, stable isotope dilution, standard addition, or addition of analyte protectants. Stable isotope dilution cannot be applied universally to multiresidue pesticide analysis in foods due to the large number of standards required, which may not be available and will greatly increase the cost. When quantitating several pesticides in a single sample, standard addition becomes very impractical and challenging to perform. Changing chromatographic conditions, more cleanup, orthogonal analysis, or high-resolution mass spectrometry (HRMS) could be used to address the issues related to isobar interference. For multiresidue pesticide analysis, there is no perfect solution to matrix effects because each analyte might be affected differently by the matrices.

Compared to GC–MS full scan or GC–MS SIM methods, GC–QqQ-MS methods that use two MRM data acquisitions within a specific RT window provide better selectivity and SNR of the target analytes in extracted ion chromatograms [39,72]. This approach is sufficient to fulfill the requirement of EU Directive 2002/657/EC [17,73] for identification of pesticide residues. Because in the course of GC–QqQ-MS analysis mass spectral fragmentation patterns are dependent on instrument, analyte concentration, and matrix effects, there are challenges using RT, two or more MRM transitions, and their relative ion intensity ratios for identification of target analytes. These include the selection of nonspecific transitions that may be the same as that of a coeluting matrix compound, inconsistent ion ratios in different matrices, or the weak response of the second MRM transition at low intensity, which could lead to false-positive/negative identification [74,75].

Ionization plays a vital role in MS. The majority of existing GC–QqQ-MS instruments uses the standard electron impact ionization (EI) source. Compared to other ionization sources such as electrospray ionization (ESI), EI is a "hard" ionization technology, using standardized conditions (e.g., 70 eV) to ionize target analyte molecules in gas phase. In the course of ionization, many fragments could be generated and molecular ions either do not have enough abundance or are absent, a fact which forces nonmolecular ions (fragments) to be chosen as precursor ions for MS/MS transitions. This raises the concerns for identification. Additionally, when molecular ions are absent, based on full scan spectra, it could be challenging and time consuming to choose multiple precursor ions for collision experiments. For example (see Figure 2), if the molecular ion (*m/z* 315) of dichlorfenthion presents with reasonable abundance, it would be much easier to choose *m/z* 315 than choose between *m/z* 279, 251, and 223 for an appropriate precursor ion. These issues prompted the need for alternative or new ionization technologies to improve existing GC–QqQ-MS methods.

Chemical ionization (CI) has been used as an alternative to EI [76,77]. Molecular ions could be generated with satisfactory abundance using CI, especially when analyzing halogenated compounds. Unlike EI, CI does not have standardized ionization conditions, which will introduce another level of complexity to the GC–QqQ-MS methods, because different reagent gases (e.g., methane, ammonia, iso-butane) and corresponding pressures can be selected and "optimized" to adjust the proton-donating ability of the reagent gas relative to the proton affinity of analytes with the ionization energies used in the analysis. New ionization technologies for GC–MS such as atmospheric pressure chemical ionization (APCI) [44] and supersonic molecular beams (SMBs) ionization [78,79] have been reported but commercially limited or unavailable. Only a few pesticides or food matrices have been tested using those new ionization technologies. Without extensive studies to evaluate the applicability of APCI or SMB, EI will continue to be the main ionization source used for GC–QqQ-MS.

Once a GC–QqQ-MS method becomes validated in a single laboratory, it is difficult to transfer the method to other laboratories. Table 2 indicates that selected MRM transitions are user dependent. The optimal parameters

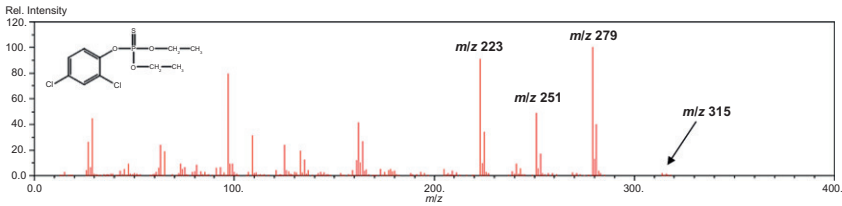

FIGURE 2 EI spectrum of dichlorfenthion. Source: *http://webbook.nist.gov/chemistry*.

TABLE 2 Comparison of QqQ-MS Parameters on Different Platforms

	MRM Transitions (Collision Energy)			
	Platform 1	*Platform 2*	*Platform 3*	*Platform 4*
Captan	149 → 79 (10 eV)	264 → 79 (10 eV)	149 → 105 (2 eV)	149 → 70 (8 eV)
	79 → 77 (10 eV)	NA	149 → 70 (12 eV)	149 → 105 (8 eV)
Cyhalothrin-lambda	181 → 152 (25 eV)	197 → 141 (10 eV)	208 → 181 (8 eV)	181 → 152 (23 eV)
	197 → 141 (15 eV)	197 → 161 (5 eV)	197 → 141 (10 eV)	208 → 181 (10 eV)
Diazinon	304 → 179 (15 eV)	304 → 179 (10 eV)	199 → 93 (16 eV)	304 → 179 (15 eV)
	179 → 137 (20 eV)	304 → 162 (10 eV)	304 → 179 (12 eV)	199 → 193 (15 eV)
Deltamethrin	181 → 152 (25 eV)	253 → 174 (10 eV)	253 → 174 (8 eV)	253 → 172 (18 eV)
	181 → 127 (25 eV)	253 → 172 (5 eV)	253 → 93 (14 eV)	253 → 93 (18 eV)
Collision gas	Nitrogen	Argon	Argon	Argon
EI source temp (°C)	320	270	250	250
References	[43]	[47]	[80]	[81]

NA, not available.

suggested by different platforms are not comparable. One cannot directly transfer one set of parameters from one GC–QqQ-MS system to another. One advantage of GC–MS is the standardized ionization so that spectra are largely transferrable among instrument platforms allowing easy library searching. GC–QqQ-MS measures a set of MRM transitions generated using conditions that are mostly nonstandardized, so the universal aspect of the EI spectrum is lost. It is clear that on different platforms, investigators have optimized different conditions for the same pesticides.

5 FUTURE OF GC–QqQ-MS

Regardless of its high capital cost and time-consuming method development requirements, GC–QqQ-MS would relieve researchers from conventional laborious sample preparation or cleanup procedures. Indeed, a large number

of pesticides have been analyzed using modified QuEChERS and GC–QqQ-MS in fresh produces and vegetables. However, increasing numbers of traditional pesticides such as carbamates and phenyl ureas and newly registered pesticides are not amenable to GC analysis, indicating a shrinking user base of GC-related technologies. A recent study shows that in some botanical matrices, approximately only 100 of the 312 target pesticides could be detected below 40 ng/g using GC–QqQ-MS [81]. This suggests that botanicals are difficult matrices and that GC–MS systems have limited capacity. Without additional sample cleanup or concentration, GC–QqQ-MS lacks the ability to separate the target analytes from isobaric interferences in those tested matrices. This limited capability with respect to analyzing pesticides in dry botanicals restricts the applicability of GC–QqQ-MS. It is not surprising that HRMS has been proposed and evaluated as an alternative to resolve isobaric interferences from analytes. Instruments equipped with new ionization sources such as LC–MS with APPI, ESI, or APCI, and especially LC–HRMS, can analyze pesticides that used to be only amenable to GC with comparable sensitivity. Without any significant improvement in the ionization source, selectivity, or decrease in cost, GC–QqQ-MS will be gradually replaced by other technologies that can perform pesticide analysis in a more practical, efficient, and cost-effective manner.

ABBREVIATIONS

APCI	atmospheric pressure chemical ionization
APPI	atmospheric pressure photo ionization
ECD	electron capture detector
EI	electron ionization
ESI	electrospray ionization
FPD	flame photometric detector
GC	gas chromatography
GC–MS	gas chromatography–mass spectrometry
GC–MS-SIM	gas chromatography–mass spectrometry single ion monitoring
GC–QqQ-MS	gas chromatography–triple quadrupole mass spectrometry
GPC	gel permeation chromatography
HRMS	high-resolution mass spectrometry
LC	liquid chromatography
LC–MS	liquid chromatography–mass spectrometry
LOD	limit of detection
LOQ	limit of quantitation
MRLs	maximum residue limits
MRM	multiple reaction monitoring
PAHs	polycyclic aromatic hydrocarbons

PBDEs	polybrominated diphenyl ethers
PCBs	polychlorinated biphenyls
PFPD	pulsed flame photometric detector
PLE	pressurized liquid extraction
PM	particulate matter
SMB	supersonic molecular beam
sMRM	supersonic molecular beam-scheduled multireaction monitoring
SNR	signal-to-noise ratio
SPE	solid-phase extraction
SRM	selected reaction monitoring
TOF-MS	time-of-flight mass spectrometry

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Chapter 4

Environmental Odor Pollution: A Complex GC–MS, Olfactometry and Diffusion Modeling Approach to Define Air Quality

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1 ENVIRONMENTAL ODOR POLLUTION

Odor, aroma, scent: Webster's thesaurus lists these terms as synonyms, but each of them has a completely different world behind it, implying different research goals and approaches. The environmental harassment that odors can cause is attracting widespread interest, and an objective/universal technology is needed to describe air quality and odors, particularly for industrial activities. The effect of odors on humans is primarily one of nuisance; however, the effects can be more problematic [1–4]. Odors can trigger nausea, headaches, and loss of

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appetite; disturb sleep patterns [5,6]; cause emotional disturbance, mental depression, and irritability, if persistent; interfere with proper working conditions; and may cause a depreciation in property values [7–9]. Besides odors' annoying impacts, which may limit some human activities, the perception of odors, especially from industrial activities, is more and more often associated with people's fear of possible health effects [10,11].

The perception of odors by humans is not completely understood because of the complex series of chemical and neurological interactions that take place in the human olfactory system. The response to an odor may provoke an emotional response [12] as well as draw on an individual's past experience [2], which may be associated with that particular odor. The nature of the odor itself may also cloud an individual's natural detection ability. The chemical composition, predominant weather conditions, and the originating source of an odor may all act in some fashion to muddle an individual's perception of the odor.

Some odorous compounds can exist and exhibit their effects at concentrations in the parts per billion (ppb) range or lower [4]. The human olfactory system, in some instances, is able to detect these compounds even at this low concentration. There are analytical methods for determining the presence of these particular odor-causing agents; however, an analytical means to determine an individual's annoyance with a particular odor does not exist.

An individual's judgment of the existence of an odorous agent is affected by the following variables:

- natural sensitivity
- age
- sex
- eating, drinking, and smoking habits
- prejudices against the source
- personal experience
- health problems
- pregnancy
- medications
- education

Humans remain the primary instrument in determining the existence of an odor problem. For this reason, the European Commission [13–15] stated that odor concentration can be determined, at least as of now, only by dynamic olfactometry. EN13725:2003 Guidelines were published in order to standardize the methodology within the Member States.

The existence and verification of an odor are complex, and analytical approaches are needed [16] to describe the environment, to characterize, among volatile organic compounds (VOCs), not only odorants that are responsible for the odor [17,18], but also other compounds, especially hazardous and potentially toxic compounds, such as hazardous air pollutants (HAPs). In this way, it is possible to describe environmental air quality in terms of both odorants [19] and possible health risks [14,20] for exposed populations.

A complex approach is presented that is used to solve complex environmental odor pollution cases. Environmental odor pollution assessment is defined by dispersion modeling of sampled odor emissions, after measuring the odor concentration by dynamic olfactometry. The same odor emissions are chemically characterized by full scan, low resolution, mass spectrometry with a sample preparation based on solid phase microextraction (SPME). With a good knowledge of the single VOCs present and their individual concentrations, it is possible to use advanced multivariate statistical approaches, such as principal components analysis (PCA) or a cluster analysis (CA), to describe sample similarities as an aid to assess the most representative odor emissions' sources in specific environments and, possibly, individual-specific markers of malodor sources.

2 ANALYTICAL METHODS

2.1 Air Sampling

Emission and environmental air sampling are performed following EN 13725:2003 guidelines, with Nalophan^{TM} bags (Tillmanns S.p.A., Italy) and a short PTFE inlet tube. Briefly, a battery-operated pump is used to evacuate a container, which allows the initially deflated bags to inflate (lung principle). As mentioned earlier, odorants and VOCs in ambient samples are usually present in traces, and it is very easy to alter their presence. In this way, the air sample does not come in contact with pump parts that might ruin sample integrity by both adsorption processes (with loss of some compounds) and pump material contamination (with sample contamination). In the bag, an isotopically labeled internal standard is added in order to increase the analytical accuracy during sample storage. Different samples are collected to accurately describe different odor emissions and ambient air, usually at the receptors' site, possibly during odor pollution episodes. Bags with sample are therefore shipped to the laboratory and analyzed [16] within 30 h as recommended by the EN method. Sampling strategy, number of samples, point location, time, etc. follows EN 13725 guidelines, while procedures used for area sources are based on a wind tunnel approach [21–23].

Nalophan bags were extracted in the lab with an SPME preconcentration [24,25] with a divinylbenzene/carboxen/polydimethylsiloxane, 50–30 μm fiber (Supelco, Bellefonte, PA, USA), preconditioned for 3 h at 250 °C. The analytes were adsorbed from the air samples by exposing the fiber [26,27] to the air samples in the bags for 30 min at room temperature (Figure 1).

2.2 Instrumental Analysis

The SPME fiber was used to inject on an Agilent 5975C Series GC/MSD. Volatiles were separated using a 60 m × 0.32 mm ID capillary column (CP 7415, Varian Inc., Lake Forest, CA, USA) with a film thickness of 1.80 μm. The carrier gas was helium at a flow rate of 1 ml/min. VOCs were desorbed exposing the fiber in the GC injection port for 5 min at 250 °C. A 2-mm glass liner was used, and the injector was set in splitless mode for 1 min. The oven

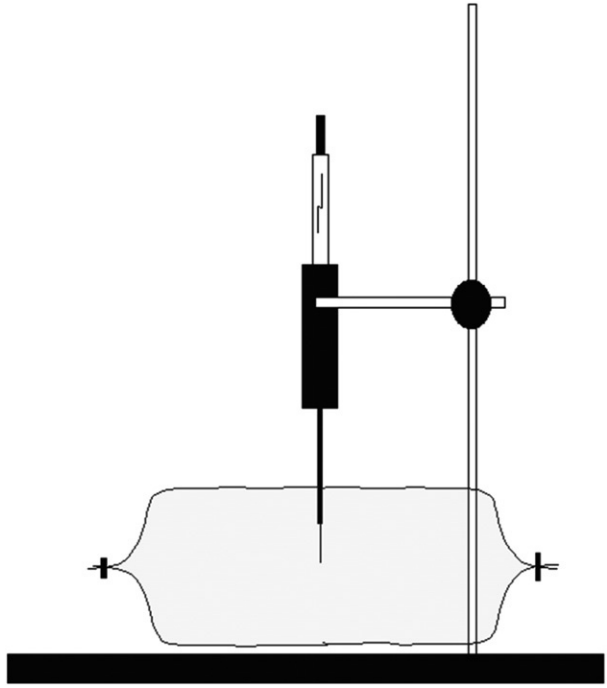

FIGURE 1 Sampling of a Nalophan bag with an SPME device in the laboratory. The SPME fiber is exposed to the airborne sample for 30 min at room temperature. An internal standard, either deuterated xylene used for semiquantitative profiling of odorants or a mixture of selected isotopically labeled compounds for quantitative analysis, is added to the sample.

temperature program was isothermal for 3 min at 35 °C, then raised to 200 °C at the rate of 8 °C/min. The transfer line to the MS was maintained at 250 °C. The mass spectra were obtained by electronic impact at 70 eV and collecting full scan data, in a mass range of 33–300 *m*/*z*. Compounds were identified by comparing their mass spectra with those contained in the NIST 98 instrumental library. The odorants and other VOCs, including HAPs, that are identified by the library software, were confirmed by an expert review and, where necessary, by pure standard comparison. Quantitative analysis on specific compounds is performed by using calibration curves with isotopically labeled analogues. Semiquantitative analysis for all the identified and unknown compounds was performed by direct comparison with the internal standard. Results were expressed as part per billion on a volume basis (ppbv).

2.3 Olfactometry

In dynamic olfactometry, diluted odorous air is presented to a panel of evaluators through a sniffing port, in order to define odor concentration. Results are

expressed as European Odor Units (OU_E) corresponding to the numerical value of the odor concentration, equal to the dilution factor that is necessary to reach the odor threshold of a specific sample. If a sample, diluted 1000 times, is detected by 50% of the test panel, its odor concentration will be 1000 OU_E, higher, for example, than a sample that needs a 100 times dilution to be detected. All analyses are performed in collaboration with external olfactometric laboratories, working under EN 13725 guidelines.

2.4 Diffusion Modeling

Pollutants and odor concentrations at receptors are estimated using American Meteorological Society/Environmental Protection Agency Regulatory Model (AMS/EPA Regulatory Model, AERMOD), a steady-state, plume atmospheric dispersion model developed by the US Environmental Protection Agency. It consists of two input data processors: a meteorological data preprocessor (AERMOD meteorological preprocessor, AERMET) that incorporates air dispersion based on planetary boundary layer turbulence structure and scaling concepts, and a terrain data preprocessor (AERMOD terrain preprocessor, AERMAP) that incorporates complex terrain using U.S. Geological Survey (USGS) Digital Elevation Data.

This model is used to predict the concentration under any weather condition from different types of sources (diffusive and punctual) and across different terrain conditions including receptor location. Site-specific inputs for the model were elaborated using meteorological and terrain data, to estimate pollutant and odor concentrations in air, and pollutant soil depositions in correspondence to the sensitive receptors identified in the specific environment.

Calpuff, developed by California Air Resources Board (CARB), is often used as an alternative model, especially in areas where calm wind conditions are important [28].

2.5 Chemometric Analysis

Data elaboration is carried out using multivariate statistics, in particular PCA and CA. The former technique allows to reduce the number of variables which characterizes a set of data in a small number of components (principal components), which are linear combinations of the original variables, orthogonal among them and ordered on the basis of the variance they subtract to the system. The first two components describing most of the system variability may be represented as orthogonal axes, and samples may be projected in a bidimensional space. The latter technique easily provides a classification into groups of objects characterized by similar properties. In this approach, it has been used as a hierarchical clustering based on Euclidian distance.

An overall flowchart for this approach is presented in Figure 2.

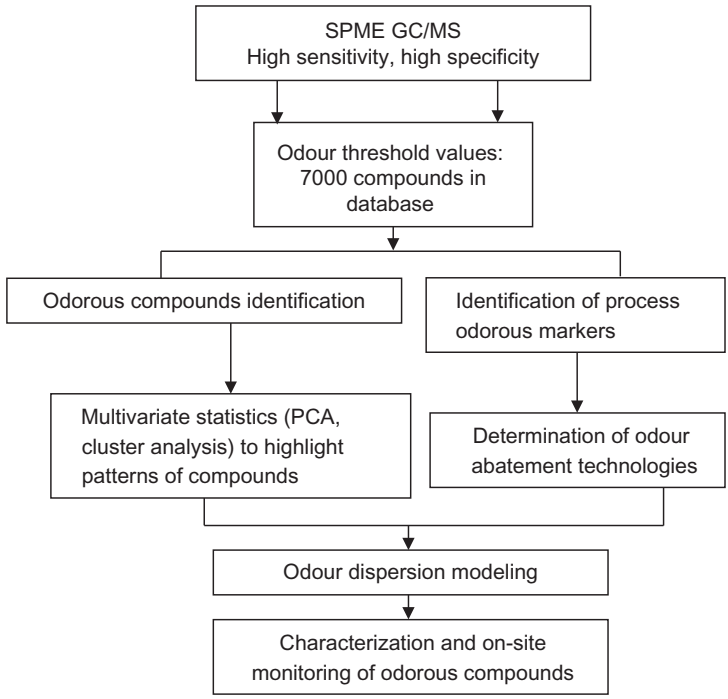

FIGURE 2 Flowchart for this approach is reported.

3 RESULTS FOR VOCs

Instrumental sensitivity allows analysis of VOC components generally well below 1 ppbv [29,30] working in full scan. When sensitivity is an issue, the MS can be used in selected ion monitoring (SIM) mode. In Figure 3, a SIM chromatogram is presented showing the results obtained after sampling a Nalophan bag with 65 ppt of dimethyl disulfide. A blank sample is overlaid in the same chromatogram.

Linearity also is good down to the low ppb region for several VOCs. In Figure 4, data are reported from a mixture of odorants and HAPs calculated versus the internal standard, perdeuterated p-xylene. Some adsorption effects can be noticed in the more polar compound, hexanoic acid, where there is a lack of linearity for low concentration standards, while all other compounds show good linearity. The dynamic range also is wide, and it has shown linearity for high concentration samples in the range of 1–100 ppm [30].

A different response factor is observed for different compounds, given that this is a well-known and commonly reported factor in SPME [31]. For this reason, the approach has to be considered semiquantitative unless homologous internal standards are used. However, precision is still good. In Table 1, results are reported from triplicate analysis of the same mixture, showing

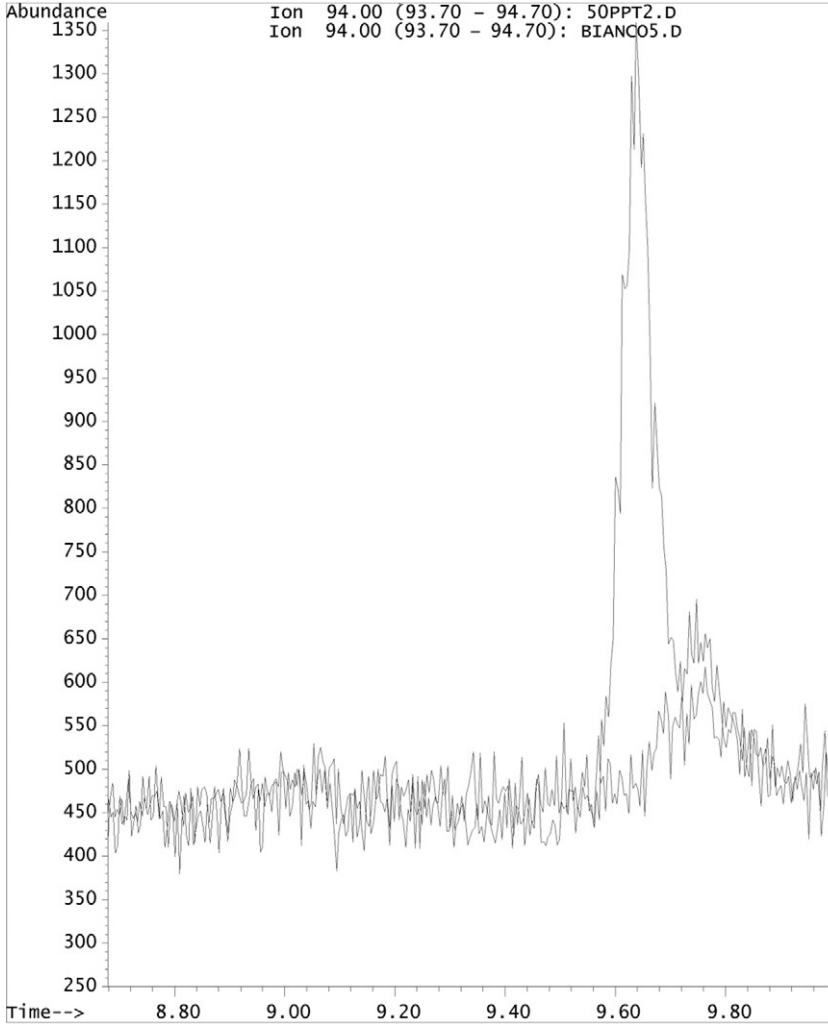

FIGURE 3 Sensitivity. SIM chromatogram is reported showing results obtained after sampling a Nalophan bag with 65 ppt of dimethyl disulfide. A blank sample is superimposed.

relative standard deviation (RSD) values generally around 20% for all compounds at all levels. Ions monitored for these compounds are listed in Table 2.

Analysis of polar compounds is possible and reveals, for example, oxygenated odorants in a composting plant emission and scrubber's effects in removing volatile fatty acids and esters (Figure 5). Air sample before the scrubber has a significant higher concentration of volatile fatty acids compared to the sample after the treatment. This abatement is not observed with esters that are not efficiently removed in this case.

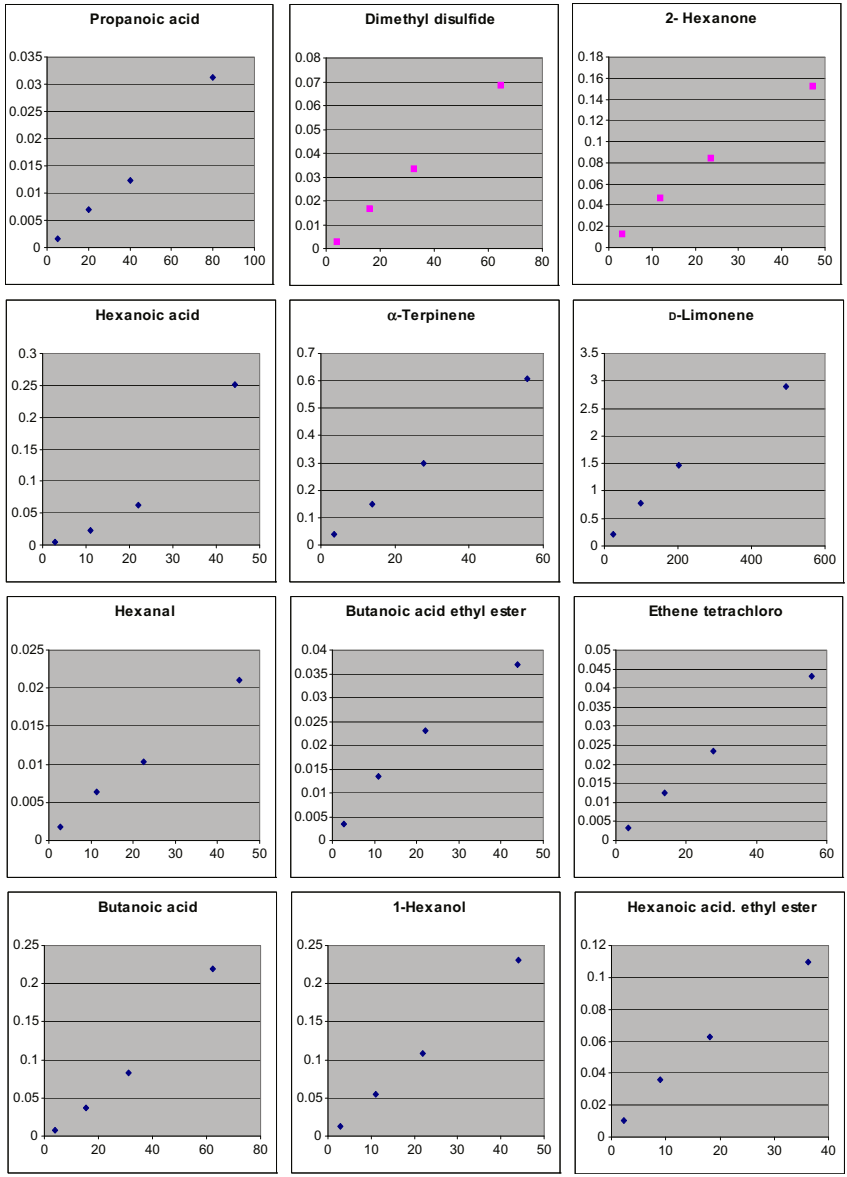

FIGURE 4 Linearity. Results in the low ppb region for several VOCs are presented for a mixture of odorants and HAPs calculated versus the internal standard, perdeuterated p-xylene.

TABLE 1 Quantitative Analysis of Odorants in an Air Sample at Low ppb Levels

	Calibration Level 1			Calibration Level 2			Calibration Level 3			Calibration Level 4		
Compounds	*ppb*	*Mean*	*RSD (%)*	*ppb*	*Mean*	*RSD (%)*	*ppb*	*Mean*	*RSD (%)*	*ppb*	*Mean*	*RSD (%)*
Propanoic acid	5	0.0017	19	20	0.0070	22	40	0.0123	10	80	0.0312	3
Dimethyl disulfide	4	0.0028	6	16.2	0.0166	25	32.4	0.0335	10	64.7	0.0688	2
2-Hexanone	3	0.0128	3	11.8	0.0466	19	23.6	0.0847	9	47.2	0.1528	3
Hexanal	2.8	0.0018	9	11.3	0.0064	20	22.5	0.0103	10	45.1	0.0210	3
Butanoic acid, ethyl ester	2.8	0.0036	3	11	0.0134	18	22	0.0231	10	44	0.0370	8
Ethene, tetrachloro	3.5	0.0034	5	13.9	0.0125	19	27.8	0.0236	9	55.7	0.0430	4
Butanoic acid	3.9	0.0078	20	15.5	0.0374	22	31.1	0.0830	4	62.2	0.2189	4
1-Hexanol	2.8	0.0130	6	11	0.0546	22	22	0.1081	8	44.1	0.2312	4
Hexanoic acid, ethyl ester	2.3	0.0104	7	9	0.0357	18	18.1	0.0630	7	36.2	0.1094	7
Hexanoic acid	2.8	0.0041	51	11	0.0226	26	22.1	0.0630	5	44.2	0.2510	8
α-Terpinene	3.5	0.0382	4	13.9	0.1490	21	27.8	0.2968	7	55.6	0.6069	1
D-Limonene	24.7	0.2131	6	98.8	0.7718	19	202.6	1.4710	9	494.2	2.8945	5

SPME must be considered semiquantitative, unless homologous internal standards are used. Here, results are reported from triplicate analysis of the same mixture in air samples, showing RSD values generally around 20% for all compounds at all levels.

TABLE 2 Ions Monitored for the Quantitative Analysis

Compound	*m/z*
Propanoic acid	73
Dimethyl disulfide	94
2-Hexanone	43
Hexanal	56
Butanoic acid, ethyl ester	88
Ethene, tetrachloro	166
Butanoic acid	60
p-Xylene d10 (internal standard)	98
1-Hexanol	56
Hexanoic acid, ethyl ester	88
Hexanoic acid	60
α-Terpinene	93
D-Limonene	93

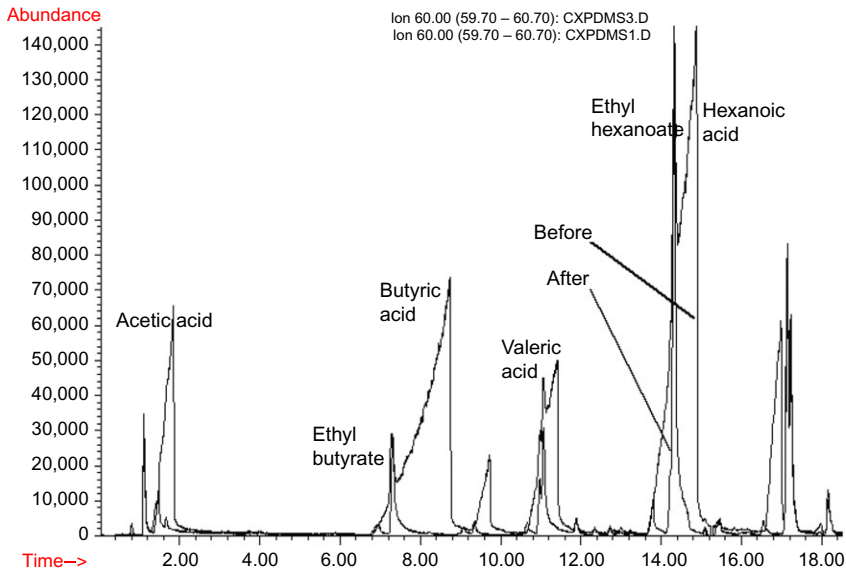

FIGURE 5 Odor abatement technologies can be compound-dependent and are characterized. For example, analysis of polar compounds reveals oxygenated odorants in a composting plant emission and scrubber's effects in removing volatile fatty acids and esters.

4 CASE STUDIES: LANDFILLS AND COMPOSTING PLANTS

In Figures 6 and 7, PCA results are presented from three different studies, performed on different environmental odor pollution cases, revealing different possible local odor sources. In Figure 6, a composting plant has very well-defined emissions from both the biofilter and diffusive emissions coming from biofilter emissions and compost curing areas (represented as B and C clusters in the figure, respectively), but they show no similarities with ambient air (represented as cluster A) during an odor pollution episode. In the score plots reported (where the transformed variable values corresponding to a particular data point are plotted), the ellipsoids group similar samples, suggesting a similarity among them. On the contrary, samples that are distant in this space are less similar. Figure 7 shows an odor pollution problem close to a composting plant. Emissions from curing compost piles (represented as B), the biooxidation area (represented as C), and the biofilter (represented as A) are very well separated in the PCA plot. In this case, ambient air samples, collected during an odor episode, are plotted inside the biofilter ellipsoid as being similar to this emission, identifying this emission as a possible source of ambient air odor pollution.

In Figure 8, results are reported from modeling of odor dispersions, with a non-steady-state Lagrangian model (Calpuff), from an animal odor source (laying hens' facility), with residents' complaints superimposed (black dots), in terms of odor units concentration per cubic meter. Data are reported in

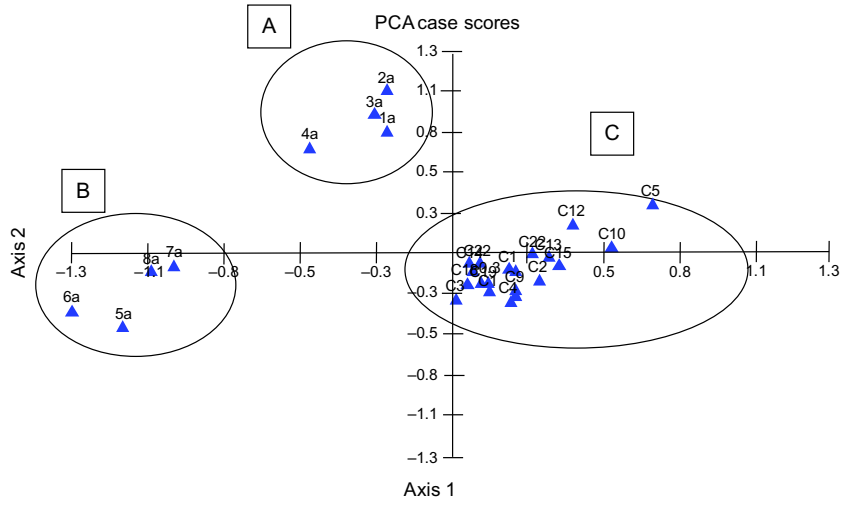

FIGURE 6 Principal component analysis (PCA) score plot for emissions ((B) biofilter emissions and (C) compost curing area) and ambient air sample (A) in a composting plant environment. Here, no similarities to ambient air are observed during an odor pollution episode.

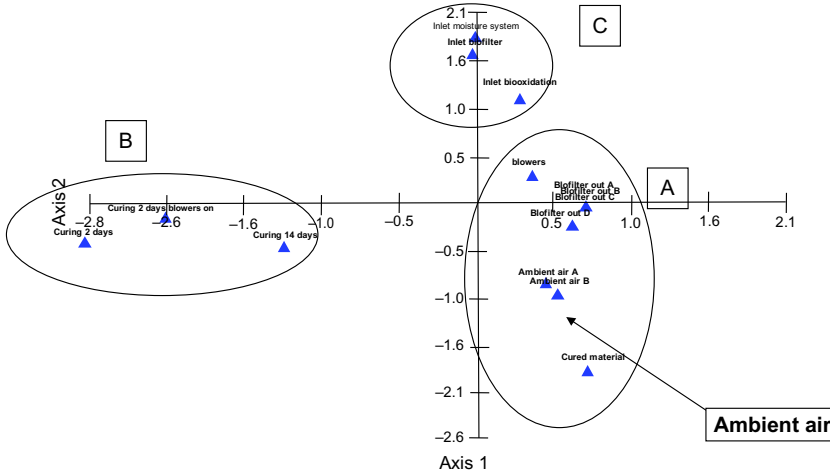

FIGURE 7 Principal component analysis (PCA) score plot shows an odor pollution problem close to a composting plant. Emissions from the biofilter (A), curing compost piles (B), and the biooxidation area (C) are very well separated in the PCA plot. In this case, ambient air samples collected during an odor episode are similar to biofilter emissions, identifying this emission as a possible source of ambient air odor pollution.

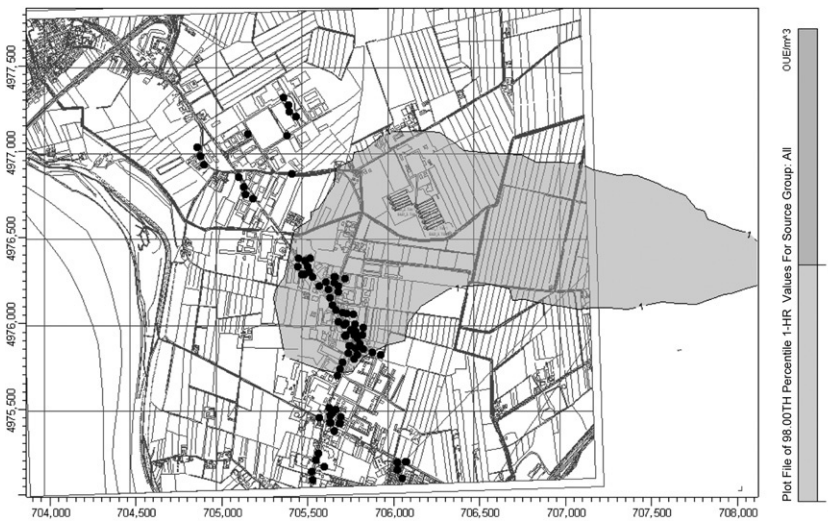

FIGURE 8 Results are reported from modeling of odor dispersions using a non-steady-state Lagrangian model (Calpuff) from an animal odor source. Dots indicate residents' complaints. The 98th percentile is reported, with isopleths reporting odor concentration in terms of OU_E/m^3.

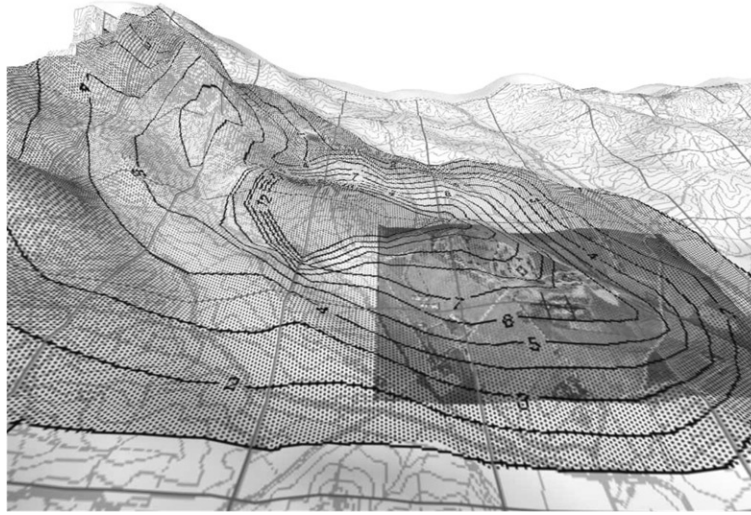

FIGURE 9 Results are reported for odor dispersion around a landfill using an Aeromod diffusion model, showing a 3D view of odor concentrations in the surrounding environment. The 98th percentile is reported, with isopleths reporting odor concentration in terms of OU_E/m^3.

terms of OU_E/m^3, as the 98th percentile of hourly values for a year ($C_{98} = x\ OU_E/m^3$). The isopleth lines report the limits of the benchmark levels, as defined by the 2011 UK Environment Agency H4 Odor Management guidelines, equal to 1.5 OU_E/m^3. In this case, the isopleths indicate that there will be an odor concentration of 1.5 OU_E/m^3 for 2% of the time, on a 1 year, hourly database, and this will be above the benchmark levels in the H4 guidelines, given this particular odor emission from the facility, indicating the likelihood of unacceptable odor pollution.

In Figure 9, results are reported for a landfill, using Aeromod software (Lakes Environmental, Canada), an U.S. EPA regulatory model based on steady-state Gaussian plume air dispersion, showing a 3D view of odor concentrations in the surrounding environment. Again, the 98th percentile is reported, with the isopleths reporting odor concentration in terms of OU_E/m^3.

In another example, seven samples were collected 0.6–1 km downwind from the entrance of a landfill (sample marked 8, Landfill IN), in the receptor's proximity. Samples 1–4 were marked as "nonodor" by the personnel involved in sampling, while samples 5–7 were marked as "odor." In Table 3, analytical data are reported for the monitoring campaign in the landfill area during an odor pollution episode, reporting only compounds found frequently in at least one of the samples. The simple analysis of air concentration is not informative about environmental sample similarities to "emission" sample 8. Total aromatics are higher in samples 8, 7, 6, and 1, but the pattern for samples 5 and 7 is more similar to that of sample 8. Terpenes are present in sample 1,

TABLE 3 Results of Samples from a Monitoring Campaign in a Landfill Area During an Odor Pollution Episode

	Sample Code							
Compound, ppbv	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8 Landfill IN*
Aromatic Hydrocarbons								
Benzene		0.19	1.07	1.03				
Toluene	3.74	1.61	4.61	2.97	6.80	2.31	24.89	40.48
Ethyl benzene/1,2-dimethyl benzene	98.54	22.54	21.85	19.75	24.10	116.08	313.04	1113.23
p-Xylene	69.56	15.24	20.26	16.78	26.53	126.43	333.44	1224.48
m-Xylene	36.46	11.54	13.71	14.85	18.26	77.47	169.,89	591.35
1,2,3-Trimethyl benzene			2.23	1.48	2.90		9.48	26.81
1-Ethyl-2-methyl benzene			0.88		0.67		3.77	4.37
1,3,5-Trimethyl benzene			1.91	2.04	2.25		7.03	24.09
Trans-decahydro naphthalene					8.47	17.13	23.07	41.92
Cis-decahydro naphthalene					11.99	25.85	37.04	56.67
Total aromatics	208.30	51.12	66.51	58.90	101.97	365.27	921.65	3123.42

Terpenes								
Alpha-pinene					1.13			
Cymene	1.26	0.48			5.45	1.33	1.75	6.98
Limonene	0.53	0.36			7.93	3.22	8.84	90.58
Total terpenes	1.79	0.84			14.51	4.55	10.59	97.56
Alcohols								
2-Butoxy ethanol				2.15	8.98	15.68	54.46	168.59
Total alcohols				2.15	8.98	15.68	54.46	168.59
Total VOC	210.09	51.97	66.51	61.05	125.46	385.49	986.69	3389.57

Data are reported only for compounds found in most of the samples. Sample numbers 5, 6, and 7 environmental odor were collected when environmental odor was perceived. Sample 8 was collected inside the landfill area.

2 and 5–8, while alcohols are present in samples 4–8 only. Why was odor perceived only in samples 5, 6, and 7? Which are the odorants among these detectable compounds that could be used as a marker? If data are analyzed with a CA (Figure 10), it can be noted that the three samples with environmental odor

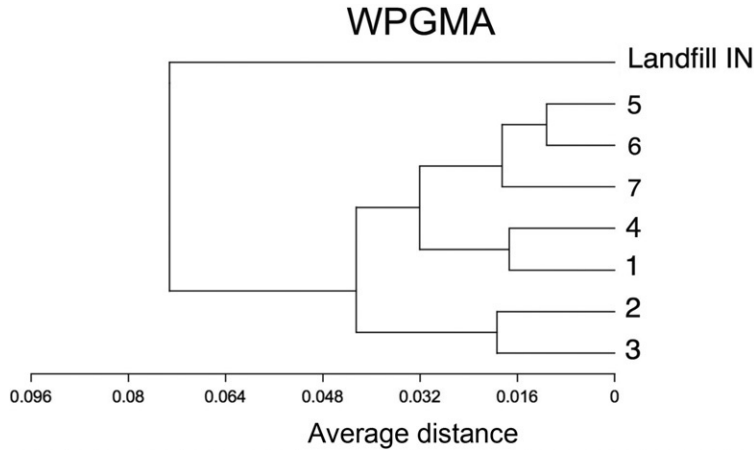

FIGURE 10 Cluster analysis (CA) during an odor episode around a landfill. The three samples with environmental odor (5–7) lie in the same cluster, being different from other samples.

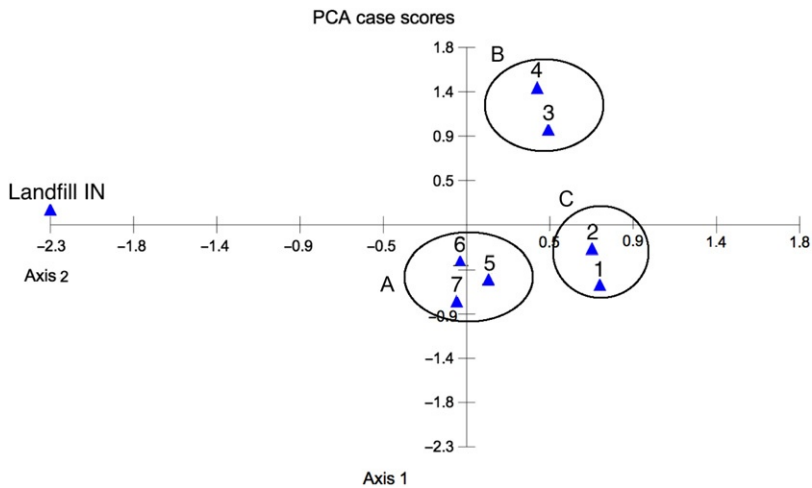

FIGURE 11 Principal component analysis (PCA) on the same sample set of Figure 10 shows that cluster A, corresponding to odorous samples 5, 6, and 7, is the closest to the sample collected at the entrance of the landfill, where significant odor was present, indicating a similarity and, again, a possible identification of the landfill facility as the source of odors. In samples 1–4, clusters B and C, no odor was noticed during sampling operations.

(samples 5–7) lie in the same cluster, being well separated from other samples (1–4), although none of them cluster with the "Landfill IN" sample. In Figure 11, PCA analysis on the same sample set shows that, while environmental samples are all close together, indicating a similarity between one another, samples in cluster A, corresponding to the samples where odor has been perceived, are the closest to the "Landfill IN" sample, supporting the fact that environmental odorants detected in these samples are similar to odorants present in the landfill area. While none of the odorants is a specific marker, the pattern is.

5 CONCLUSIONS

The use of instrumental analysis in conjunction with sensorial and chemometric analysis presents several advantages in this field. First, a chemical characterization of the emissions is an important tool to detect malfunctions of specific subprocesses or odor abatement technologies. Second, it gives important clues regarding the potential toxicity of the emissions and ambient air during odor pollution episodes. Finally, more objective data could be acquired for long periods and also automatically [29], once VOC tracers have been identified. The complementary sensorial evaluation allows dispersion modeling of odor to be performed, following standardized guidelines. The proposed approach is relatively simple and requires analytical instrumentation that is widely available in many laboratories. There is the need to have a deeper knowledge of odor characterization from different facilities on a more uniform level, in order to have a better understanding for regulatory purposes of ambient air odor concentrations. In Italy, the Regione Lombardia recently proposed guidelines for odor emissions [32], but ambient air acceptability criteria will be defined only in a new release, after 3 years of ambient air odor data acquisition and after a reliable odor emission inventory. Odor nuisance will be controlled by setting emission rates at the emission source. Moreover, acquiring knowledge of odorants and potentially toxic compounds from sources of odor emissions is an important step during the authorization procedures involving regulation of odor.

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Chapter 5

Injection Port Derivatization for GC/MS–MS: Analysis of Hormones in Water

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1 INTRODUCTION

Hormones have been a growing concern among the types of emerging contaminants since the late 1990s when it was first reported that fish were experiencing feminization from exposure to several hormones, including: 17-β-estradiol and 17-α-ethinylestradiol [1]. During the last decade, endocrine disruption in fish has been observed, including both wild- and caged fish [1–4]. Wastewater input has been implicated in these cases, and as a result, there has been a concerted effort to track down the compounds that cause feminization in fish, as well as other types of endocrine disruption. In particular, feminine hormones, such as 17-β-estradiol and 17-α-ethinylestradiol [1–4], have been implicated as prime targets for endocrine disruption. In

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one study, 17-α-ethinylestradiol (a human birth control pill) was added to a lake in Canada, and endocrine disruption and collapse of the fish population were observed at exposure concentrations of 5 ng/L [5].

Furthermore, the hormone 17-β-estradiol is produced and excreted by humans each day at levels of 2–100 μg per person [6]. Pregnant women may excrete much more than this, up to 30 mg/day [7]. Thus, between natural excretion and oral contraceptive use of 17-α-ethinylestradiol, wastewater receives large inputs of possible endocrine active substances. Agricultural wastes are another source of endocrine active substances. This includes feedlots for cattle, sheep, hogs, and other livestock. In these cases, both the addition of growth hormones and natural excretion of hormones, such as 17-β-estradiol, are involved [8]. Thus, the analysis of hormones in wastewater effluents, surface water that is impacted by wastewater, and groundwater that may receive these compounds is of widespread importance. Other compounds that are not hormones, but that may have the potential to behave as endocrine disruptors because of their chemical structure, include compounds such as nonylphenol and bisphenol A [9]. Thus, methods that address these compounds are environmentally important and significant.

Many methods have appeared over the past decade that address these compounds in water samples. These methods have been reviewed by several researchers [10,11]. They include extraction steps by solid-phase extraction or liquid/liquid extraction followed by mass spectrometry. There are basically two approaches for mass spectrometry, either gas chromatography/mass spectrometry or liquid chromatography/mass spectrometry. There are positive aspects for each approach. Gas chromatography/mass spectrometry offers the power of excellent separation because of the long columns of fused silica that have literally hundreds of thousands of theoretical plates, which allow excellent separation of hormones from their isomers or interfering substances. Nowadays, with the increase in the sensitivity of instrumentation and the use of mass spectrometry/mass spectrometry with multiple reaction monitoring (MRM), the instruments are both sensitive and reliable. The two major drawbacks with gas chromatography/mass spectrometry, however, are that the solvents used for GC/MS are not compatible with the most popular extraction method, which is solid-phase extraction or SPE. Second, the hormones themselves are not volatile in the GC/MS instrument. In past times, liquid–liquid extraction (LLE) was used, which is compatible with GC/MS. However, LLE is an environmental health hazard that requires large volumes of toxic solvents such as methylene chloride, which is also toxic to our atmosphere. Thus, SPE has nearly replaced LLE as the method of choice for sample preparation for hormone analysis and endocrine-disrupting substances in general.

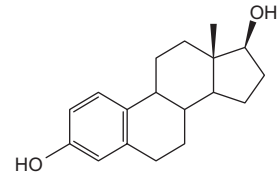
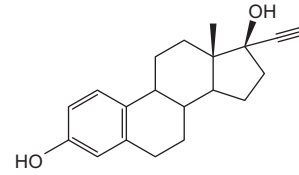
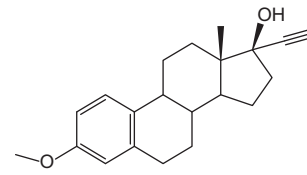
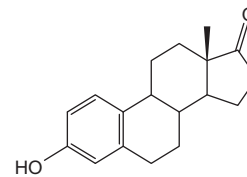
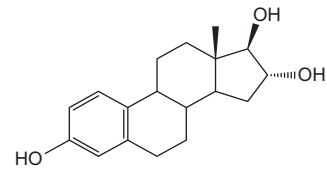
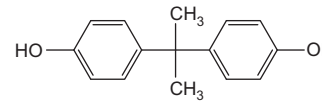
Because SPE directly allows the water sample to be stripped of hormones onto the solid support, it is a popular technique. Typically, 1–5 g of SPE material is used to remove hormones from a 200- to 1000-mL water sample.

Different types of SPE support have been used, the most popular being either C-18 or the hydrophobic lipophilic polymer, Oasis-HLB. Both are effective at the enrichment of the hormones from water and wastewater. The hormones are then removed from the sorbent by elution with a polar solvent, such as methanol or acetonitrile. These two solvents are not readily compatible with GC/MS analysis but are easily analyzed by liquid chromatography/mass spectrometry (LC/MS). Thus, the emergence of LC/MS has occurred with electrospray ionization to analyze hormones in water samples. Electrospray is a technique invented in the 1980s [12] to analyze proteins and works by the addition of either a proton to the molecule or the removal of a proton from the molecule, working on either weak acids or bases. Hormones, because of their phenolic hydroxyl, work in negative ion. In cases where there is no phenolic group, positive ion electrospray is used. Because of the structure of the hormones, the sensitivity of ionization is low, making it difficult to reach the low concentrations of endocrine disruption that have been observed in wild fish, that is, levels of 5–10 ng/L. Thus, at the moment, there is no simple method for hormone analysis that has all the attributes of an ideal method.

Given the fact that SPE is now the preferred choice for sample preparation, there is the problem of how to use this method if one chooses the option then of using GC/MS or GC/MS–MS for hormone analysis. How does one overcome the shortcoming of the volatilization of the hormones? This problem is exacerbated by the fact that although there are derivatization reagents that are effective for hormones, there is the issue of poor derivatization caused by the matrix, in this case, the wastewater organic compounds, and the salts and water associated with the isolation of the hormones themselves. Analysts have tackled this problem with the use of deuterated internal standards of each of the compounds that they are analyzing. This is an effective method, typically, but does increase the cost and labor of analysis. It would be quite useful if that were not necessary, as a typical deuterated standard costs 10 times more than the nonlabeled standard.

The common derivatization reagents that are used for the hormones are silylating reagents, such as BSTFA (*N*,*O*-bis(trimethylsilyl)trifluoroacetamide) or *N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide (MSTFA). These reagents silylate the hydroxyl groups of the hormones. Table 1 shows five commonly analyzed hormones and one endocrine disruptor in wastewater samples. They include: 17-β-estradiol, 17-α-ethinylestradiol, mestranol, estriol, estrone, and bisphenol A. Compounds such as testosterone and progesterone do not contain hydroxyl groups and do not need derivatization and will chromatograph on the GC/MS directly. The silylation reaction is shown in Figure 1. Here, the silylating reagent adds 72 mass units to the molecular mass of the hormone. This increase in mass is then used to target the precursor ion for GC/MS–MS analysis, or if one is doing GC/MS only, to find the molecular ion of the hormone. These silylation compounds are easily

TABLE 1 List of Hormones and Bisphenol A Used in this Study of On-Column Derivatization

Compound Name	Elemental Composition	Molecular Exact Mass (M)	Chemical Structure
17-β-Estradiol	$C_{18}H_{24}O_2$	272.1776	
17-α-Ethinylestradiol	$C_{20}H_{24}O_2$	296.1776	
Mestranol	$C_{21}H_{26}O_2$	310.1933	
Estrone	$C_{18}H_{22}O_2$	270.1620	
Estriol	$C_{18}H_{24}O_3$	288.1725	
Bisphenol A	$C_{15}H_{16}O_2$	228.1150	

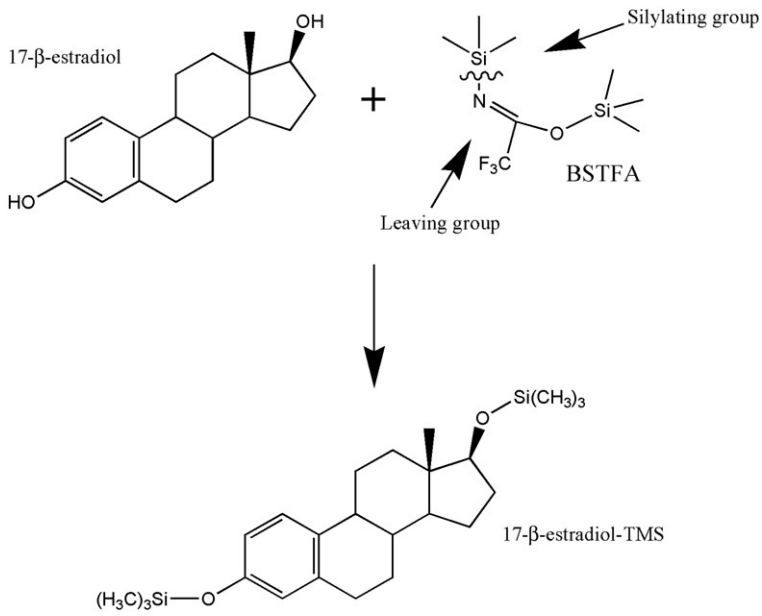


FIGURE 1 Derivatization of 17-β-estradiol with BSTFA, mechanism of action.

degraded by water or salt that may be present in the extracts. Because the natural organic matter is present in the extracts due to co-isolation by SPE, they will be present during the derivatization process.

Typically, the derivatization process involves the extraction of the compound by SPE and elution by a solvent such as methanol and then the blowing to dryness of the solvent extract. The dried sample is then solubilized in a solvent such as pyridine and the derivatization reagent, let us say, BSTFA is added. The extracts are then heated to a temperature of 65°C for approximately 4 h. The extract is then transferred to a vial for analysis by GC/MS–MS or GC/MS. If vials are stored or left on the instrument, they have the tendency to degrade. The idea of the deuterated internal standard is to correct for this loss of analyte or, in some cases, for the poor derivatization that occurs in the first place.

For these reasons, analysts are always looking for better and easier ways to develop their methods. Also there are safety concerns for the analysts when they have to handle the derivatization reagents several times, as these compounds are toxic when on the skin or inhaled. This is where the idea of derivatization on the GC column comes into play. The idea of on-column derivatization is not a new idea. Recent papers in the 2000s [13–15] showed that on-column derivatization of pesticides was an effective technique for analyzing heat-labile compounds by GC/MS. Recently, papers have appeared that use on-column derivatization for a suite of organic compounds, including

organic acids, bases, and pesticides [13–15]. However, to date, hormones have not been included in this list of compounds.

This chapter describes the research that has been carried out to eliminate the tedious nature of the derivatization step so that the hormones may be analyzed by a robust method that is not analyst-dependent, that is, requiring special care during the derivatization step by the analyst so that the method has good recoveries. Once the reagents are added to the sample in the vial, the derivatization occurs in the injection port of the GC/MS. We have tested this method on a number of standards using GC/MS–MS analysis and compared our results with the standard method used by the U.S. Geological Survey for hormone analysis of water and wastewater samples to find good agreement. This chapter describes that work in detail.

2 EXPERIMENTAL METHODS AND SAMPLE PREPARATION

2.1 On-Column Derivatization Method for Hormones and Bisphenol A

2.1.1 Sample Preparation

Water samples were collected in baked glass containers as grab samples from Boulder Creek and the wastewater treatment plant in Boulder, Colorado. The samples were processed by solid-phase extraction using the Horizon automated solid-phase extraction disk system with C-18 disks [16]. This procedure allows for the total analysis of the water sample and is not plagued by problems with plugging of the solid-phase extraction cartridges. The Horizon disk system consists of an SPE-DEX 4790 automated extraction system with controller. The disks are Atlantic^{TM} C18 SPE disks 47 mm in diameter. The automated method is described in an Application Note of Horizon [16] that essentially consists of first wetting the disks and preparing them with a rinse of methanol followed by water. The disk is left wet in water so that there is good and even application of the water sample. A 1-L water sample can be applied to this disk with good recoveries, greater than 50–80%. This is an advantage of the disks over SPE cartridges in that the amount of sample applied can be 5–10 times larger, with fast processing times. The disks can process a 1-L sample with a 15-mL/min flow rate, or more, so that the sample is applied in approximately 1 h. The disks were eluted with 15 mL of methanol and evaporated to dryness in a Turbovap concentration workstation. The dry test tubes then received the derivatization solvents that consisted of the following.

2.1.2 Derivatization Reagents

The derivatization reagents BSTFA and trimethylchlorosilane (TMCS) were obtained from Sigma-Aldrich (St. Louis, MO). An aliquot of 500 μL of TMCS was added to 5 mL of BSTFA in order to have a 10% TMCS

derivatization reagent. In the same way, 2 mL of pyridine was mixed with 8 mL of BSTFA/TMCS to form a (BSTFA/TMCS)/pyridine (4:1 v/v) solvent mixture for addition to the dry extracts. This combination of BSTFA, TMCS, and pyridine was the mixture used not only for solubilizing the dry extract from solid-phase extraction but also for derivatizing in the GC/MS–MS instrument.

2.1.3 On-Column Derivatization

The (BSTFA/TMCS)/pyridine solvent was used for injection. Two hundred microliters of the reagent was used to solubilize the dry extracts after evaporation under nitrogen in 10-mL test tubes. The tubes were vortexed for 15 s, and the extracts were transferred to 2-mL vials for analysis by GC/MS–MS. The injection port of the GC/MS–MS system was set at 280 °C, and 1 microliter was injected on the column.

2.1.4 GC/MS–MS Instrumentation and Analysis

The identification of the hormones and bisphenol A was carried out on an Agilent 7890 gas chromatograph coupled to a triple quadrupole mass spectrometer, Agilent 7000 series (Agilent Technologies, Inc., Santa Clara, CA). The chromatographic separation was performed using an Agilent J&W HP-5 column (5% phenyl, 95% methylpolysiloxane), 30 m × 0.25 mm i.d., fused-silica capillary column. The carrier gas was helium at a constant flow rate of 1.2 mL/min held by electronic pressure control. Injector temperature was 280 °C, and a splitless injection mode was used. The oven temperature was programmed for 100 °C (held for 1 min) to 240 °C at 40/min and held for 1 min, then to 300 °C at 10/min and held for 4 min. The MS operating conditions were the following: positive electron ionization mode (EI+) using automatic gain control with an electron energy of −70 eV. The ion source temperature was 300 °C. Gain voltage was set to 30 V. A dwell time of 50 ms was used for each MRM transition. One microliter of the extracts was injected on the system. Mass Hunter software was used for instrument control and data analysis. Details of MRM and analysis are given in Section 3 of this chapter.

2.2 Comparison Method: U.S. Geological Survey Method for Hormones

2.2.1 Sample Preparation

Stream water samples were analyzed for hormones at the U.S. Geological Survey National Water Quality Laboratory in Denver, Colorado [17]. Filtered water samples were fortified with deuterated analogs of 13 analytes as isotope dilution standards, and the samples were poured into stainless steel extraction tubes equipped with a multigrade GFF over a 47-mm C-18 solid-phase

extraction (SupelcoENVI) disk [17]. The sample was passed through the GFF/C18 disk under pressure, as needed. Following compound isolation, the GFF/C18-disk was rinsed with 25% methanol in reagent water and dried with nitrogen, and the compounds were eluted with methanol. The methanol eluent was evaporated to dryness and reconstituted in a mixture of 5% methanol in dichloromethane (DCM/MeOH). The extract was passed through a 1-g Florisil SPE column and eluted with the DCM/MeOH solution. The eluent was reduced in volume and transferred to a 5-mL reaction vial, then evaporated to dryness.

2.2.2 Derivatization and Analysis

Ketone and alcohol groups on the analytes were derivatized to trimethylsilyl or trimethylenol ether analogs to make them stable for analysis by gas chromatography. Derivatization was accomplished by adding 200 μL of MSTFA activated with 2(trimethylsilyl)ethanethiol and ammonium iodide (NH_4I), then heating the MSFTA solution to 65 °C for 1 h. The analytes were separated by gas chromatography and quantified by tandem quadrupole mass spectrometry using an isotope dilution procedure. This procedure allowed for quantitation of 17 hormones and their deuterated analogues [17].

3 RESULTS AND DISCUSSION

3.1 Derivatization by BSTFA/TMCS/Pyridine

Six analytes were chosen for this method, which included the five hormones l7-α-ethinylestradiol, 17-β-estradiol, estriol, estrone, and mestranol and bisphenol A. Table 1 shows the structure, formula, and molecular mass of the investigated compounds. The five hormones are similar in structure with the four-membered ring system of the android hormones. Because of their structure, they contain between one to three hydroxyl groups, are not volatile in the gas chromatograph, and require derivatization. The reagent, BSTFA (Figure 1), was used as the reagent for this process. This reagent is highly reactive toward either amine or hydroxyl groups and reacts to form the derivative. Figure 1 shows the reaction for the natural human hormone, 17-β-estradiol. Both the hydroxyl groups are derivatized, which increases the volatility of the 17-β-estradiol so that it chromatographs easily for analysis by GC/MS–MS.

Silylation, which is one of the most widely used forms of derivatization, works on active or exchangable hydrogens, such as those in either amines, hydroxyls, and phenolic hydroxyls. Thus, they are useful for the hormones since they contain both hydroxyl and phenolic hydroxyl groups. Silyl derivatives replace the active hydrogen with a trimethylsilyl group called TMS. Figure 1 shows an example for 17-β-estradiol, where both of the hydroxyl groups are replaced by a TMS group. The mechanism is thought to be a nucelophilic attack of the hydroxyl group upon the BSTFA molecule with the

leaving group shown in Figure 1, which is the larger half of the molecule with the bond breaking between the silicon and nitrogen atoms.

The typical procedure for derivatization involves adding an excess of BSTFA along with a catalyst, which is typically TMCS, between 1% and 10%. Ten percent was used in this work to help speed up the derivatization of hindered groups, such as the hydroxyl group next to the ethine group of 17-α-ethinylestradiol. Pyridine was also added to further help with the solubilization and catalysis of the reaction. The mixture used was 10% TMCS and 20% pyridine, as described in Section 2 of this chapter. The derivatization reagent does create a background peak in the chromatogram when the mixture is analyzed by GC/MS–MS. Figure 2 shows the full scan chromatogram for the derivatization of 17-β-estradiol. Notice that the first peak in the chromatogram is at 9.5 min, which has the mass spectrum also shown in Figure 2. This peak is most likely a phthalic acid contaminant based on the mass spectrum shown in Figure 2 with two major ions at *m/z* 149 and 167. This is not a surprising result since plastic pipette tips were used for transferring reagents during derivatization processes. Because of the reproducibility of this peak, it could be used as an internal reference for chromatography. This peak appeared in all of the derivatization reactions of each hormone standard. However, because it was not monitored in the MRM transitions of hormones, it is not seen in any of the MRM chromatograms. BSTFA and its by-products, including TMCS and pyridine, are volatile and appear early in the chromatogram, typically before the mass spectrometer is turned on and do not show up in the chromatograms, which makes this reagent a popular one for derivatization reactions. The hydrofluoric acid by-product of the BSTFA reaction is also not a problem and keeps the detector of the mass spectrometer from fouling.

The ease of derivatization of active hydrogen atoms follows the following order: alcohol > phenol > carboxylic acid > amine > amide. Steric hindrance also plays a role with alcohols in the following order: primary > secondary > tertiary; and for amines, the order is as follows: primary > secondary. As mentioned earlier, the use of TMCS can help with hindered sites. This will be addressed again as the various hormones are examined. BSTFA is a flammable and water-sensitive liquid. If properly stored, this reagent is stable indefinitely and can therefore be used quite easily over and over for on-column derivatization making the analyst work quite effortless.

The most likely problem with the BSTFA reagent is water that can decompose the reagent. However, when it is stored in a tight vial in the refrigerator with pyridine present the derivatization reagent does have a long half life. Typically, it is important to analyze a test compound with each day's analysis to test for repeatability. The addition of TMCS, which is a relatively weak silyl donor, to BSTFA will enhance the donor strength of the stronger donor, BSTFA, making the reaction go more quickly and efficiently for derivatization. The by-product of TMCS is hydrochloric acid. Thus, it is important to use a glass liner in the inlet of the GC/MS rather than metal. It is also

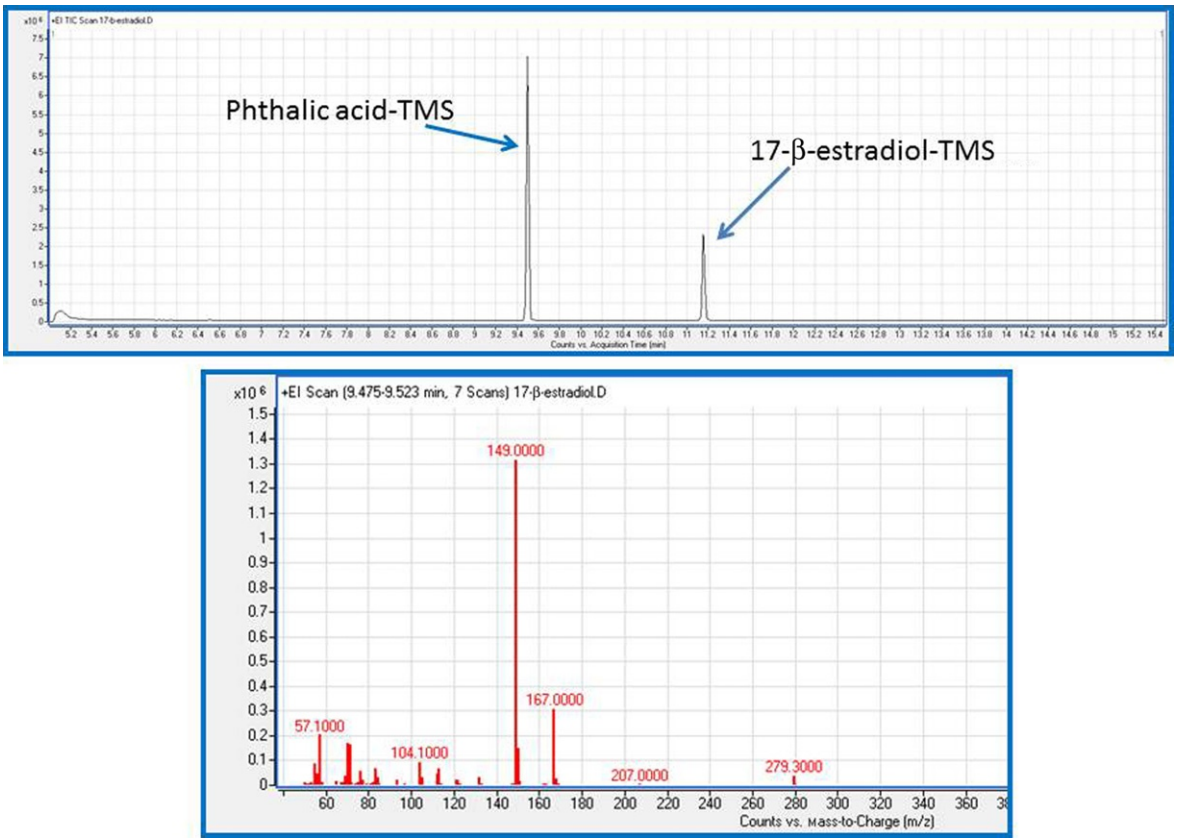


FIGURE 2 Chromatogram and mass spectrum of BSTFA phthalic acid eluting before 17-β-estradiol.

important to replace liners more often when doing on-column derivatization, as well as to use a relatively hydrophobic column such as the 5% phenylsilicone columns recommended in Section 2 of this chapter.

3.2 Derivatized Hormone Mass Spectra

The five hormones shown in Table 1 have different reactivities toward derivatization by BSTFA, which will be shown individually in the following sections. To summarize, several of the hormones derivatize completely with all hydroxyl groups being derivatized by the TMS group (trimethylsilane). This includes 17-β-estradiol and estrone. Several of the hormones have one peak that consists of a single TMS group because of the steric hinderance mentioned earlier, and a second smaller peak when both hydroxyl groups are derivatized. This includes 17-α-ethinylestradiol and mestranol. Estriol contains two major derivatization products, one with two TMS groups and one with three TMS groups. The fact that there is variable reaction suggests the importance of using a labeled standard for each of the hormones that undergo partial derivatization. The five hormones will be discussed in separate sections, beginning with the most effective derivatizations and progressing toward those that have multiple products. However, it is possible to easily derivatize and analyze the suite of hormones, as shown in Figure 3, with the extracted ion chromatogram for the set of five hormones, with their major precursor ion.

3.2.1 17-β-Estradiol

Figure 4 shows the mass spectrum for 17-β-estradiol. The major ion in the chromatogram is the *m*/*z* 386. This ion is the result of a loss of 30 mass units from the 17-β-estradiol with two TMS moieties, as shown in Figure 4.

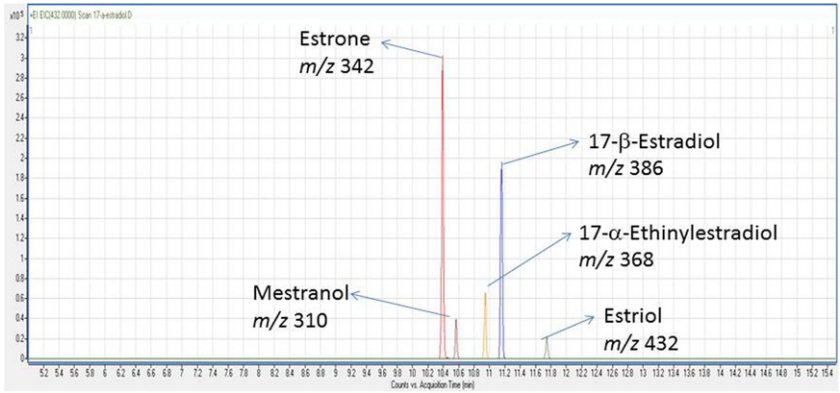


FIGURE 3 Extracted ion chromatogram of a hormone mixture with the major precursor ions shown.

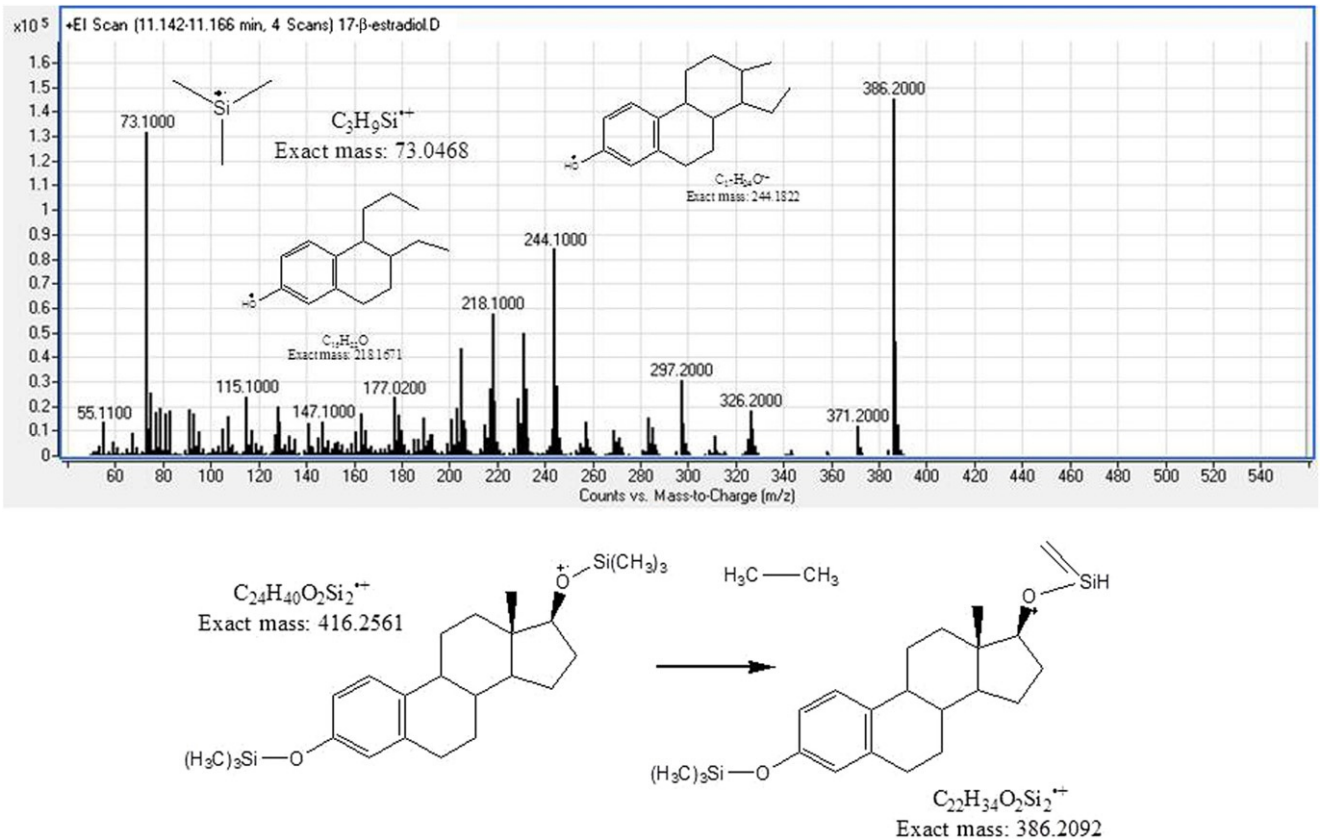


FIGURE 4 The mass spectrum of 17-β-estradiol as a TMS derivative.

Basically, there is a loss of two methyl groups from the *m/z* 416 ion to give rise to a stable and strong ion at *m/z* 386. The *m/z* 386 ion is then chosen as the precursor ion for the MRM transitions. The product ions include: the *m/z* 244 and 218 ions, which are structural to the hormone. It is never wise to choose the *m/z* 73 ion since it is the TMS derivatization reagent, which could originate from any compound that contains an active hydrogen atom. The *m/z* 73 ion is common and occurs in all of the derivatized hormones, often as a major component of the mass spectrum. Putative structures are shown for the major fragment ions at *m/z* 244 and 218. These two transitions from *m*/z 386 to 244 and from *m*/z 386 to 218 are the two transitions chosen for the analysis of 17-β-estradiol in MRM mode for GC/MS–MS analysis and give rise to a robust method for the 17-β-estradiol.

3.2.2 Estrone

Figure 5 shows the mass spectrum for estrone. The major ion in the chromatogram is the *m/z* 342. This ion is the result of a single TMS derivative to the estrone structure. Notice that the carbonyl group is not derivatized by the BSTFA, which is an expected result. The *m/z* 342 is chosen then as the precursor ion for estrone for the MRM transitions. The product ions include: *m/z* 257 and 218 ions, which again are structural to the hormone. Putative structures are shown for the two major fragment ions at *m/z* 257 and 218. The *m/z* 218 again occurs in the estrone structure because of its similarity to 17-β-estradiol. In fact, it differs only by the conversion of the hydroxyl group of the 17-β-estradiol to a ketone. These two transitions from *m/z* 342 to 257 and from *m/z* 342 to 218 are the two transitions chosen for the analysis of estrone in MRM mode for GC/MS–MS analysis and give rise to a robust method for estrone.

3.2.3 17-Alpha-Ethinylestradiol

Figure 6 shows the total ion chromatogram and mass spectrum for the major chromatographic peak at 10.9 min for 17-alpha-ethinylestradiol. This compound shows a different derivatization pathway than the first two hormones, 17-β-estradiol and estrone, which had only one chromatographic peak each. The 17-α-ethinylestradiol shows two chromatographic peaks because it has one for a single TMS group and a smaller peak at a longer retention time when there are two TMS groups. The derivatization favors the single TMS derivatization of the phenolic hydroxyl group. The alcoholic hydroxyl group is highly hindered by the ethyne group attached to the same carbon as the hydroxyl (Table 1). The ethyne group blocks the BSTFA from reacting with the alcoholic hydroxyl, and the product is the smaller chromatographic peak at 11.3 min with its highest mass being the *m/z* 425 ion, which is consistent with two TMS groups (Figure 7).

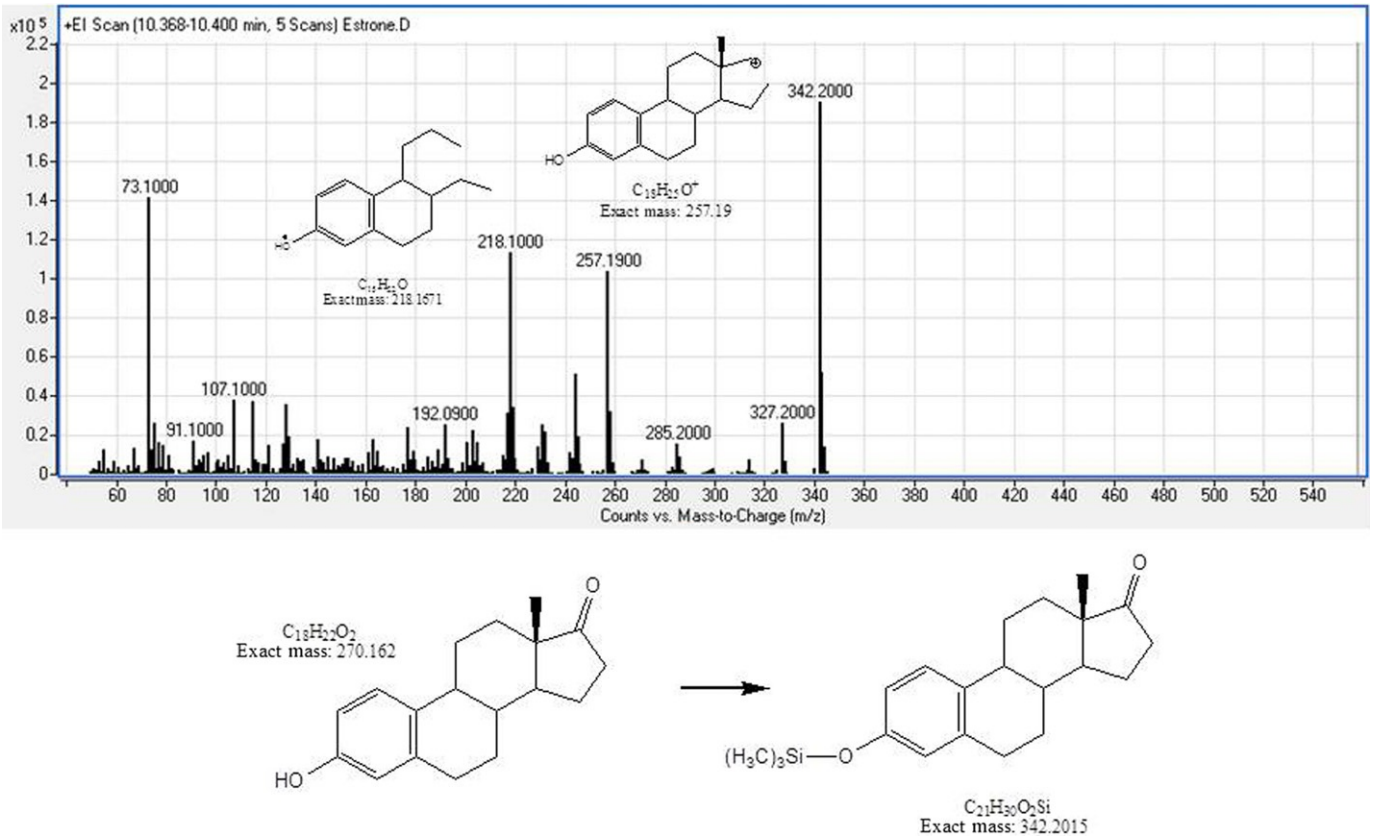


FIGURE 5 The mass spectrum of estrone as a TMS derivative.

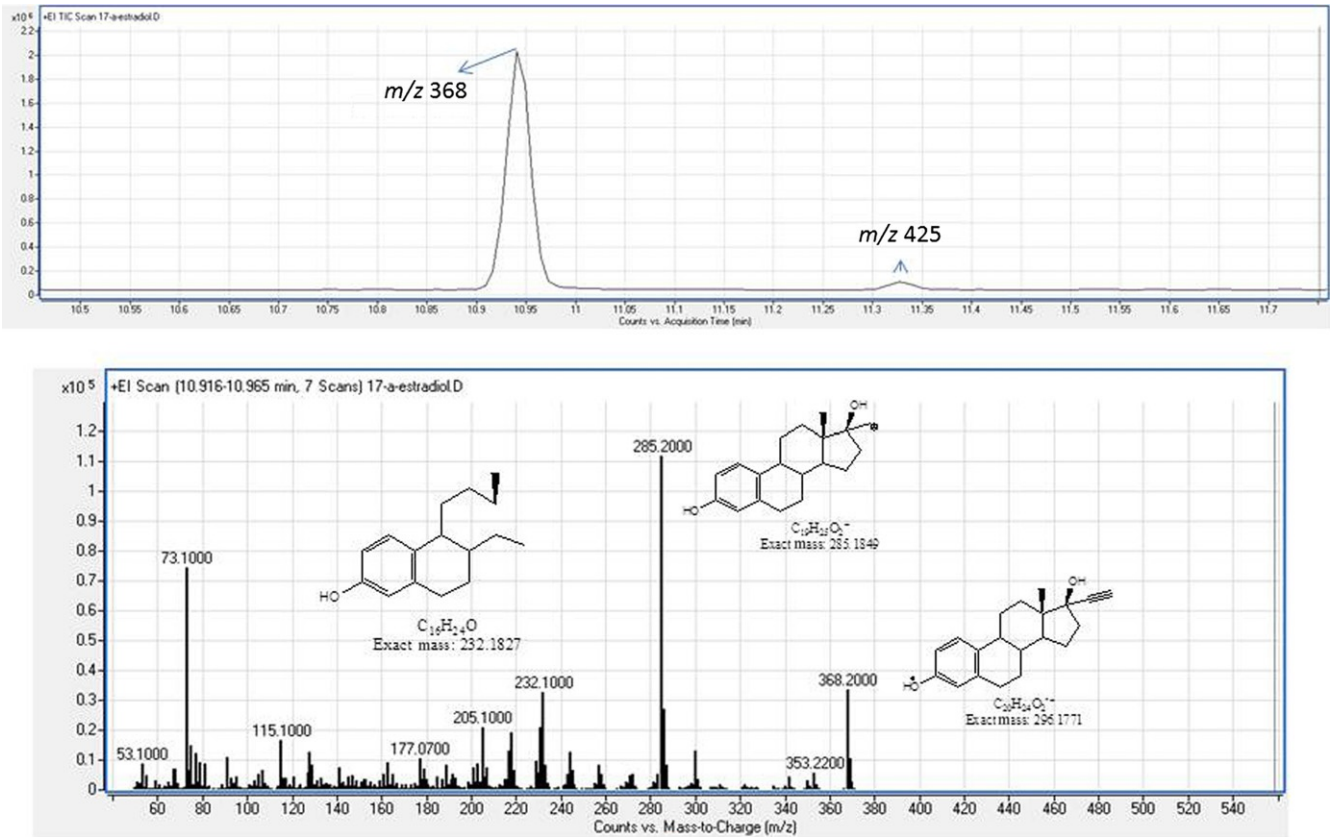


FIGURE 6 The mass spectrum of 17-α-ethinylestradiol as a TMS derivative.

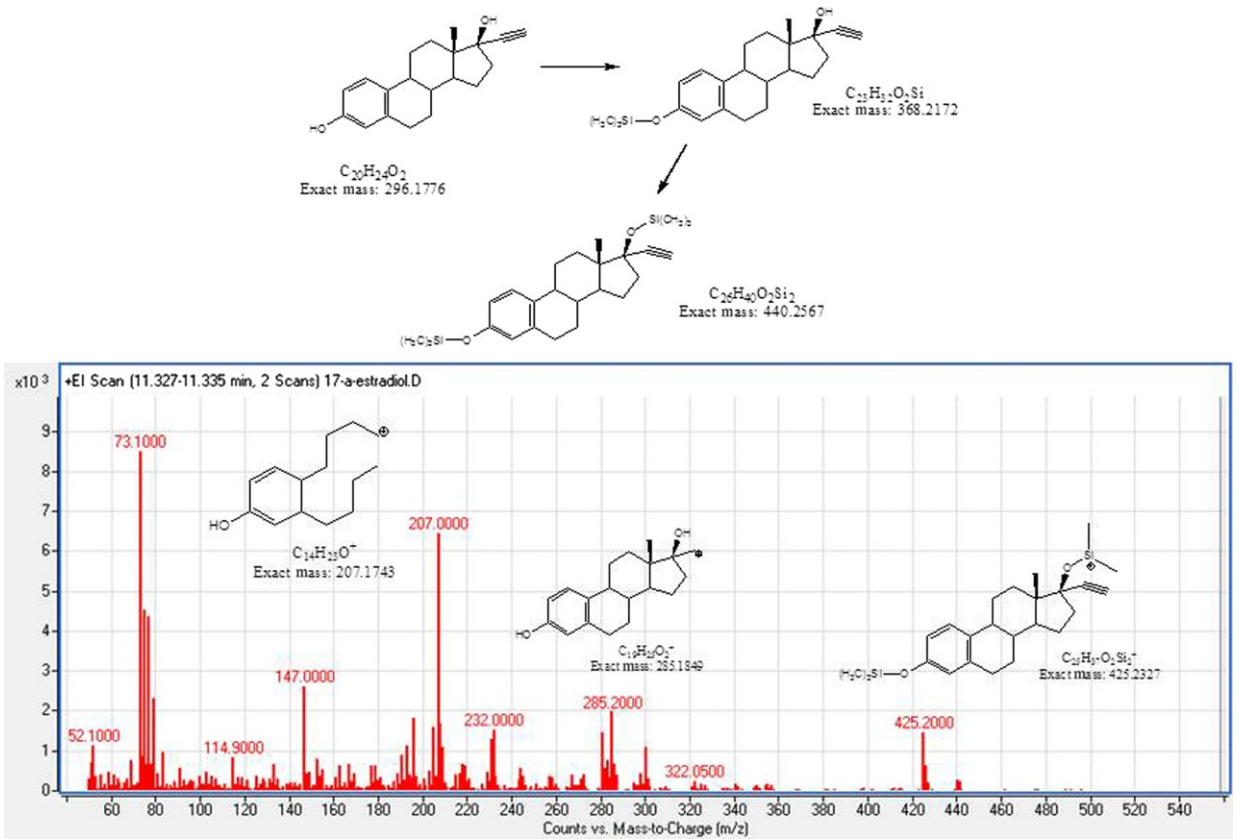


FIGURE 7 The mass spectrum of the two TMS deivatives of 17-α-ethinylestradiol with two TMS groups.

The larger chromatographic peak has the highest mass of *m/z* 368, which is the correct mass for the addition of one TMS group (Figure 7 pathway at the top of the figure). The *m/z* 368 is not the base peak ion; rather, the *m/z* 285 ion is the major peak in the mass spectrum, with the putative structure shown in Figure 6. Again, it is consistent with the core part of the hormone and is a good product ion for the MRM transition, *m/z* 368–285. The second MRM comes from the transition of *m/z* 368–232, which is again a structural component of 17-α-ethinylestradiol.

Figure 7 shows the mass spectrum for the second smaller peak at 11.3 min with the highest mass of *m/z* 425. This mass is consistent with the addition of two TMS groups followed by the loss of 15 mass units, a $—CH_3$ group. In this case, the *m/z* 425 ion is not the base peak ion but the *m/z* 207 ion (the *m/z* 73 ion is not considered here because it is the silyl ion). The putative structure for the *m/z* 425 ion is shown in Figure 7. This ion looses both TMS groups to give rise to its two products ions, *m/z* 285 and 207. The *m/z* 285 ion was found also in the preceding example of the *m/z* 368 ion where there is one TMS group attached to the 17-α-ethinylestradiol. The putative structures for both of the product ions are shown in Figure 7 and consist of the basic core of the hormone structure.

3.2.4 Mestranol

Figure 8 shows the total ion chromatogram and mass spectrum for the major chromatographic peak at 10.5 min for mestranol. This compound also shows a different derivatization pathway, which is actually similar to 17-α-ethinylestradiol. Because the phenolic oxygen is not present, rather there is a methoxy group, the major chromatographic peak derivatized by the TMS corresponds to the mass at *m/z* 310. The ethyne group again blocks the derivatization of the alcoholic hydroxyl group. But not only does it block derivatization it also sterically hinders the hydroxyl group from reacting and sorbing to the inlet of the GC, which creates a large tailing chromatographic peak or complete retention in the inlet. Thus, the dual role of the ethyne group leads to an underivatized chromatographic peak for mestranol as the major component of the hormone.

The second chromatographic peak at 10.95 min is the case where there is one TMS group derivatized to the alcoholic hydroxyl group that is protected by the ethyne group. It is a small peak since it is poorly reactive, which is nearly identical to the result that was seen for 17-alpha-ethinylestradiol in Figure 6. The single TMS derivative of mestranol gives rise to a ion at *m/z* 367, which is consistent with the addition of one TMS group followed by the loss of a $—CH_3$ group (Figure 9). This is the same reaction that was seen in the previous hormone, 17-α-ethinylestradiol, for the *m/z* 425 ion. Apparently, the similarity in structure gives rise to similar pathways of fragmentation. The base peak in Figure 9 was the *m/z* 207 ion, again a similarity to

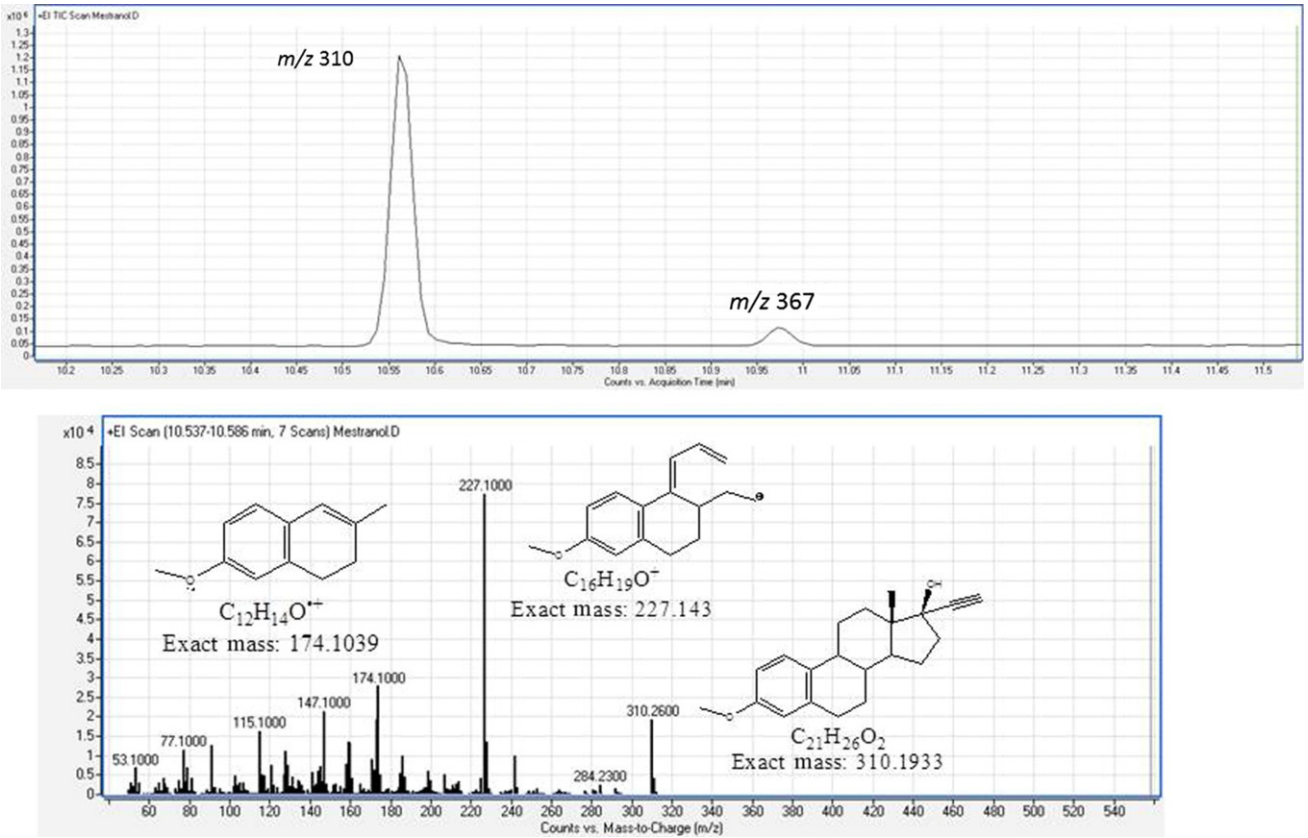


FIGURE 8 Total ion chromatogram for mestranol showing two chromatographic peaks at 10.55 and 10.97 min.

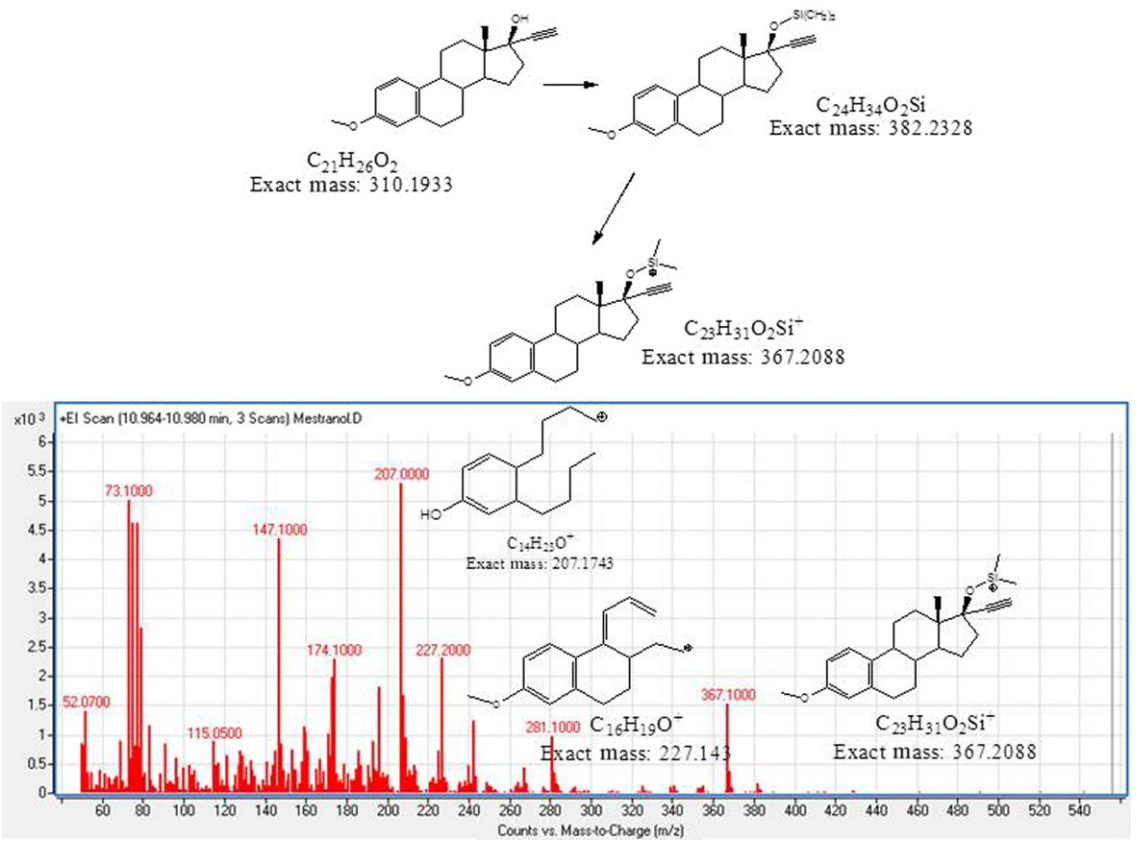


FIGURE 9 The mass spectrum and pathway of derivatization for a single TMS derivative of mestranol.

17-α-ethinylestradiol. The putative structures are shown for both the product ions for MRMs of mestranol with MRM transitions of *m/z* 367–207 and *m/z* 367–227.

3.2.5 Estriol

Figure 10 shows the total ion chromatogram and mass spectrum for two major chromatographic peaks at 11.75 and 11.85 min for estriol. This compound has three alcoholic hydroxyl groups, two of which are positioned on adjacent carbon atoms and apparently experience some steric hinderance. The first chromatographic peak has a mass of *m/z* 432, which is the addition of two TMS groups to the molecule, taking it from a mass of *m/z* 288 to 432 with the addition of two groups of 72 mass units each. The molecular ion at *m/z* 432 is not the base peak, rather the ion at *m/z* 345 and 129 are larger. The putative structures for each of these two product ions are shown on Figure 10. Thus, the two transitions chosen for MRM study are the *m/z* 432–345 and *m/z* 432–129. The *m/z* 345 ion is simply the basic structure of estriol and one TMS group that has subsequently lost a —CH_3 group. The *m/z* 129 ion is a highly fragmented component of the estriol structure for which a structure can be easily drawn, but without accurate mass data, it is not possible to be sure of its exact formula or structure.

The second chromatographic peak at 11.85 min is the larger of the two peaks and has a molecular ion at *m/z* 504, which is consistent with three TMS groups each derivatizing one of the three hydroxyl groups of estriol. This derivative has ions at *m/z* 345 and 129, which are the same ions that occur in the previous derivative containing two TMS groups.

3.2.6 Bisphenol A and d-16 Bisphenol A

Bisphenol A is an important endocrine-disrupting compound and is included in this chapter along with the hormones that have been implicated in endocrine disruption in fish. Along with the bisphenol A is an example of the use of a labeled standard, which is quite useful for quantitative analysis of derivatized hormones and endocrine-disrupting compounds. These two compounds are shown as examples of the approach to use for labeled standards by the method called isotope dilution.

Figure 11 shows the mass spectra for both the derivatized bisphenol A and its d-16 label. Note that all of the hydrogen atoms of the bisphenol A have been replaced by deuterium atoms. However, the mass increase is 14 mass units rather than 16 mass units because two of the deuterium atoms are active and replaced by TMS groups. Bisphenol A is unstable as a derivative and fragments by the loss of the CH_3 group in the center of the molecule. This is easily determined because of the loss of CD_3 on the labeled compound, where the mass of the molecular ion goes from *m/z* 386 to 368 with the loss of 18, while the nonlabeled compound goes from *m/z* 372 to 357, the loss

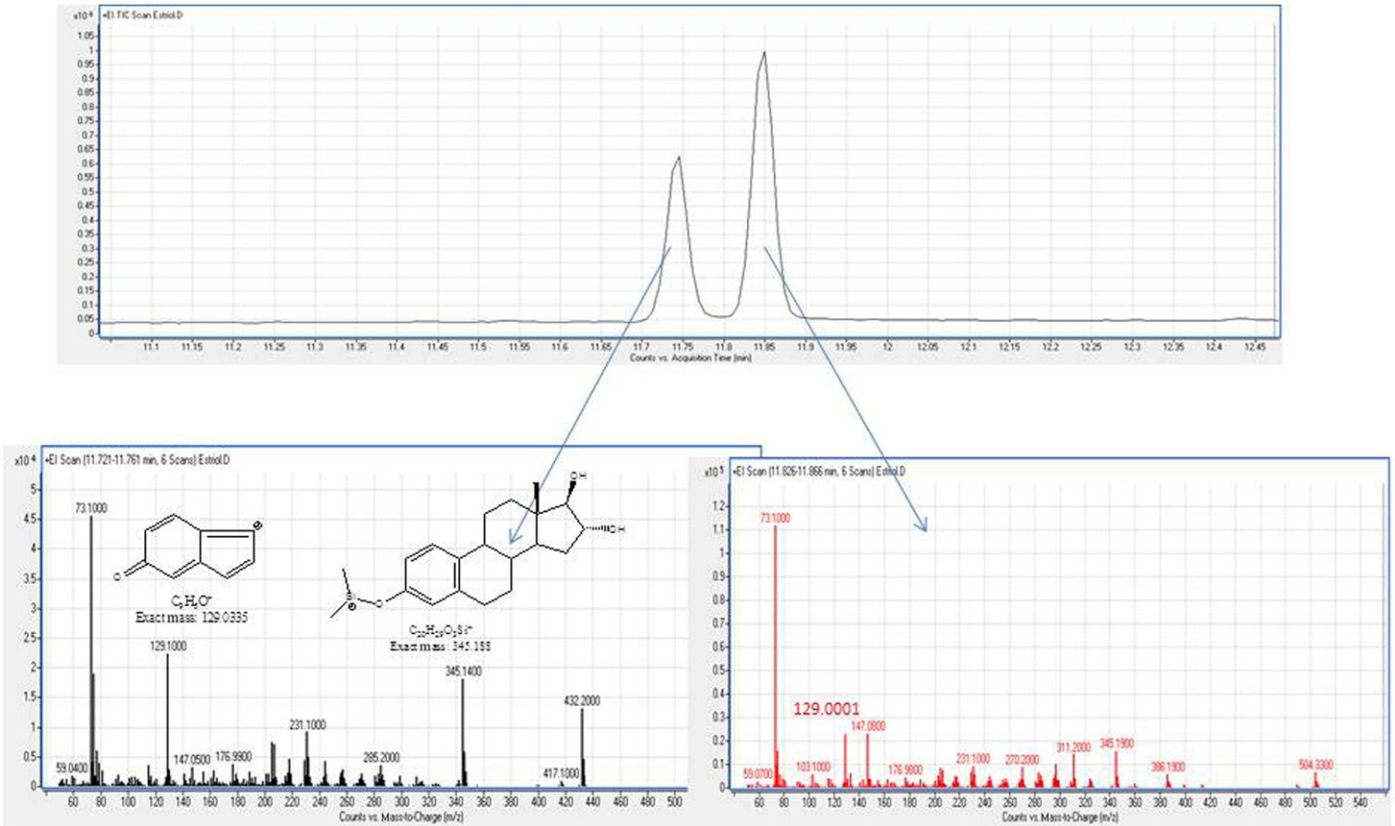


FIGURE 10 The mass spectrum and major ions for both the two and three TMS derivatives of estriol.

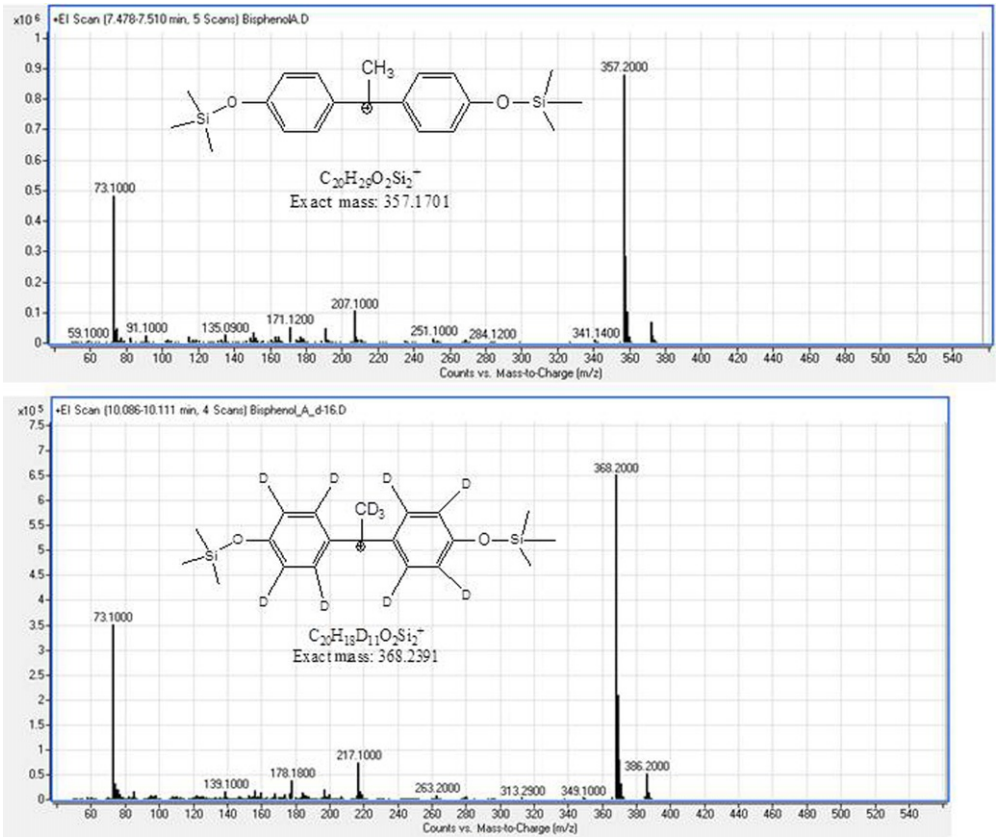


FIGURE 11 The mass spectra and major ions for both bisphenol A and its d-14 deuterium label as TMS derivatives.

of 15 mass units. Thus, we know that the methyl group lost must contain three deuterium atoms. The chromatography of the two compounds is different. Bisphenol A elutes at 7.5 min and the deuterium bisphenol A at 10.1 min. The addition of 14 deuteriums increases the boiling point of the compound considerably. The MRM transitions were from *m/z* 357 to 207 and 357 to 171 for bisphenol A and from *m/z* 368 to 217 and 368 to 178 for the d-14 bisphenol A.

3.3 Building the MRM Table for Hormones and Endocrine Disruptors

The MRM table for the five hormones and bisphenol A and its deuterated analogue is shown in Table 2. The larger intensity ion (in bold) is used for quantitation and the smaller intensity ion for confirmation. These MRM transitions give a robust method for the analysis of hormones and endocrine disruptors, such as bisphenol A. For a complete method, it is important to also obtain labeled standards for each of the hormones. This is our future work using the on-column derivatization technique, similar to what was done for bisphenol A. With the isotope dilution method, one has only to add a known amount

TABLE 2 MRM Transitions for Hormones and Bisphenol A

Compound Name	Precursor Ion	Product Ions	Retention Time (min)
17-β-Estradiol	**386**	**244** 218	11.1
17-α-Ethinylestradiol	**368**	**285** 232	10.9
Mestranol	**310**	**227** 174	10.5
Estrone	**342**	**257** 218	10.4
Estriol peak 1	**432**	**345** 129	11.7
Estriol peak 2	**504**	**345** 129	11.8
Bisphenol A	**357**	**207** 171	7.5
d-16 Bisphenol A	**368**	**217** 178	10.1

Quantitation ion is in bold and Confirmation Ions are in regular type.

of the labeled compound and use this to develop a standard curve for each of the hormones, although it is possible to identify hormones and to be semi-quantitative using the on-column method without labeled standards.

3.4 Analysis of Water Samples

3.4.1 Sample Collection

Surface water samples were taken from Boulder Creek (Boulder, CO) upstream and downstream of a WWTP. A U.S. Geological Survey spiking mixture was used for the SPE recovery experiment, which contained four-labeled hormones ($^{13}C_6$-estradiol, $^{13}C_6$-estrone, D_4-ethynylestradiol, and D_4-mestranol). No additives were added to the water samples, and no filtration of samples was needed. This part of the study involved the use of the U.S. Geological Survey Laboratory in Denver, Colorado, and the use of their hormone method described in Section 2 [17]. The purpose was to evaluate both the SPE isolation procedure and to compare the on-column derivatization for hormones with the U.S. Geological Survey published method.

3.4.2 Hormone Recovery by SPE

The SPE extracts were analyzed at the U.S. Geological Survey Laboratory by GC/MS–MS using a Quattro-micro-GC® instrument (Waters Corp., Milford, MA) with an Agilent 6890 gas chromatograph. Chromatography was on a 30 m × 0.25 mm internal diameter Rxi XLB gas chromatography column with a 0.25-μm film thickness (Restek Corp., Bellefonte, PA) and a helium flow rate of 1 mL/min with the injection port maintained at 275 °C. The gas chromatograph was programmed on a variable temperature gradient from 100 to 310 °C. For each target compound, the most abundant diagnostic ion in the full scan spectrum was selected as a precursor, and appropriate conditions were selected to maximize the signal for three precursor-product transitions. The recoveries for all five hormones were obtained by comparing the chromatographic areas to an external labeled standard. The other hormones were quantified relative to the isotopic dilution standards based on the absolute method recovery of the isotopic standards (i.e., U.S. Geological Survey method in Ref. [17]). The recoveries for the five hormones and bisphenol A are presented in Table 3. In general, acceptable recoveries of extraction were obtained for the compounds studied, approximately 47–80%. Also, the recoveries were very consistent in the three different water matrices studied, showing that the Horizon automated disk solid-phase extraction procedure is reliable, reproducible, and comparable to the U.S. Geological Survey method [17].

3.4.3 Hormone Analysis in Water Samples

Three surface water samples (downstream, upstream, and near a wastewater source) were analyzed with the on-column derivatization method described

TABLE 3 Recovery Results for Five Hormones Using the Horizon Automated Solid-Phase Extraction Disk with C-18 Disks

Recoveries for Hormones by SPE % Using Solid-Phase Extraction Disks			
Compound Name	*Surface Water Upstream*	*Surface Water Downstream*	*Canyon Water Site*
17-β-Estradiol	60	59	54
Estrone	72	80	63
17-α-Ethinylestradiol	64	70	57
Estriol	47	66	49
Mestranol	72	74	63

TABLE 4 Concentrations in Nanogram per Liter for Five Hormones Identified in Surface Water Samples By the On-Column Derivatization Method, Which Compared Well with the U.S. Geological Survey Method for the Five Hormones Below (Values Within ±25% Data not Shown)

Compound Name	Surface Water Upstream (ng/L)	Surface Water Downstream (ng/L)
17-α-Ethinylestradiol	–	0.2
17-β-Estradiol	–	0.5
Estriol	–	–
Estrone	0.6	9.5
Mestranol	–	–

in this work coupled to disk solid-phase extraction, and several hormones were successfully identified. The results are shown in Table 4. These results are preliminary at this time and show that the on-column derivatization method coupled to solid-phase disk extraction is a viable method for the five commonly studied hormones and bisphenol A. Future work will examine the five labeled hormones (deuterated analogues of the five compounds studied herein) to complete a robust method. Given the results at this time, this should be a straightforward method and allow complete quantitation from sample concentration to final analysis.

4 CONCLUSIONS

Hormones are an important class of emerging contaminants that are not easily analyzed by either LC/MS or GC/MS. They have been implicated in the feminization of fish, especially in water that receives wastewater downstream. They do not ionize easily by electrospray LC/MS and have a low sensitivity. With GC/MS, they are not volatile unless they are derivatized. Derivatization creates another step in analysis that is difficult and time-consuming, and often there are losses of analyte in the process. Thus, methods that make this task easier are important for the analysis of these compounds in water and wastewater. We have developed and tested an on-column derivatization method for five major hormones that is quite simple and takes the work out of derivatization for GC/MS analysis. The method consists of concentrating the hormones by solid-phase extraction from water followed by transferring the extract to a tube, drying the extract, and adding derivatization reagent to the tube. The reagent and sample are injected into the GC/MS injection port, where the derivatization occurs, and then the hormones are chromatographed. This on-column derivatization removes the tedious steps from off line and puts them online for higher recoveries. The hot temperature of the inlet, 280 °C, is sufficient for instantaneous derivatization of the hormones. The method will also work with phytoestrogens and bisphenol A, two other classes of compounds that fit the emerging contaminants list and show possible feminization potential. The combination of C-18 disk solid-phase extraction followed by on-column derivatization was successful for the analysis of five hormones, 17-α-ethinylestradiol, 17-β-estradiol, estrone, estriol, mestranol, and the endocrine disruptor, bisphenol A.

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Chapter 6

High-Throughput Analysis of PPCPs, PCDD/Fs, and PCBs in Biological Matrices Using GC–MS/MS

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1 INTRODUCTION

There is an increasing demand for high-throughput analytical methods specifically designed for the quantitation of organic contaminants in biological matrices. This demand exists in the broad fields of environmental chemistry and toxicology as well as medicine, food safety, drug discovery, and criminal forensics. Analytical chemists seek to develop techniques to reduce or eliminate method bottlenecks, which will improve the flow of information and downstream decisions. Incorporation of full automation and/or semiautomated analytical methods minimizes bottlenecks by reducing sample preparation time and increasing analyte sensitivity. A holistic approach is required to develop high-throughput analytical methods, especially regarding analytically challenging analytes and matrices. High-throughput analytical methods seek

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to improve the laboratory's capacity, preparedness, and efficiency for time-sensitive projects, such as environmental disasters, while still providing the required analytical precision and accuracy.

Biological matrices often present significant analytical challenges, which regularly require elaborate cleanup strategies and/or powerful mass spectrometry (MS) techniques, such as MS^n. Lipid-rich biological matrices, such as liver or blubber tissue, may require multiple labor-intensive cleanup steps prior to contaminant analysis. If insufficient cleanup strategies are performed, a more rigorous and selective MS strategy may be utilized. However, it is important to recognize that the goal should be to reduce the mass of matrix introduced to the MS, regardless of its capabilities. As a result, trends in analytical chemistry have focused on developing methods and techniques that reduce the cost associated with sample analysis (i.e., time, money, labor, training, safety, and laboratory space), yet are highly sensitive and selective. In recent years, there have been significant advances in the analysis of organic compounds in biological matrices with improvements in sample preparation and optimization of MS^n strategies.

In the following pages, two case studies are presented: case study #1 highlights an automated method capable of measuring a wide range of polychlorinated dibenzo-*p*-dioxins and furans (PCDD/Fs) and polychlorinated biphenyls (PCBs) in fish, clams, and crab tissue; case study #2 describes the advances in analysis of pharmaceutical and personal care products (PPCPs) in fish tissue, which required the development of a novel sample preparation technique as well as additional MS/MS optimization. These analytical methods improve the laboratory's ability to provide routine analysis and scale up its capability for larger, time-sensitive projects. In addition, these advances reduce labor, training, consumption of and exposure to hazardous organic solvents, and laboratory space, thus reducing the time and cost of analysis.

2 SAMPLE PREPARATION OVERVIEW

Sample preparation typically includes sample pretreatment, sample extraction, and extract cleanup. Sample pretreatment is designed to prepare a consistent and uniform sample ready for extraction. This pretreatment step should reduce the sample-to-sample variability that is common in biological samples. Sample extraction should serve to draw out target analytes from the matrix into solution. However, other compounds present in the sample may be simultaneously extracted along with the target analytes. These compounds, often referred to as extract impurities or interferences, can potentially hinder or prevent the analysis of target analytes. Extract cleanup often requires exhaustive strategies with multiple cleanup steps.

2.1 Pretreatment

Sample homogenization is usually the first sample pretreatment step and can employ both mechanical and chemical approaches. Mechanical homogenization

is often the preferred pretreatment method for organic contaminant analysis and includes grinding, blending, shearing, beating, and shocking. The optimal homogenization step for a particular analysis is largely dependent on the preferred characteristics of the final homogenate. Homogenization can increase the overall surface area contact between the extraction solvent(s) and the sample during extraction, thereby improving the method's extraction efficiency [1].

Biological samples often require a drying or dehydrating step to remove excess moisture. Dehydration helps to reduce extraction variability, particularly when nonpolar extraction solvents are utilized [2]. Dehydrating agents used include anhydrous sodium sulfate and diatomaceous earth. Other techniques, including freeze-drying or lyophilization, physically remove water from the sample homogenate [3,4]. Freeze-drying allows a sample homogenate to freeze under a vacuum where the water is removed through sublimation. In the following case studies, the dehydrating agent is homogenized with sample in order to retain water.

2.2 Extraction

The analysis of organic contaminants, such as dioxins, furans, pesticides, and polybrominated diphenyl ethers (PBDEs), in complex biological matrices, typically requires exhaustive extraction strategies. The practice of extracting organic compounds from solid matrices using solid–liquid extraction is described in our earliest recorded history [5]. Over the last 130 years, Soxhlet extractors have been heavily relied upon to extract organic compounds from biological samples that typically require large volumes of organic solvents [5]. Historical extraction techniques are often time-, cost-, space-, training-, and labor-intensive. Over the past decade, there have been significant advances in organic contaminants extraction from solid matrices, specifically pressurized liquid extraction (PLE).

2.2.1 Pressurized Liquid Extraction

PLE is a solid–liquid extraction technique capable of extracting a wide range of organic contaminants from a wide variety of biological matrices [6]. PLE offers improved extraction efficiency, sample throughput, and automation [7]. PLE can be viewed as an extension of supercritical fluid extraction, utilizing organic solvents instead of CO_2 [8]. PLE holds solvents near their supercritical region where solvents have elevated extraction properties, while remaining in a liquid state. PLE utilizes solvents at elevated temperature and pressure, which builds on Soxhlet, sealed and unsealed microwave-assisted extraction, and subcritical water extraction techniques.

PLE-based analytical methods have been well received, and to date over 1000 research methods and application articles utilizing PLE and techniques that combine PLE and cleanup, referred throughout this chapter as enhanced PLE (ePLE), have been published (Figure 1). Throughout the literature,

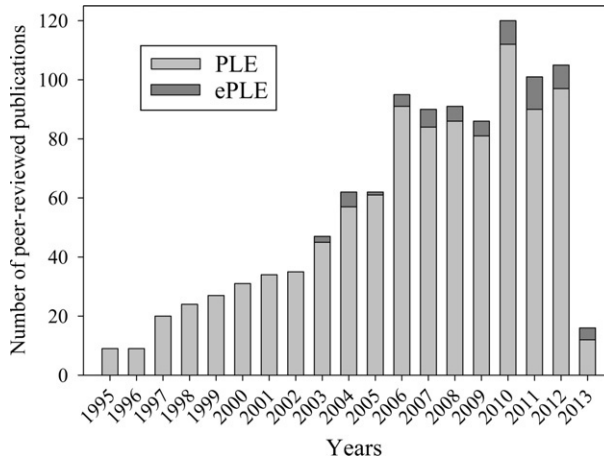

FIGURE 1 Frequency of peer-reviewed research and application articles of PLE and ePLE from 1995 to 2013, February. Source*: SciFinder® [9].*

PLE is also described as pressurized fluid extraction, pressurized hot solvent extraction, high-pressure solvent extraction, and subcritical solvent extraction [10]. In 1996, Dionex launched an automated PLE system trade-named accelerated solvent extractor (ASE™) [7]. The ASE system was utilized in U.S. EPA method 3545 for the extraction of volatile and semivolatile substances from soil, sludge, clay, sediments, and waste solids [11]. Within the literature, there are several review articles describing, in detail, the application of the automated PLE system [6,10,12].

A brief description of the attributes and benefits of an automated PLE system are described below. Typically, cellulose or glass fiber filters are placed at the bottom of the extraction cell to prevent bulk matrix from damaging the PLE system. Sample homogenates are packed into the extraction cells and the empty space (above the packed homogenate) can be filled with dispersant matrix or dehydrating agents, such as anhydrous sodium sulfate. PLE parameters include extraction temperatures (25–200 °C), pressure (500–3000 psi), solvents (organic to weak acids), number of extraction cycles, duration of static cycles, and rinse volume.

PLE of target analytes from solid matrices is kinetically and thermodynamically driven. Improved performance at elevated temperature and pressure is mainly due to the disruption of surface equilibrium as well as solubility and mass transfer effect. The disruption of surface equilibrium occurs when thermal energy overcomes cohesive (solute–solute) and adhesive (solute–matrix) energy by lowering the activation energy required for the desorption processes. The increased solubility of water in organic solvents at elevated temperatures can facilitate the extraction of analytes found inside water-sealed pores of the sample matrix [7]. Faster diffusion rates at elevated temperatures

and the addition of fresh solvent during static extraction increase the mass transfer in PLE similar to the mass transfer in Soxhlet [8]. In addition, increased temperatures result in decreased surface tension and solvent viscosity, which enhance the solvent's ability to wet and penetrate the matrix. Elevated pressure allows the use of liquid solvent at temperatures above their boiling points. The high pressure also allows solvent to solubilize air bubbles, which improves the solvents capability to come in contact with the entire sample (especially analytes trapped within matrix pores) [7,8].

PLE techniques reduce solvent consumption and extraction time, with increased efficiency and analyte recovery precision compared to previous methods [7,13]. On average, Soxhlet extractors consume 100–500 mL of organic solvents over a 12- to 24-h extraction period. Typically, PLE extractions require between 50 to 150 mL of solvent per sample over a 15- to 40-min total extraction time. As a result of these improvements, PLE has been well received by the scientific community (Figure 1).

2.3 Cleanup

Biological tissues often contain a wide range of potential polar and nonpolar interferences, such as proteins, fatty acids, oils, and resins [6]. As a result, many analytical methods utilize one or more cleanup steps, including gel permeation chromatography (GPC) and/or column chromatography techniques, such as silica gel chromatography cleanup. Specific sorbents are often used to retain specific types of interference and/or target analytes. Silica gel is typically used to retain polar interferences [14]. Florisil® can be used to retain large molecular weight interferences and, in some cases, can eliminate the need for further GPC cleanup [15]. Graphitized black carbon has been used to retain planar compounds, such as PCDD/Fs. The use of multiple cleanup techniques may increase the loss of target analytes during sample preparation, which can increase variability within the dataset and negatively affect interpretations. In addition, multiple organic solvent extraction and cleanup steps can increase human exposure to hazardous chemicals.

3 NEXT-GENERATION PLE TECHNIQUES

The next generation of PLE techniques was designed to reduce or eliminate extraction and cleanup bottlenecks. These techniques combine the extraction (i.e., PLE) with one or more of the necessary cleanup steps, such as silica gel, alumina, and celite column chromatography, in a single automated step (Figure 2) [15–17]. By incorporating commercially available cleanup sorbents into the extraction cell, it is possible to reduce errors associated with extensive sample preparation. Typically, precleaned sorbent(s) are placed beneath or incorporated into the sample homogenate prior to extraction. This allows the target analytes and interferences extracted from the sample homogenate to

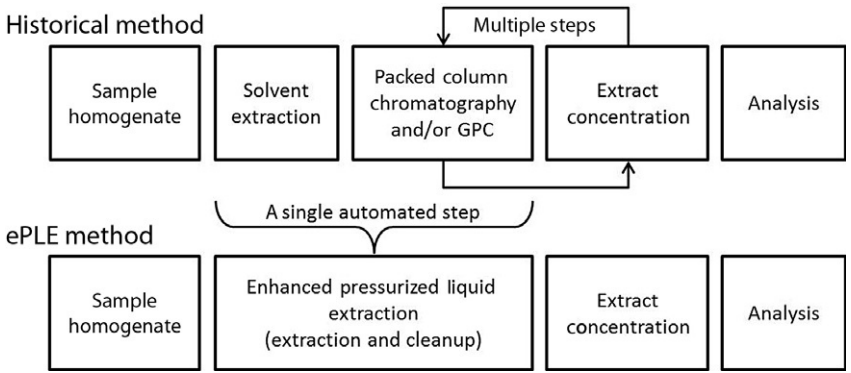

FIGURE 2 General comparison of an ePLE method with combined extraction and cleanup step(s) to a typical analytical method with independent extraction and cleanup step(s).

interact with the cleanup sorbent(s) during the extraction process [15]. Analogous to PLE, these more advanced techniques are described in the literature using many terms including ePLE, selective PLE, in-cell cleanup, and *in situ* cleanup [4,15,17–21]. In this chapter, the term ePLE is broadly used to describe techniques that combine the extraction and cleanup step(s).

Soxhlet or PLE methods can be used as a starting point and help guide the selection of extraction solvents, cleanup strategies, and extraction parameters for ePLE methods (Figure 3). In some cases, historical PLE methods were directly developed and validated into ePLE methods [15]. Overall, ePLE techniques require less time to develop, optimize, and validate than previous methods, due to the reduction in the time associated with extraction and

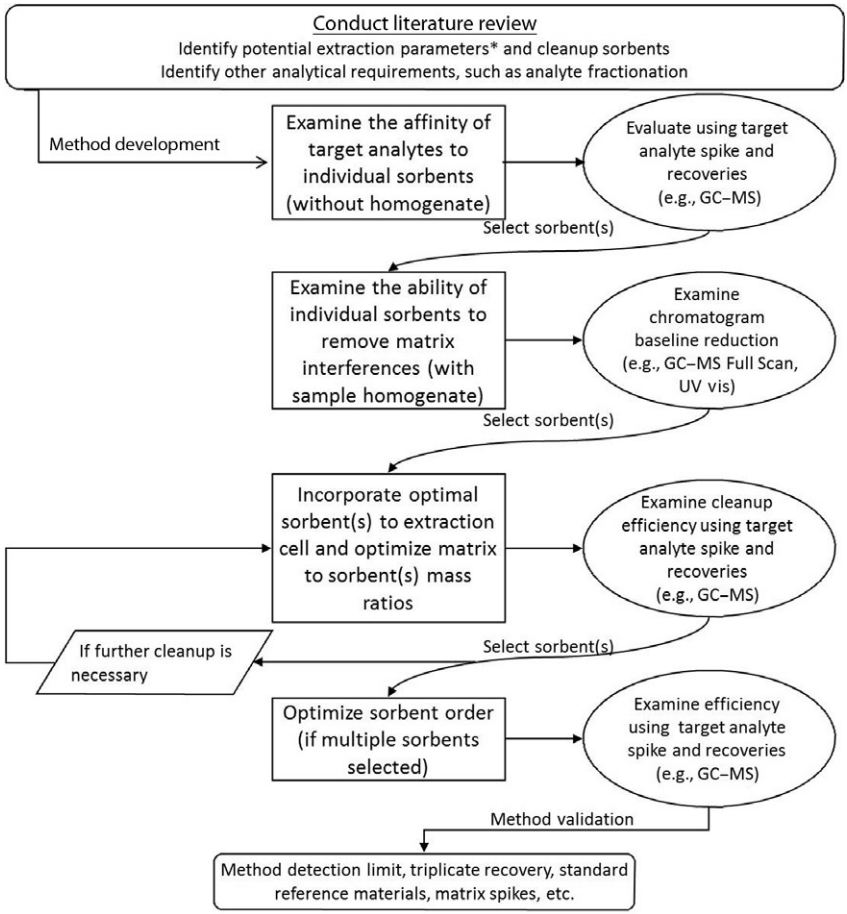

FIGURE 3 A proposed flowchart for ePLE method development and validation. *Extraction parameters such as temperature, pressure, number and duration of cycles, and solvent(s) may need to be optimized for certain analytes and/or matrices.

cleanup. ePLE techniques are also paving the way for the analysis of emerging contaminants [14] and novel biological matrices [22].

ePLE is a cost-effective sample preparation technique in terms of time, labor, and solvent consumption, as compared to PLE followed by independent cleanup step(s). Haglund *et al*. [23] compares the cost of PCDD/Fs analysis using ePLE to a previously developed PLE method. Typically, the routine analysis of PCDD/Fs costs $700 per sample including labor, consumables, instrument investment and depreciation, and service costs [23]. Approximately, 70% of the total cost of analysis was associated with the PLE extraction, cleanup, and fractionation steps. The total cost of analysis using an ePLE technique was ~$170 per sample or about 25% of the cost associated with the PLE method [23].

There are several advantages of ePLE, which often go unmentioned. First, with analytical laboratory space at a premium, particularly hood space, any reduction in required space can significantly improve the laboratory's overall capacity. The use of an ePLE technique can reduce the square footage required, especially because cleanup step(s) can be combined with the extraction method. Second, using ePLE can reduce the personnel, training, and/or instrumentation required to perform otherwise needed cleanup techniques, such as GPC. Third, the advantages of reducing human exposure to hazardous organic solvents are obvious and should be taken into consideration. Finally, reducing the time required for sample preparation can minimize inadvertent sample contamination within the laboratory.

As previously mentioned, the potential matrix interferences for the analysis of organic contaminants can be removed by (1) combining extraction and cleanup (i.e., ePLE) and/or (2) MS optimization (i.e., interference discrimination). The following case studies illustrate the aforementioned strategies used to eliminate potential interferences for contaminant analysis in biological samples.

3.1 Case Study #1: San Jacinto River Waste Pits

This case study highlights an ePLE technique capable of measuring PCDD/Fs and PCBs in fish, clam, and crab tissues. Using U.S. EPA method 1613 as a guide, Subedi and Usenko [15] developed an ePLE method that incorporated the extraction with all of the necessary cleanup steps as well as a fractionation of PCBs from PCDD/Fs. This ePLE method combines PLE with alumina, celite:carbopack™, Florisil®, and silica gel column chromatography into a single automated step (Figure 4). Prior to analysis, extracts were concentrated down to 250 μL and required no additional cleanup.

Specifically, this method was able to reduce the intrinsic costs associated with the sample preparation protocol: time (~95%), solvent (~65%), labor, laboratory space and training, and loss of analytes as compared to previous U.S. EPA methods. GPC was eliminated through the use of Florisil® (Figure 5). Surrogate recoveries ranged from 75% to 125% and showed significant improvements as compared to U.S. EPA methods (reported surrogate

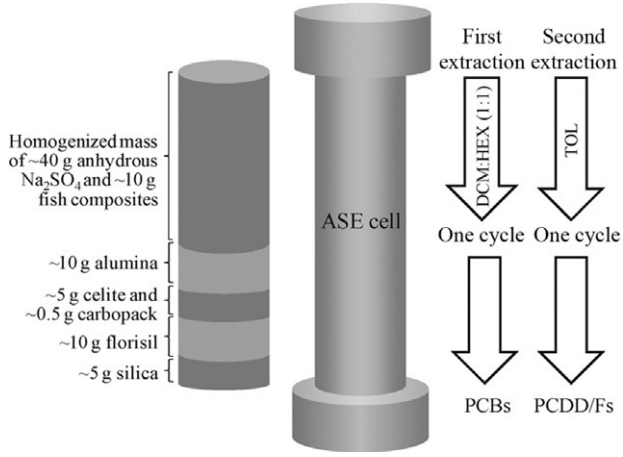

FIGURE 4 Schematic of the finalized ePLE technique (Subedi and Usenko [15]).

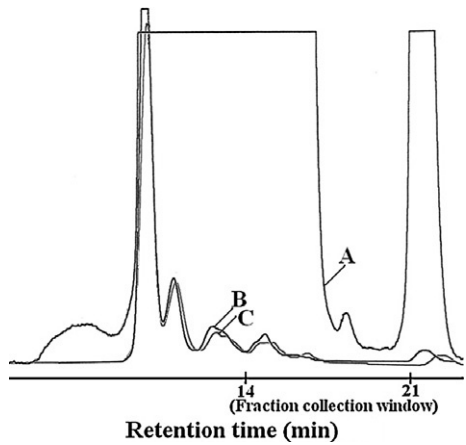

FIGURE 5 GPC–UV chromatograms following simultaneous extraction and cleanup (Subedi and Usenko [15]): (A) fish composites (~5 g), silica, and celite (1:1:1); (B) fish composites (~5 g), silica, and Florisil® (1:1:1); and (C) fish composites (~5 g), silica, celite, and Florisil® (1:1:1:1).

recoveries ranged from 17% to 197%). These improvements can be attributed to the use of automation and the overall reduction in the number of sample preparation steps.

Subedi and Usenko [15] were also able to utilize celite:carbopack™ fractionation, multiple extraction cycles, and multiple extraction solvents to effectively separate ~95% of the dioxin-like PCBs (dl-PCBs) from the PCDD/Fs (Figure 6). As a result of this analytical separation, there was a reduction in potential molecular interferences (i.e., dl-PCBs) in the PCDD/F extract.

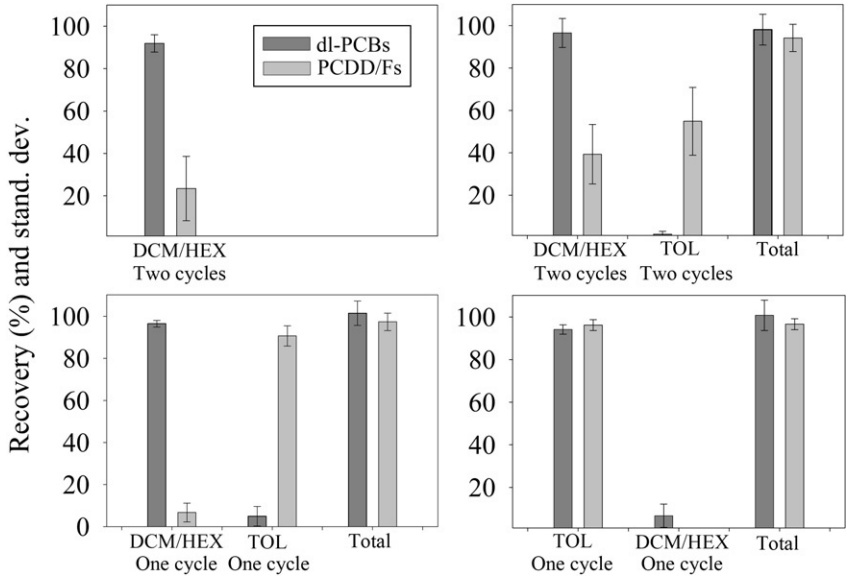

FIGURE 6 Separation of PCDD/Fs and dl-PCBs through solvent optimization (Subedi and Usenko [15]): (A) two-cycle DCM:HEX (1:1) extraction; (B) two-cycle DCM:HEX (1:1) extraction followed by two-cycle TOL extraction; (C) one-cycle DCM:HEX (1:1) extraction followed by one-cycle TOL extraction; and (D) one-cycle TOL extraction followed by one-cycle DCM:HEX (1:1) extraction. 'C' represents the finalized extraction strategy.

In addition, the exposure of personnel to hazardous organic solvents was reduced through the use of automation and the elimination of GPC and column chromatography. The overall precision and accuracy of the method was demonstrated with triplicate target analyte spike and recovery experiments. Percent recoveries of PCDD/Fs and relative standard deviation (RSD) measured in fish, clam, and crab tissue were $86 \pm 3.0\%$, $87 \pm 2.2\%$, and $93 \pm 2.8\%$, respectively. Percent recoveries and RSD for PCBs in fish, clam, and crab tissue were $93 \pm 2.4\%$, $76 \pm 5.4\%$, and $92 \pm 2.5\%$, respectively [24]. Overall, the method detection limits were similar to those of U.S. EPA method 1613 (2.0–44 pg/g wet weight). This high-throughput analytical method was used to measure PCDD/F and dl-PCB concentrations in fish (Figure 7), clam, and crab tissue as part of a San Jacinto River Waste Pits Superfund project conducted in 2012 [24].

4 GAS CHROMATOGRAPHY TANDEM MS FOR TRACE ANALYSIS IN BIOLOGICAL MATRICES

Tandem MS, a technique proposed by McLafferty in the 1980s has been well received and employed for the analysis of trace environmental contaminants

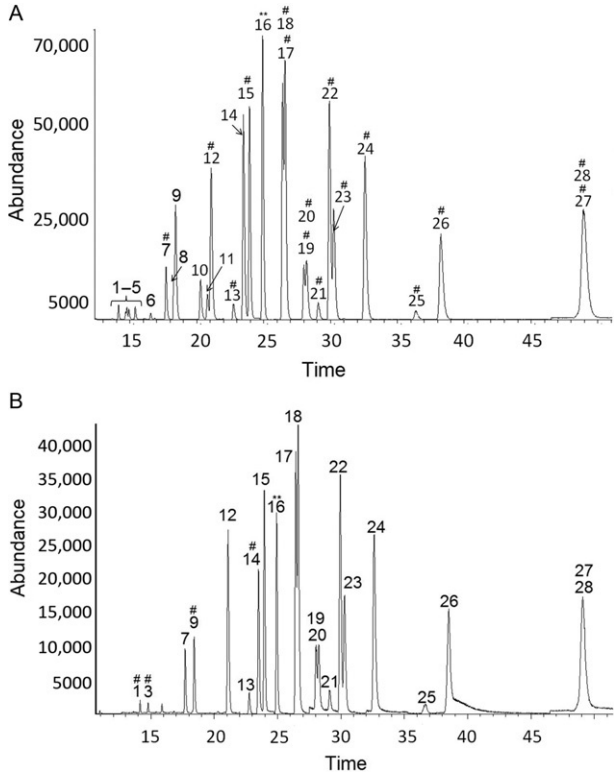

FIGURE 7 (A) DCM:HEX extraction of dl-PCBs from fish tissue, (B)Toluene extraction of PCDD/Fs from fish tissue. Peak identification: #Analyte not detected, **Internal standard, (1) PCB-81 and ^{13}C-PCB-81, (2) PCB-123, (3) PCB-77 and ^{13}C-PCB-77, (4) PCB-118, (5) PCB-114, (6) PCB-105, (7) 2378-TCDF and 13C-2378-TCDF, (8) PCB-167, (9) PCB-126 and ^{13}C-PCB-126, (10) PCB-156, (11) PCB-157, (12) 12378-PeCDF and ^{13}C-12378-PeCDF, (13) 12378-PeCDD and ^{13}C-12378-PeCDD, (14) PCB-169 and ^{13}C-PCB-169, (15) 23478-PeCDF and ^{13}C-23478-PeCDF, (16) PCB-189 and ^{13}C-PCB-189, (17) 123478-HxCDF and ^{13}C-123478-HxCDF, (18) 123678-HxCDF and ^{13}C-123678-HxCDF, (19) 123478-HxCDD and ^{13}C-123478-HxCDD, (20) 123678-HxCDD and ^{13}C-123678-HxCDD, (21) 123789-HxCDD and ^{13}C-123789-HxCDD, (22) 123789-HxCDF and ^{13}C-123789-HxCDF, (23) 234678-HxCDF and ^{13}C-234678-HxCDF, (24) 1234678-HpCDF and ^{13}C-1234678-HpCDF, (25) 1234678-HpCDD and ^{13}C-1234678-HpCDD, (26) 1234789-HpCDF and ^{13}C-1234789-HpCDF, (27) OCDD and ^{13}C-OCDD, (28) OCDF and ^{13}C-OCDF.

in diverse environmental biotic and abiotic matrices [25–27]. GC–MS/MS is capable of filtering coeluting matrix components, [28] resulting in a low background and baseline spectra [29]. MS/MS ionic discrimination has been accompanied with different mass analyzers such as quadrupole, magnetic sector, and ion-trap [14,30,31]. Ion-trap is the most commonly used MS/MS strategy due to its relatively small size, cost, weight, and pumping requirements [28,32].

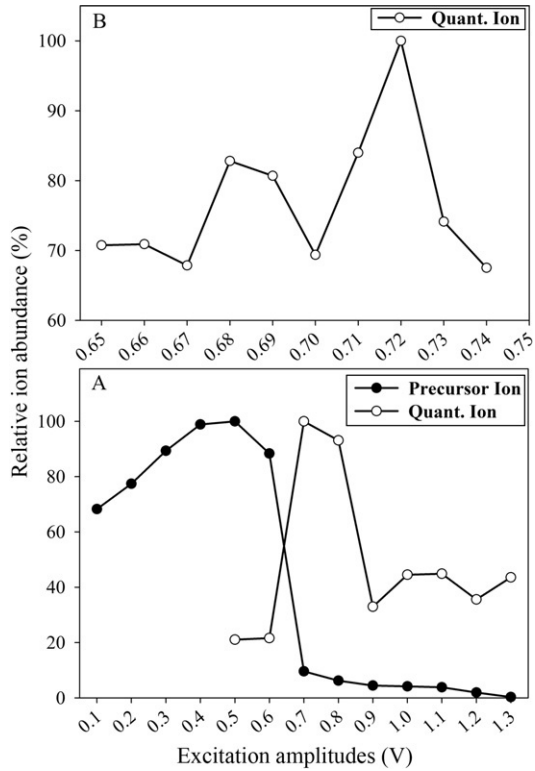

FIGURE 8 Differential ion response (normalized to maximum peak area counts) for musk ketone: (A) first step excitation amplitude optimization by 100 mV and (B) second step excitation amplitude optimization by 10 mV (Subedi *et al*. [14]).

Typically, MS/MS requires precursor ion selection, ion isolation and storage, collision-induced dissociation (CID), and radiofrequency (RF) signal optimization [28]. Appropriate RF signal traps the precursor ion or range of ions of desired *m*/*z* between hyperbolic electrodes and ejects other unstable ions. The precursor ion typically has the highest possible *m*/*z* ratio and ionic abundance. As a result, it would have the least interference from background ions and provide the highest abundance of product ions during secondary fragmentation [28]. The trapping field created by resonant or nonresonant RF storage voltage isolates and stabilizes the precursor ion. RF storage levels are calculated using *m*/*z* and "*q*" values. RF storage levels are optimized automatically utilizing a section in analysis software called Automated Method Development [33–35]. Isolated precursor ions are then subjected to a secondary fragmentation by CID using inert gas (He or Ar) at $\sim 10^{-4}$ torr. The effective collisions between selected *m*/*z* ion(s) and buffer gas results in the formation of product ions; hence, determine the fragmentation efficiency [26].

The ionic storage capabilities of ion-trap MS/MS technique provide increased sensitivity (up to subpicogram level) and stability of performance for organic analysis in biological matrices. The complete ion spectra that can be recorded, even at the lowest ionic concentration levels, are more informative than single ion monitoring quadrupole techniques. Quadrupole ion-trap MS/MS systems offer decreased sensitivity by $\geq 5\times$ than high-resolution mass spectrometry (HRMS); however, the optimized MS/MS parameters for secondary fragmentation, such as resonant or nonresonant excitation amplitude, improve their specificity and sensitivity [36]. Typically, excitation amplitudes during MS/MS can be optimized to the tenths of millivolts (first step optimization, 0.1 V). The analytical response may be further optimized for every hundredth of millivolts (second step optimization, 0.01 V) over the range of amplitudes optimized in the first step (Figure 8) [14,37]. Optimization of the excitation amplitude for PCDD/Fs in biological matrices using quadrupole ion-trap MS/MS improved the LOD, similar to that by HRMS [36]. MS/MS sensitivity and selectivity have also been enhanced using different structural designs of collision cells. For example, SCION Triple Quadrupole GC–MS/MS (Bruker Daltonics Inc., MA) employed a curved $q_{0,}$180 °C curved collision cell and multiaxis neutral noise canceling technology for enhanced sensitivity and/or S/N ratio [30].

Selective and sensitive GC–MS/MS analytical methods have been used for the analysis of diverse chemical functionalities in complex human matrices. For example, drugs of abuse in human hair samples (LOD of 6–52 pg/mg) [30] and leukotriene B4 were analyzed by GC–MS/MS in human plasma [38]. GC–MS/MS also demonstrated a capability of analyzing a wide range of chemical functionalities in diverse environmental matrices. GC–MS/MS resulted in a significant reduction in matrix interferences and LODs comparable to GC–MS with negative chemical ionization (NCI) for PBDE, pentabromotoluene, and pentabromoethylbenzene analysis in fish tissue [39]. However, the GC–MS/MS method quantification limit was ~1 order of magnitude lower compared to that from GC–MS (electron impact ionization or NCI), particularly to higher congeners [39]. Similarly, GC–MS/MS determination of PAHs provided a $5\times$ lower LOD in smoked salmon fish (particularly for high mol. wt. PAHs, such as benzo(g,h,i)perylene) compared to that by GC–MS [40]. The matrix interference discriminating capabilities of GC–MS/MS and GC–SIM–MS were also compared for the analysis of select personal care products (PCPs) in fish tissue. PCP analysis in fatty fish tissue (4.9% lipid) required GPC cleanup followed by GC–MS/MS whereas GC–SIM–MS could only be utilized to analyze PCPs from lean fish tissue (0.4% lipid) in order to maintain acceptable quality assurance parameters [31].

GC–MS/MS exhibit more selectivity and sensitivity in complex matrices such as fish, milk, and fly ash; however, HRMS provided improved precision and accuracy [41–43]. HRMS analysis of PCDD/Fs encountered interferences from chlorinated dibenzothiophenes; however, using MS/MS,

these interferences were selectively overlooked by the system [36,44,45]. GC–ECD analysis provided a nonlinear response within PCB homologues and could not differentiate PCBs from coeluting matrix interferences [43,46–48]; however, GC–MS/MS was capable of selective determination of trace PCBs and polychlorinated naphthalene in harbor seal tissues [43]. In addition to having a higher sensitivity, MS/MS was also capable of isomer differentiation. For example, the selective self/ion monitoring reaction modes were capable of isomer differentiation of aniline (aniline, 2-picoline, 3-picoline, and 5-hexynenitrile) [49].

4.1 Case Study #2: PPCP Nationwide Study

PPCPs are pseudo-persistent contaminants that enter the environment through the direct discharge of wastewater effluent from wastewater treatment plants (WWTP) [14]. PPCPs are considered contaminants of environmental concern because of their (1) high production volume, (2) widespread and continuous use, and (3) biochemical interactions in the environment. Despite their relatively short environmental half-lives, PPCPs have been measured in a wide variety of biota, [50,51].

Due to these environmental concerns, a U.S. EPA pilot study was initiated in 2006 to examine PPCP concentrations in fish tissue in the United States [52]. Ramirez *et al.* [52], reported results from a pilot study examining PPCPs concentrations in fish tissue collected from five sites located downstream from WWTPs and one reference site (without WWTP effluent). The study found that pharmaceuticals and PCPs both accumulate in fish tissue from wastewater and that the level of treatment matters. The analysis of 24 pharmaceuticals and 12 PCPs utilized LC–MS/MS [53] and GC–MS/MS [14], respectively. The extraction, cleanup, derivatization, and analysis of PCPs in fish tissue have been previously described [14]. Briefly, tissue samples were sonicated with acetone for 15 min, centrifuged, the supernatant evaporated to dryness, and then reconstituted in 200 μL of 65:35 (v/v) hexane–acetone. Extracts were then passed through silica gel column chromatography, derivatized, and analyzed using GC–MS/MS. This pilot study highlighted the need for high-throughput analytical methods in order to support large scale analysis studies.

In 2007–2008, there was a nationwide study of PPCPs in bream fish from 14 German Environmental Specimen Bank (GESB) sites [37]. The reference site was a lake with no WWTP effluent present, while the remaining 13 of the 14 sampling sites were river sites downstream from WWTPs. The rivers examined in the study included the Rhine, Danube, and Elbe rivers along with their tributaries. This German study utilized ePLE with an optimized MS/MS technique [14].

As a result of this 2007 German nationwide study, Subedi *et al.* [14] developed an analytical method capable of measuring two pharmaceuticals and 12 PCPs in fish tissue. Using Mottaleb *et al.*, [31] as guide, this ePLE method was able to combine PLE with silica gel cleanup. However, due to the

insufficient cleanup of the single sorbent ePLE, GPC and an additional MS/MS ion discrimination optimization were utilized. Optimizing the MS/MS's resonant CID energy to the hundredths place (0.01 V) improved the overall sensitivity of the analytical method through improved ion production (Figure 8). Typically, resonant CID energy is optimized to the tenths place (0.1 V). This second optimization step increased ion production for six of the 12 PCPs (ion production improvements ranged from 24% to 122%). This ePLE method reduced the inherent cost associated with PCP analysis in fish tissue and increased the target analysis list to include two pharmaceuticals.

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Chapter 7

GC–MS Applied to the Monitoring of Pesticides in Milk and Blackberries and PAHs in Processed Meats of Colombia

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1 INTRODUCTION

The analytical know-how for toxic residues in Colombia is still very limited, given the inadequate infrastructure of the assay labs to attend to the growing demand. As the international regulations of pesticides get tighter, the demand gets higher. Despite this, there is only one accredited assay laboratory within the ISO/IEC 17025:2005 regulation in Colombia.

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In order to protect the consumers, many countries have developed and established several regulation mechanisms, such as maximum residue limits for pesticides (MRLs), compound banning, and many others. These regulations are directed toward maintaining the equilibrium between consumer protection and crop protection.

The agricultural food market has grown in Colombia during the last few years. The inner demand is just one factor, as new markets have been opened with the United States and Canada (Free Market Trades); Honduras, Nicaragua, and El Salvador (Merconorte); Chile, South Korea, Trinity and Tobago, Russia, Israel, and the European Union (Trade Agreements). The health safety of the foods has to be guaranteed in order to trade, and this includes meeting the MRLs as a requirement of the major international buyers. Monitoring of pesticide residues is usually done to each lot of crop produced for export, which also increases the residue analysis demand.

At present, demonstrating the quality of the results reported by assay laboratories has become a requirement, as it must meet the ISO/IEC 17025:2005 regulations. The diagnosis and control of pollution research group (GDCON) is actually accredited by the IDEAM (Hydrology, Meteorology, and Environmental Studies Institute), which comes under the Colombian Environment and Sustainable Development Ministry. This enables the GDCON to successfully analyze pollutants with high quality results.

In the last few years, GDCON has evaluated the phytosanitary status in Colombian agricultural products and the presence of pollutants in high-demand products such as milk, fruits, and meat. This chapter shows the results obtained through the monitoring of these matrices. Pesticide residues were monitored in milk and blackberry (*Rubus glaucus* sp.). Polycyclic aromatic hydrocarbons (PAHs) were monitored in processed meats.

The QuEChERS procedure was used as the sample preparation method, as it is appropriate for polar and pH-dependent analytes. Two official methods are available to date: AOAC 2007.01 [1] and UNE-EN 15662 [2]. The QuEChERS method consists of a series of easy steps. First, there is a solvent extraction step using acetonitrile, and then the mixture is separated by a salting-out effect. The extract is then purified using a dispersive solid phase extraction (d-SPE) [3] step. The extract obtained can be analyzed by either a gas chromatography (GC) technique or a liquid chromatography (LC) technique.

Determination of the xenobiotics in this study was made using gas chromatography coupled to mass spectrometry in selective ion monitoring mode (GC–MS-SIM). The SIM mode enables high sensitivity and selectivity methods, as it detects specific ions or fragments even to the trace level. The injection system used was a programmed temperature vaporizer in solvent vent mode (PTV-SV), in order to meet Colombian MRLs. Method validation proceeded according to SANCO/12495/2011 guidelines, which clearly complement the NTC-ISO/IEC 17025 requirements.

2 MATERIALS AND METHODS

2.1 Equipment

- Ultra-turrax T 10 basic (Ika Laboratory and Analytical Equipment, Staufen, Germany).
- HOBART Food Processer (Troy, OH, USA).
- Agilent Technologies 7890A gas chromatograph coupled to a mass spectrometer detector (MSD) 5975C (Palo alto, CA, USA).

2.2 Sampling

Cow raw milk samples were obtained during 6 months between January and July 2011. Up to 13 milk herds in the Antioquia department (Colombia) were monitored. The milk herds were located in the countryside of the following towns: San Pedro de los Milagros, Santa Rosa de Osos, Belmira and Entrerríos (north region); La Unión and La Ceja (east region), and Medellín (capital city of Antioquia). The northern and eastern regions are within a radius of 100 km of Medellín. The samples were taken from the reservoir tanks in each milk herd, by trained personnel of a big milk company in the state. This company also has operative processing plants in San Pedro de los Milagros, Santa Rosa de Osos, and Medellín towns. One hundred and forty seven samples were taken during this monitoring.

Blackberry samples were obtained during 2 months between november and december in 2012. Four markets within Antioquia were monitored: one in Medellín and three rural markets in Guarne, Marinilla, and San Félix towns. Ten fresh blackberry samples were taken during this monitoring.

Processed meats consisted of several kinds of meat products: nine sausages, three cerveroni pepperoni, six beer pepperoni, three hams, six mortadellas, three smoked ribs, six Antioquia's sausages, and six bacons. All of them were collected in Medellín city. Forty two samples were taken during this monitoring.

The collected samples in the towns aforementioned (Figure 1) were carried to the GDCON laboratory, under controlled conditions of temperature (2–4 °C). Blackberries and processed meats were immediately homogenized after their arrival using a HOBART Food Processer (Troy, OH, USA). After the homogenization step, all the samples (milk, blackberries, processed meats) were stored in a freezer (−20 °C) in containers providing adequate protection until the day of the analysis.

2.3 Reactives and Reference Materials

The pesticide standards used were high purity (≥99.5%) compounds supplied by Dr. Ehrenstorfer (Augsburg, Germany) and Chemservice (West Chester, PA, USA). Triphenylphosphate was used as the internal standard (≥99.5%, Dr. Ehrenstorfer) in the validation methods for milk and blackberry.

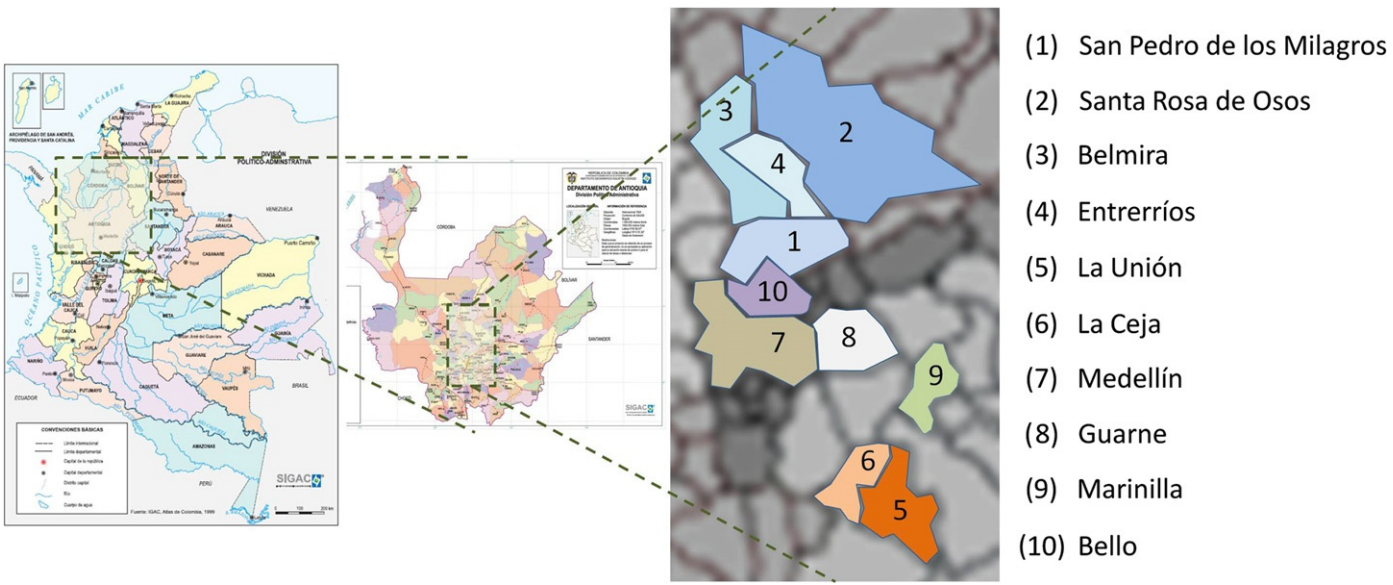

FIGURE 1 Department of Antioquia and its monitored towns. Source*: Maps gathered from the Geographic Institute Agustin Codazzi*.

Naphthalene D-8, pyrene D-10, and benzo(ghi)perylene D-12 (Dr. Ehrenstorfer) were used as internal standards in the validation method for processed meats. Anthracene D-10 (Dr. Ehrenstorfer), PCB 138, and PCB 158 (Chemservice) were used as quality control standards. Acetonitrile and acetone (Honeywell Burdick & Jackson; Muskegon, MI, USA) were the solvents of choice for pesticide residue analysis. Glacial acetic acid (>99.8%, Sigma–Aldrich; Steinheim, Germany) was also used. Acetonitrile and acetone were used for stock solution preparation. Acetonitrile was also used in the working solutions and sample preparation for all the matrices. Glacial acetic acid was used in the QuEChERS AOAC 2007.01 sample preparation method.

Extraction kits of the QuEChERS original method are prepackaged with 4.0 g of magnesium sulfate ($MgSO_4$) and 1.0 g of sodium chloride (NaCl). Those of the QuEChERS UNE-EN 15662 method are prepackaged with 4.0 g $MgSO_4$, 1.0 g NaCl, 1.0 g of sodium citrate, and 0.5 g of disodium citrate sesquihydrate. Extraction kits of the QuEChERS AOAC 2007.01 method are prepackaged with 6.0 g $MgSO_4$ and 1.5 g of sodium acetate.

SampliQ QuEChERS d-SPE prepackaged tubes (Agilent Technologies; Wilmington, DE, USA) were used for the cleanup process. According to the method, the 2-mL tubes contained 25 mg of primary secondary amines (PSA), 2.5 mg of graphitized carbon black (GCB), 25 mg of octadecylsilane (C_{18}), and 150 mg of $MgSO_4$; 50 mg of PSA, 50 mg of C_{18}, and 150 mg $MgSO_4$.

The Protecting Agents mix was prepared by weighing 50 mg of sorbitol, 100 mg of D-(+)-gluconic acid δ-lactone, and 1.0 g of 3-etoxy-1,2-propanediol in a 10-mL volumetric flask, using acetonitrile:H_2O (7:3 v:v) as solvent, and stored in cold conditions (4 °C) until needed.

Individual pesticide stock solutions were prepared at a final concentration of ~1000 mg/L, by weighing 10 mg of each pesticide in a 10-mL volumetric flask and diluting to volume using acetonitrile or acetone. Standard solutions of pesticides (Chemservice) had concentrations of 100 mg/L or 1000 mg/L, according to the pesticide. Internal standards (triphenylphosphate and naphthalene D-8, pyrene D-10, and benzo(ghi)perylene D-12) and quality control standards (Anthracene D-10, PCB 138, and PCB 158) were individual stock dilutions. Individual working solutions of 10 mg/L for the standards were made from the stock solutions and stored in a freezer (−20 °C) until needed.

A mix solution was made for each analysis method at the appropriate concentration. The mix solutions contained only the analytes and were prepared by mixing and further diluting known volumes of the stock solutions. The mix solutions were used during the validation to build calibration curves and spiked samples, and later for the quality control in the laboratory analyses. The quality control standards were used to verify the recovery of the analytes in the samples.

2.4 Methods

- Sample preparation. The schematic procedure used for the sample preparation process is depicted in Figure 2 for each of the matrices.
- Instrumental analysis by PTV–GC–MS-(SIM). The chromatographic conditions used for the instrumental analyses are summarized in Table 1 for each of the matrices.

3 VALIDATION

3.1 Validation Process

Validations were carried out for each of the methods developed following the SANCO/12495/2011 guidelines. They describe the minimum requirements for method validation and its analytical control procedure. This document is a comprehensive complement to the NTC ISO/IEC 17025 [4] regulation. Fresh raw cow's milk and blackberries from organic farms with good agricultural practices and free of pesticides were obtained as blank matrices.

Common sausages had demonstrated in a previous study to be free of PAH contamination, so they were used as the blank matrix for the processed meats. This was the only type of processed meat validated according to the SANCO/12495/2011 guidelines for analyzing similar matrices, without the requirement of further validation, if a representative matrix had been previously validated.

The method was tested to assess for sensitivity, mean recovery (as a measure of trueness), precision, and specificity. This was done performing recovery experiments with spiked blank samples to estimate the accuracy of the method. A minimum of five replicates is required (to check precision) at the limit of quantitation (to check the sensitivity) and at least another higher level.

For the quantitative analysis of pesticides in milk and blackberries, and PAHs in processed meat, a matrix-matched calibration was used, instead of simple solvent calibration. The matrix-matched calibration allows unbiased quantification as it includes the possible matrix effects in the analysis. Pesticides were analyzed in the range of 1–200 μg/L in milk and 20–200 μg/L in blackberries. PAHs were analyzed in the range of 0.5–50 μg/L in processed meats. Each of the standards was prepared by a careful dilution of the appropriate volume of mixed solution (1.0 mg/L) in a 1-mL volumetric flask. The solvent used for matrix-matched calibration was the extract obtained after sample preparation of blank matrices, as specified in Figure 2.

Mean recoveries and precision (repeatability, expressed as relative standard deviation in percent) were determined by analyzing spiked milk, blackberries, and common sausage samples in quintuplicate at two spiking levels each. The results obtained are shown in Tables 2–4.

The limit of quantification (LOQ) was established as the lowest concentration assayed, which gave satisfactory recovery (70–120%) and precision (<20% RSD) for most of the analyzed compounds.

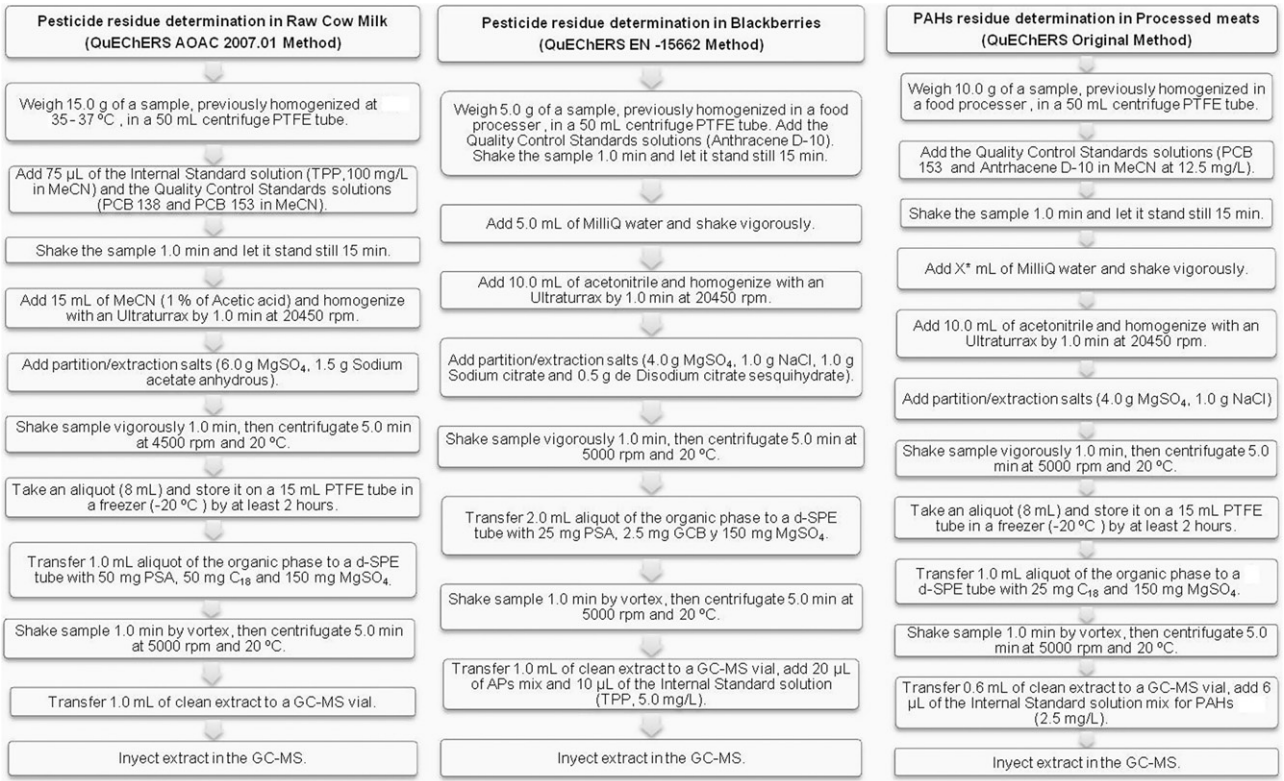

FIGURE 2 Flux diagrams of the sample preparation methods. Source*: Diagrams made by the authors.*

TABLE 1 Chromatographic Parameters of the Residue Analysis Methods

Parameter	Raw Cow's Milk	Blackberry (*Rubus glaucus* sp.)	Processed Meats
PTV injector	Solvent vent mode	Solvent vent mode	Solvent vent mode
Cooler gas	CO_2	CO_2	CO_2
Vent time	0.40 min	0.10 min	0.20 min
Vent flow	180 mL/min	100 mL/min	153.5 mL/min
Vent pressure	3.5 psi	0 psi	0 psi
Purge	50.0 mL/min held for 2.50 min	100 mL/min held for 1.0 min	50.0 mL/min held for 2.0 min
Injector temperature	30.0 °C held for 0.40 min	50.0 °C held for 0.10 min	35.0 °C held for 0.20 min
	30–350 °C at a rate of 650 °C/min	50–340 °C at a rate of 500 °C/min	30–350 °C at a rate of 700 °C/min
	350 °C held for 5 min	340 °C held for 5.0 min	350 °C held for 5 min
	350–250 °C at a rate of 10 °C/min	340–300 °C at a rate of 10 °C/min	350–250 °C at a rate of 10 °C/min
	250 °C held until end	300 °C held until end	250 °C held until end
Injection volume	10 µL	10 µL	6 µL
Injection speed	30,000 µL/min	30,000 µL/min	30,000 µL/min
Column	HP-5MS (30 m × 0.25 mm × 0.25 µm) (Agilent Technologies, Palo alto, CA)	ZB-5HT Inferno (30 m × 0.25 mm × 0.25 µm) (Phenomenex)	Varian select PAH (30 m × 0.25 mm × 0.15 µm) (Agilent Technologies, Palo alto, CA)

Oven program	40 °C for 2.50 min	80 °C for 4.0 min	40 °C for 4.0 min
	40–170 °C at a rate of 30 °C/min	80–180 °C at a rate of 20 °C/min	40–130 °C at a rate of 20 °C/min
	170–190 °C at a rate of 5 °C/min	180 °C at a rate of 6 °C/min	130–280 °C at a rate of 15 °C/min
	190 °C held for 5.0 min	180–255 °C at a rate of 10 °C/min	280–320 °C at a rate of 5 °C/min
	190–280 °C at a rate of 5 °C/min	255 °C held for 5.0 min	320 °C held for 10.0 min
	280 °C held for 5.0 min	255–280 °C at a rate of 5 °C/min	
		280 °C held for 5.0 min	
Carrier gas	Helium at a rate of 1.5 mL/min	Helium at a rate of 1.0 mL/min	Helium at a rate of 1.0 mL/min
Detector	Electron impact 70 eV	Electron impact 70 eV	Electron impact 70 eV
	Selective ion monitoring (SIM)	Selective ion monitoring (SIM)	Selective ion monitoring (SIM)
Interphase	280 °C	280 °C	280 °C
Ionization source	250 °C	250 °C	250 °C
Quadrupole	150 °C	150 °C	150 °C

TABLE 2 LOQ, MRLs, and Recoveries of Residues in Raw Cow's Milk

			% Recovery, %RSD (*n*: 5)			
Pesticide Residue	**LOQ (mg/kg)**	**MRLs[a] (mg/kg)**	***0.02 mg/kg***		***0.06 mg/kg***	
Dichlorvos	0.02	0.02	105	6	103	3
α-BHC (lindane)	0.02	0.01	82	6	91	5
γ-BHC	0.02	–	87	5	89	5
β-BHC	0.02	–	79	7	88	7
δ-BHC	0.02	–	82	4	86	4
Tefluthrin	0.02	–	78	8	82	5
Chlorothalonil	0.02	–	123	10	135	11
Methyl parathion	0.02	–	95	5	103	8
Heptachlor	0.02	0.006	56	9	62	16
Transfluthrin	0.02	–	109	10	118	12
Fenitrothion	0.02	0.001	96	6	103	10
Malathion	0.02	–	87	9	83	17
Aldrin	0.02	0.006	58	5	61	9
Fenthion	0.02	–	106	16	114	11
Ethyl parathion	0.02	–	96	10	103	13
Chlorpyrifos	0.02	0.02	83	14	91	14
Heptachlor epoxide	0.02	–	85	4	89	5
Chlorfenvinphos	0.02	–	110	16	121	5
Allethrin	0.02	–	71	12	81	10
Trans-γ-chlordane	0.02	–	63	4	66	4
Endosulfan I	0.02	–	107	10	95	11
Cis-α-chlordane	0.02	–	63	4	66	5
4,4′-DDE	0.02	–	53	5	56	4
Endosulfan II	0.02	–	96	14	117	18
4,4′-DDD	0.02	–	71	4	73	5
Ethion	0.02	–	108	3	94	16
Endosulfan sulfate	0.02	–	84	5	85	4
4,4′-DDT	0.02	–	60	3	59	7

TABLE 2 LOQ, MRLs, and Recoveries of Residues in Raw Cow's Milk—Cont'd

			% Recovery, %RSD (*n*: 5)			
Pesticide Residue	**LOQ (mg/kg)**	**MRLs (mg/kg)**	*0.02 mg/kg*		*0.06 mg/kg*	
Resmethrin	0.02	–	91	8	102	5
Endrin ketone	0.02	–	75	4	78	5
Iprodione	0.02	–	113	7	123	5
Bifenthrin	0.02	0.05	83	6	84	4
Methoxychlor	0.02	–	107	14	99	18
Fenpropathrin	0.02	–	84	7	81	4
Phenothrin	0.02	–	78	5	81	3
Lambda-cyhalothrin	0.02	–	91	7	103	7
Permethrin	0.02	0.10	89	4	108	11
Cypermethrin	0.02	0.05	89	9	109	11
Fenvalerate	0.02	0.10	95	4	99	3
Deltamethrin	0.02	0.05	80	11	81	12

[a]*MRLs without reported values belong to pesticide residues not included yet in Colombian regulation.*

3.2 Validation Results

Solvent calibration and matrix-matched calibration in each matrix showed an acceptable linearity ($r^2 \geq 0.95$), as shown in Tables 5–7. Figures 3 and 4 show the chromatographic profile of the pesticide standard mix in their corresponding matrices for raw cow's milk and blackberries, respectively. Linear regression equations and matrix effects were calculated and are also shown in the aforementioned tables.

To guarantee specificity, a mass spectrometer was used as a detection device. When possible, a quantification ion and two confirmation ions were chosen in order to ensure confirmatory identification of the pollutant. The analytes could be successfully detected above the LOQ. The ions used are shown in Tables 5–7.

Pesticide monitoring in raw cow's milk represents an analytical challenge as the matrix interferes heavily with the usual confirmatory ions of the pesticides monitored. In this particular case, the identification can only be done in a

TABLE 3 LOQ, MRLs, and Recoveries of Residues in Blackberries

Pesticide Residue	LOQ (mg/kg)	MRLs[a] (mg/kg)	% Recovery, %RSD (*n*: 5)			
			0.04 mg/kg		*0.12 mg/kg*	
Diazinon	0.04	0.1	118	(2)	119	(5)
Fenhexamide	0.04	15	77	(6)	78	(8)
Fludioxonil	0.04	5	76	(4)	72	(10)
Iprodione	0.04	30	75	(2)	79	(5)
Permethrin 1	0.04	1[b]	73	(2)	77	(6)
Permethrin 2	0.04		68	(3)	74	(5)
Vinclozolin	0.04	5	72	(2)	80	(5)

[a]*MRLs established in Social Protection Ministry Resolution N. 2906 of 2007.*
[b]*MRL expressed as the sum of concentration of both isomers.*

TABLE 4 LOQ and Recoveries of PAHs in Processed Meats

Pollutant[a]	LOQ (mg/kg)	% Recovery, %RSD (*n*: 5)			
		0.0005 mg/kg		*0.005 mg/kg*	
Acenaphthylene	0.0005	55	(22)	61	(14)
Acenaphthene	0.0005	71	(16)	102	(11)
Fluorene	0.0005	68	(13)	65	(6)
Anthracene	0.0005	89	(7)	95	(20)
Fluoranthene	0.0005	61	(11)	90	(12)
Pyrene	0.0005	72	(8)	61	(8)
Benzo(a)anthracene	0.0005	62	(13)	66	(16)
Chrysene	0.0005	75	(9)	110	(5)
Benzo(b)fluoranthene	0.0005	116	(5)	87	(5)
Benzo(k)fluoranthene	0.0005	104	(11)	82	(6)
Benzo(a)pyrene	0.0005	89	(20)	117	(9)
Dibenzo(a,h)anthracene	0.0005	99	(16)	79	(13)
Indene(1,2,3-cd)pyrene	0.0005	105	(5)	84	(18)
Benzo(ghi)perylene	0.0005	65	(8)	96	(11)

[a]*Colombia does not have MRLs established for PAHs in foods; nevertheless, according to Commission Regulation (EC) No. 1881/2006, an MRL of 0.005 mg/kg may be applicable for benzo (a)pyrene, considered a marker for PAHs.*

TABLE 5 Calibration Data for raw cow's milk

			Internal Standard Calibration						
			Solvent			*Matrix-Matched*			
Compound	$t_{R(min)}$	**Qtfy. Ion**	*Slope*	*Intercept*	r^2	*Slope*	*Intercept*	r^2	**% ME**
Dichlorvos	7.13	185	0.0084	0.0056	0.995	0.0033	0.0002	0.989	−60.7
α-BHC (lindane)	10.72	219	0.0137	0.1669	0.973	0.0091	−0.0313	0.984	−33.6
γ-BHC	11.31	219	0.0124	0.1313	0.976	0.0077	−0.0274	0.995	−37.9
β-BHC	11.47	219	0.0106	0.1634	0.972	0.00065	−0.0198	0.990	−93.9
Anthracene D-10 (QC)	11.84	188	0.0897	0.3687	0.992	0.0654	−0.2295	0.995	−27.1
δ-BHC	12.11	219	0.0120	0.0931	0.983	0.0079	−0.0161	0.996	−34.2
Tefluthrin	12.18	177	0.0109	0.0407	0.995	0.0083	0.6487	0.992	−23.9
Chlorothalonil	12.27	266	0.0255	0.0497	0.995	0.0206	−0.0578	0.994	−19.2
Methyl parathion	13.45	263	0.0060	−0.0692	0.981	0.0062	−0.0203	0.996	3.3
Heptachlor	13.72	272	0.0107	0.0227	0.996	0.0051	−0.0053	0.994	−52.3
Transfluthrin	13.61	165	0.0094	−0.0147	0.999	0.0066	−0.0043	0.998	−29.8
Fenitrothion	14.61	277	0.0077	−0.0989	0.979	0.0069	−0.0192	0.998	−10.4
Malathion	15.15	173	0.0112	−0.1358	0.980	0.0085	−0.0042	0.999	−24.1
Aldrin	15.21	263	0.0107	0.0844	0.991	0.0069	−0.0147	0.995	−35.5
Fenthion	15.55	278	0.0275	−0.1242	0.997	0.0161	−0.015	0.997	−41.5

Continued

TABLE 5 Calibration Data for raw cow's milk—Cont'd

			Internal Standard Calibration						
			Solvent			*Matrix-Matched*			
Compound	$t_{R(min)}$	**Qtfy. Ion**	*Slope*	*Intercept*	r^2	*Slope*	*Intercept*	r^2	**% ME**
Ethyl parathion	15.70	291	0.0075	−0.1049	0.972	0.0054	−0.0271	0.995	−28.0
Chlorpyrifos	15.66	314	0.0184	−0.0671	0.998	0.0045	0.0030	0.997	−75.5
Heptachlor epoxide	17.23	353	0.0129	0.0621	0.996	0.0085	−0.0156	0.997	−34.1
Chlorfenvinphos	17.86	123	0.0068	−0.0677	0.991	0.0069	−0.0024	0.999	1.5
Allethrin	17.96	267	0.0128	−0.1117	0.991	0.0153	−0.0523	0.997	19.5
Trans-γ-chlordane	18.40	375	0.0199	0.1060	0.995	0.0122	−0.0222	0.996	−38.7
Endosulfan I	18.96	241	0.0036	0.0276	0.995	0.0023	0.0213	0.997	−36.1
Cis-α-chlordane	19.13	375	0.0177	0.0504	0.998	0.0112	−0.0231	0.996	−36.7
4,4′-DDE	20.27	318	0.0277	0.1175	0.996	0.0173	−0.0143	0.999	−37.5
Endosulfan II	21.57	195	0.0041	0.0178	0.993	0.0027	−0.0001	0.999	−34.1
4,4′-DDD	22.14	235	0.0529	−0.0329	0.996	0.0383	−0.022	0.999	−27.6
Ethion	22.50	231	0.0200	−0.3731	0.969	0.0165	−0.0125	0.998	−17.5
PCB 138 (QC)	22.72	360	0.0288	0.1446	0.996	0.0176	−0.0222	0.998	−38.9
Endosulfan sulfate	23.43	387	0.0042	−0.0071	0.996	0.0024	−0.0002	0.999	−42.9
4,4′-DDT	23.71	235	0.0386	−0.2463	0.996	0.0209	0.0185	0.999	−45.9

PCB 153 (QC)	23.87	360	0.0243	0.1089	0.996	0.0147	−0.0084	0.999	−39.5
Triphenylphosphate (IS)	24.66	326	–	–	–	–	–	–	–
Resmethrin	25.03	123	0.0119	−0.1276	0.981	0.0133	−0.0559	0.998	11.8
Endrin ketone	25.27	317	0.0098	0.0110	0.999	0.0060	0.0126	0.999	−38.8
Iprodione	25.58	314	0.0082	−0.0896	0.983	0.0085	0.0039	0.999	3.7
Bifenthrin	26.17	181	0.0735	−0.5297	0.995	0.0554	−0.0008	0.999	−24.6
Methoxychlor	26.17	227	0.0565	−0.5424	0.991	0.0329	−0.0079	0.999	−41.8
Fenpropathrin	26.36	181	0.0124	−0.0833	0.995	0.0091	−0.0074	0.999	−26.6
Phenothrin	27.17	123	0.0156	−0.1892	0.9761	0.0166	−0.0019	0.999	6.4
Lambda-cyhalothrin	28.33	181	0.0121	−0.1339	0.983	0.0143	−0.0034	0.999	18.2
Permethrin	29.96	183	0.0137	−0.0841	0.994	0.0237	0.0336	0.999	73.0
Cypermethrin	31.56	163	0.0054	−0.0496	0.992	0.0084	0.4411	0.995	55.6
Fenvalerate	33.33	125	0.0069	−0.0628	0.987	0.0117	0.2046	0.998	69.6
Deltamethrin	34.84	181	0.0027	−0.0388	0.976	0.0059	−0.0186	0.999	118.5

% ME, Matrix Effect Percentage $= \frac{\text{Matrix slope} - \text{Solvent slope}}{\text{Solvent slope}} \times 100$.
IS, internal standard; QC, quality control standard.

TABLE 6 Calibration Data for Blackberries

				Internal Standard Calibration						
				Solvent			*Matrix-Matched*			
Compound	$t_{R(min)}$	Qtfy. Ion	Conf. Ions	*Slope*	*Intercept*	r^2	*Slope*	*Intercept*	r^2	% ME
Anthracene D-10 (QC)	13.63	189	187	0.688	−0.758	0.999	0.573	2.882	0.995	16.77
Diazinon	13.77	179	199.304	0.571	−0.404	0.999	0.407	−1.546	0.997	−28.74
Vinclozolin	15.84	198	187.285	0.384	−0.175	0.999	0.341	0.205	0.998	−11.23
Fludioxonil	20.34	248	154.182	1.869	1.445	0.999	1.345	−2.567	0.998	−28.06
Fenhexamide	22.15	177	179.301	0.656	−3.993	0.999	0.604	1.036	0.998	−7.92
TPP (IS)	22.63	326	325	–	–	–	–	–	–	–
Iprodione	23.09	314	316.189	0.321	−2.401	0.998	0.356	0.078	0.998	10.94
Permethrin 1[a]	26.32	183	163.184	1.163	−13.825	0.998	1.320	1.044	0.998	13.54
Permethrin 2[a]	26.60	183	163.184	0.756	−10.143	0.996	0.874	0.887	0.998	15.55

% ME, Matrix Effect Percentage $= \frac{\text{Matrix slope} - \text{Solvent slope}}{\text{Solvent slope}} \times 100$.

IS, internal standard; QC, quality control standard.

[a]*Permethrin 1 and 2 are cis/trans isomers but their respective percentages were not confirmed.*

TABLE 7 Calibration Data for Processed Meats

				Internal Standard Calibration						
				Solvent			*Matrix-Matched*			
Compound	$t_{R(min)}$	**Qtfy. Ion**	**Conf. Ions**	*Slope*	*Intercept*	r^2	*Slope*	*Intercept*	r^2	**% ME**
Naphthalene D-8 (IS 1[a])	10.40	136	137.134	NA	NA	NA	NA	NA	NA	NA
Acenaphthylene	13.07	152	151.153	0.055	0.012	0.989	0.085	0.250	0.996	54.6
Acenaphthene	13.28	153	154.152	0.037	0.012	0.987	0.052	0.096	0.983	40.5
Fluorene	14.13	166	165.163	0.050	0.012	0.982	0.065	0.852	0.994	30.0
Pyrene D-10 (IS 2)	18.55	212	211.208	NA	NA	NA	NA	NA	NA	NA
Anthracene D-10 (QC)	15.98	188	189.187	0.042	−0.028	0.999	0.044	0.003	0.999	4.8
Anthracene	16.02	178	176.179	0.056	−0.023	0.998	0.058	0.121	0.995	3.6
Fluoranthene	18.08	202	200.203	0.069	−0.051	0.999	0.062	0.093	0.999	−10.1
Pyrene	18.58	202	201.200	0.062	0.012	0.999	0.064	0.089	0.999	−6.0
Benzo (a)anthracene	21.02	228	226.229	0.055	−0.030	0.999	0.054	0.019	0.999	−1.8
Chrysene	21.22	228	226.229	0.046	−0.011	0.998	0.044	0.011	0.999	−4.3
Benzo(ghi)perylene D-12 (IS 3)	30.50	288	289.144	NA	NA	NA	NA	NA	NA	NA
Benzo(b)fluoranthene	24.06	252	253.250	0.077	−0.032	0.997	0.063	0.021	0.999	−18.2
Benzo(k)fluoranthene	24.13	252	253.250	0.083	−0.097	0.992	0.075	0.010	0.999	−9.6

Continued

TABLE 7 Calibration Data for Processed Meats—Cont'd

				Internal Standard Calibration						
				Solvent			*Matrix-Matched*			
Compound	$t_{R(min)}$	**Qtfy. Ion**	**Conf. Ions**	*Slope*	*Intercept*	r^2	*Slope*	*Intercept*	r^2	**% ME**
Benzo(a)pyrene	25.35	252	253.250	0.070	−0.050	0.998	0.064	0.001	0.999	−8.6
Dibenzo(a,h)anthracene	29.04	278	279.139	0.057	−0.045	0.998	0.057	0.006	0.999	0.0
Indene(1,2,3-cd)pyrene	29.10	276	277.274	0.079	−0.059	0.998	0.075	0.025	0.999	−5.1
Benzo(ghi)perylene	30.58	276	277.274	0.072	−0.028	0.999	0.065	−0.019	0.999	−9.7
PCB 153 (QC)	30.43	360	362.358	0.029	0.015	0.998	0.025	0.006	0.999	−13.8

% ME, Matrix Effect Percentage $= \frac{\text{Matrix slope} - \text{Solvent slope}}{\text{Solvent slope}} \times 100$.
IS, internal standard; QC. quality control standard.
[a]*Three internal standards were used. They correct the analytes that elute after them and before the next IS.*

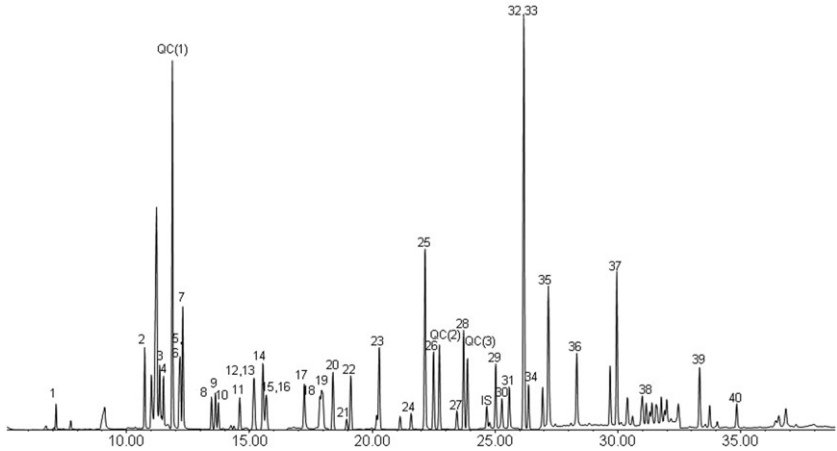

FIGURE 3 Pesticide standard mix in raw cow's milk. Chromatogram at 0.2 mg/L (matrix-matched standard) for each pesticide (1, Dichlorvos; 2, α-BHC (lindane); 3, γ-BHC; 4, β-BHC; 5, δ-BHC; 6, Tefluthrin; 7, Chlorothalonil; 8, Methyl parathion; 9, Heptachlor; 10, Transfluthrin; 11, Fenitrothion; 12, Malathion; 13, Aldrin; 14, Fenthion; 15, Chlorpyrifos; 16, Ethyl parathion; 17, Heptachlor Epoxide; 18, Chlorfenvinphos; 19, Allethrin; 20, *Trans*-γ-Chlordane; 21, Endosulfan I; 22, *Cis*-α-Chlordane; 23, 4,4′-DDE; 24, Endosulfan II; 25, 4,4′-DDD; 26, Ethion; 27, Endosulfan Sulfate; 28, 4,4′-DDT; 29, Resmethrin; 30, Endrin ketone; 31, Iprodione; 32, Bifenthrin; 33, Methoxychlor; 34, Fenpropathrin; 35, Phenothrin; 36, Lambda-cyhalothrin; 37, Permethrin; 38, Cypermethrin; 39, Fenvalerate; 40, Deltamethrin). Source*: Chromatogram obtained by André's Gallo during method validation*.

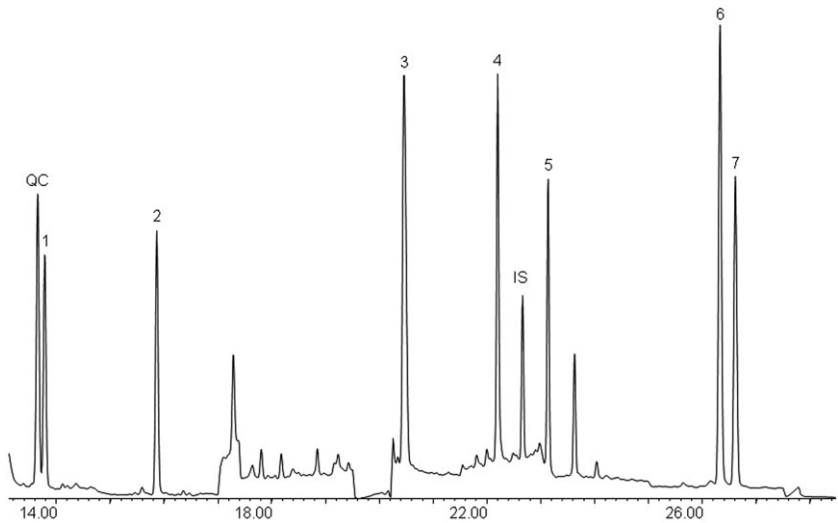

FIGURE 4 Pesticide standard mix in Blackberries. Chromatogram at 0.2 mg/L (matrix-matched standard) for each pesticide (1, Diazinon; 2, Vinclozolin; 3, Fenhexamide; 4, Fludioxonil; 5, Iprodione; 6, Permethrin 1; 7, Permethrin 2). Source*: Chromatogram obtained by Duvan Hoyos during method validation*.

presumptive way using the SIM for a single characteristic uninterfered ion for each compound. This decision undertakes sensitivity in exchange of selectivity. This is not an odd occurrence in the validation process of chromatographic methods as equilibrated merit figures are preferable to outweighed ones.

Mean recoveries (as a measure of the method trueness) were determined by analyzing spiked milk, blackberries, and common sausage samples in quintuplicate at two spiking levels each. The results were within 70–120% for recoveries, and the relative standard deviations were below 20% for most of the analytes. The results are shown in Tables 2–4.

The LOQ is the lowest concentration at which precision and trueness are met. Tables 2–4 show each analyte's LOQ. As the LOQs are equal or below the MRLs, the monitoring can be used to verify national or international requirements of pollutants in the products analyzed.

The overall data for the methods validation are shown in Tables 2–7.

4 MONITORING

4.1 Raw Cow's Milk

Several pesticides were analyzed in raw cow's milk. The samples were obtained from 13 different herds in the north and east of Antioquia in 12 monitoring campaigns carried out over a 6-month lapse of time. Chlorpyrifos was the most common residue, found in 31.6% of the samples in concentrations between 0.001 and 0.179 mg/kg. Some of the contamination cases (16%) were atypically high (almost 10 times higher) than the average contamination cases. Iprodione was found in 44 samples (27%) with only one outlier measure. Ethion was found in 35 samples. Permethrin was found in 22 samples. Thirteen samples had fenitrothion residues while only two had bifenthrin and fenthion residues. Ethyl parathion and deltamethrin were found in only one sample.

These monitoring results provide evidence for the widespread use of chlorpyrifos in the control of pests in foodstuffs, which in turn has a direct effect on the milk that cows produce. The presence of this pollutant does not represent a risk in the milk consumer's health, as its concentration is considerably lower than the EPA reference dose; nevertheless, its presence needs to be controlled.

4.2 Blackberry

Pesticides or their residues could not be found in the analyzed blackberries from the rural as well as the city areas. Even so, it is important to continue with the pesticide monitoring in this product.

It is known that some blackberry producers use pesticides containing cypermethrin, which is not included in the Colombian legislation. Its indiscriminate use, especially in Medellín city, suggests that cypermethrin should be monitored as a possible indicator for risk assessment. Chronic

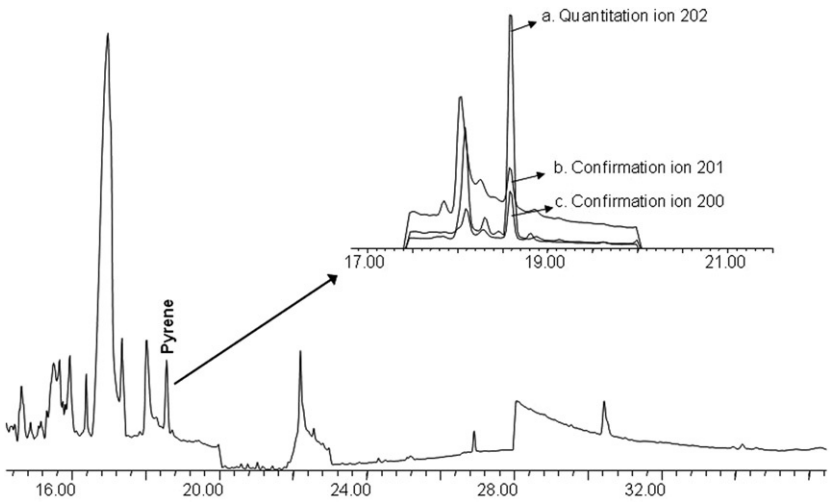

FIGURE 5 Pyrene (PAH residue) found in sausages, a sample of the processed meat monitoring. Source*: Chromatogram obtained by Duvan Hoyos during sample analysis.*

disease or even acute poisoning could be a probable consequence. Further research will be needed to determine the impact of the nonregulated pesticides, especially cypermethrin.

4.3 Processed Meats

Regulated PAHs in processed meats were not found above the LOQ of the method in any of the samples. This would mean, according to the regulations, that the processed meats monitored met the specifications and were safe for human consumption. However, pyrene (a PAH not included in the regulation) was found in several kinds of meats with a frequency of 45%. One of the chromatograms obtained for sausages, showing the pyrene and its confirmation ions, is shown in Figure 5. Its occurrence was sporadic, with concentrations ranging between 0.006 and 0.010 mg/kg, and an outlier in smoked ribs of 0.017 mg/kg. Consistent occurrences were detected in some kinds of hams, sausages, and pepperoni in concentrations between 0.007 and 0.024 mg/kg. The most contaminated samples were cocktail sausages, as their contamination reached the EPA reference dose (0.03 mg/kg). The residues found suggest that pyrene should be a regulated PAH in processed meats, and it may represent a chronic risk of poisoning for the consumers of the products.

5 CONCLUSIONS

The GC–MS technique is a useful tool to diagnose and control the regulated pesticide residues in foods and foodstuffs, especially in countries where

state-of-the-art instruments (such as LC–MS/MS or GC–Q-TOF) are not available. The results obtained are proof of a direct relationship between the overuse of pesticides and their presence in the fumigated crops. The monitoring must continue in developing countries in order to protect national and international consumers. Ensuring the safety of agro-alimentary products has a variety of impacts. An exported lot may be considered dangerous if residues are found in the products, which would have associated economic issues. On the other hand, a lot may be considered safe, but if it is in fact contaminated, it would put its consumers at risk, and health issues would be consequent.

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Chapter 8

Applications and Strategies Based on Gas Chromatography–Low-Resolution Mass Spectrometry (GC–LRMS) for the Determination of Residues and Organic Contaminants in Environmental Samples

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1 INTRODUCTION

Environmental pollution has become a worldwide issue because of the occurrence of different residues and organic contaminants in the environment and the risks that they entail for humans and ecosystems. As a result, international organizations and governments have shown an increasing concern in the last decades, developing legislation with the aim of reducing or avoiding the presence of such compounds in the environment. For instance, the United Nations established a list of persistent organic pollutants (POPs) [1] in the Stockholm Convention in 2001 consisting of different annexes that regulate the production and use of determined substances and the inclusion of new contaminants. More locally, the European Union (EU) [2] and the United States Environmental Protection Agency (US-EPA) [3] have released different lists of priority pollutants of environmental interest, including pesticides and organic contaminants. Recently, the EU has also published the so-called REACH regulation related to Registration, Evaluation, Authorization, and Restriction of Chemicals and their safe use to protect human health and the environment [4]. These documents are being updated continuously whenever new compounds are found that show adverse effects on humans and ecosystems. This is the case, for instance, of some emerging contaminants that have been recently included in such lists [5].

At this point, it may be necessary to clarify the difference between "residue" and "contaminant." A "residue" is a substance that is added intentionally by humans to reach an objective; however, as a result of the influence of different parameters, this substance can remain in the environment as a residue. A "contaminant" is a substance that has not been added intentionally by humans but reaches the environment through different ways. Pesticide residues are the most common residues found as a consequence of their wide application in agriculture to fight pests and diseases and preserve crop yields. They can occur through contamination of agricultural soils, and further through leaching to ground waters [6–8] or volatilization to atmosphere [9]. The occurrence of these residues is a global concern because some of them are considered as potential carcinogens and endocrine-disrupting compounds (EDCs), and pose a potential risk for humans and ecosystems.

In the case of organic contaminants, the range of families that can be found is wider than in residues; the contaminants are usually classified in different subgroups, such as priority and emerging contaminants. In this sense, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and phenolic compounds are commonly included in the group of priority pollutants, while pharmaceuticals, personal care products (PCPs), and brominated flame retardants (BFRs) are normally included within the group of emerging contaminants. However, some of them could also be considered as priority contaminants or as residues. PAHs and PCBs show a high occurrence and they are considered as ubiquitous contaminants following the high number of monitoring and survey studies that have reported their occurrence

worldwide [10–15]. PAHs are formed in pyrolitic and combustion processes of both anthropogenic and natural origin and the main sources of these compounds are coal processing, the incomplete combustion of organic matter, pollution by vehicles, burning of forests, and volcanic activity. They have a nonpolar and lipophilic character that makes them prone to bioaccumulation, showing carcinogenic effects [16]. On the other hand, PCBs have been widely used in industry since the 1920s in the manufacturing of electronic equipment, and as plasticisers or as additives in pesticide formulations. Because of their high stability and resistance to degradation, oxidation, and the action of other agents, they posed a high risk for humans and the environment and were included in the list of POPs [1]. In the case of phenolic compounds, their occurrence is mainly due to their presence in detergents, the reuse of wastewater effluents treated with aerobic or anaerobic microorganisms, their presence in pesticide formulations, or as a result of different industrial processes. The harmful effects of these contaminants on the environment are well-known, especially in the case of alkylphenols (APs) that are considered EDCs [17].

In the field of emerging contaminants, pharmaceuticals are the most important compounds. Their main source is the release of wastewaters (WW) subjected to incomplete or deficient treatments. Nowadays, concern over their occurrence is increasing on account of their estrogenic properties and their capability to develop bacterial resistance to antibiotics in organisms. Other compounds that are released to the environment through the re-utilization of wastewater and also labeled as emerging contaminants are PCPs. These compounds comprise a wide range of phenolic compounds, such as chlorophenols (CPs), triclosan, and parabens, and are used in shampoos, bath gels, creams, and related compounds. Consideration of some of them as EDCs supposes a threat for the ecosystems as well. Finally, BFRs are also included in this group and have been used for years in computers, electronic devices, plastics, and other products to avoid flame propagation. They have been found even in areas far away from the areas where they were used or manufactured. The most common BFRs used are polybrominated diphenylethers (PBDEs), and because of their persistence and lipophilic character, it makes them prone to bioaccumulation in human and animal tissues as well as to persistence in the environment. Moreover, some of them are considered hormonal disruptors and potential carcinogens [18].

The aforementioned compounds occur at very low concentration levels in a wide range of matrices, which are often complex matrices (e.g., soils or wastewater). In this sense, the development of sensitive, selective, and multiresidue/multicontaminant analytical methodologies for the analysis of these compounds is greatly in demand to carry out comprehensive pollution monitoring. These analytical methodologies are based on sample pretreatment, derivatization (if necessary), and final determination of the analytes. During the sample pretreatment, the extraction procedure is a key step to achieve successful results. In the last years, an increasing demand has been noticed in the development of new extraction techniques that allow automation, shorter

extraction times, and the use of lower volumes of organic solvent, as well as the possibility of the simultaneous extraction of different families of compounds. For instance, in water samples, traditional liquid–liquid extraction (LLE) has been gradually replaced by solid-phase extraction (SPE), which uses less amounts of organic solvent and allows automation of the extraction procedure. In addition, research in microextraction techniques in this field is currently increasing, and it could be considered as a step forward in terms of automation and organic solvent consumption. On the other hand, traditional Soxhlet extraction, which is widely used for solid samples, such as soils and sediments, is losing strength versus other safer (lower solvent consumption), automated and less time-consuming techniques, such as pressurized liquid extraction (PLE) or matrix solid-phase dispersion (MSPD). However, it must be pointed out that Soxhlet extraction is still a common technique used in analytical laboratories, and it remains an extraction technique of the US-EPA reference method (Method 1699) for the analysis of pesticides in soils and sediments [19] among others.

For the final determination, gas chromatography techniques coupled to low-resolution mass spectrometry analysis (GC–LRMS) are widely used. The high suitability of GC for the analysis of a wide range of compounds, such as nonpolar pesticides, PAHs, PCBs, APs, or PBDEs makes it an essential technique in research and routine analysis laboratories, with a clear application field in spite of the fast implementation of liquid chromatography (LC). Moreover, the GC analysis of medium-highly polar, thermolabile, and low volatile compounds, such as polar pesticides, pharmaceuticals, or PCPs (commonly analyzed by LC) is possible by performing derivatization steps of the compounds and alternative injection approaches, which further increases the application field of this technique. For the detection of compounds, the high sensitivity and selectivity provided by mass spectrometry (MS), especially using tandem mass spectrometry (MS/MS), make the coupling of GC–LRMS a preferred technique in comparison to traditional detectors.

This chapter reviews the most recently reported applications and strategies for the analysis of residues and organic contaminants, such as pesticides, PAHs, PCBs, phenolic compounds, pharmaceuticals, PCPs, and PBDEs in environmental samples by GC–LRMS. Special attention is paid to extraction methodologies for water and solid matrices and the derivatization procedures applied to highly polar compounds, as well as the main GC–LRMS strategies utilized, such as, type of GC injection, type of analytical column, MS detection, and some of the most interesting identification/confirmation strategies recently reported. Finally, conclusions and future trends are addressed.

2 ADVANCED EXTRACTION TECHNIQUES IN ENVIRONMENTAL ANALYSIS

Environmental analysis comprises the analysis of a wide range of matrices that can be classified in two main groups: water and solid matrices. The study

of water pollution is a well-known field that covers the monitoring of a wide range of water matrices, such as wastewater or river, lake, sea, surface water, and groundwater. On the other hand, the number of reported studies regarding the pollution of solid matrices, such as soils and sediments, is considerably lower than in waters. However, in the last years, an increasing concern has been experimented because both soils and sediments can act as sink and diffusers of pollution to other systems, such as ground and surface waters.

A good number of developed analytical methodologies have been published for the aforementioned matrices based on GC–LRMS. Sample pretreatment is commonly characterized by an extraction step selected from among a wide range of extraction methodologies (depending on matrix and analyte natures). Desirable characteristics include versatility (e.g., simultaneous extraction of compounds of different families and physicochemical properties), use of lower organic solvent volumes, reduction of extraction time, improvements on analyst safety, automation of the extraction process, and increase in sample throughput. However, the successful development of extraction methodologies that cover all these features is a challenging task in analytical chemistry.

In this section, the most important strategies and advanced applications for the extraction of residues and organic contaminants from water and solid matrices will be reviewed regarding the most important advantages and disadvantages of each technique and their main features.

2.1 Extraction of Water Samples

2.1.1 LLE-Based Extraction Techniques

The most important approach in LLE-based extraction techniques is the possibility of miniaturization of the classical LLE technique by using liquid–liquid microextraction techniques (LLME), such as ultrasound-assisted emulsification microextraction (USAEME) or vortex-assisted LLME (VALLME), which allow the use of extremely low organic solvent consumption (at μL levels). USAEME has been successfully applied for the extraction of volatile organic compounds (VOCs) [20], PAHs [21], PCBs [22], and PCPs [23]. However, it has an important drawback because of the use of ultrasounds to disperse the organic solvent (Figure 1A), which could cause the degradation of certain analytes. In this sense, the VALLME technique is similar to USAEME, but a vortex shaker instead of an ultrasonic bath performs the dispersion. VALLME has been applied for the extraction of PCBs in wastewater with adequate results [24]. Despite the simplicity of USAEME and VALLME, both techniques present some drawbacks. For instance, the organic solvent used is restricted to those showing a higher density than water (i.e., chloroform, 1-bromoctane, or 1,1,1-trichlorethane) making difficult their adequate dispersion in the aqueous bulk. Furthermore, a centrifugation step is needed to obtain a final solution separated into two phases. These disadvantages

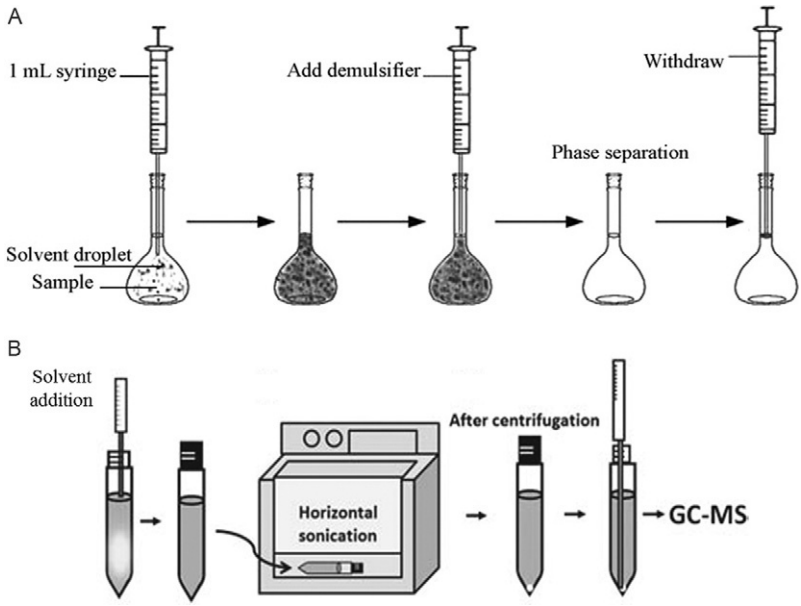

FIGURE 1 Illustrations of the (A) USAEME and (B) LDS-SD-DLLME extraction procedures. *Reproduced from Refs. [24] and [25] with permission of John Wiley and Sons and Elsevier, respectively.*

can be overcome by using low-density solvent-based solvent demulsification dispersive LLME (LDS-SD-DLLME) as extraction technique. In this extraction technique, an organic solvent with a lower density than water is mixed with the sample, and a disperser solvent is added, usually methanol or acetonitrile (AcN), in order to form a water-organic-disperser solvent emulsion. After the required extraction time, another aliquot of disperser solvent is added and the emulsion is broken up, separating the solution in two phases without centrifugation as can be seen in Figure 1B. Chen *et al.* firstly reported LDS-SD-DLLME in 2010 for the extraction of carbamate pesticides in lake waters [25], and after that, it has been applied for the extraction of organochlorine pesticides (OCPs) [26] and PAHs [27] in waters.

As described, the miniaturization of LLE techniques provides several advantages versus classical LLE, such as the very low amount of organic solvent used, the very low sample volumes required, and the simplicity of the extractions. Nevertheless, it presents some drawbacks. For instance, the automation of these techniques seems to be a difficult task, and the development of methodologies for the simultaneous extraction of different families of organic residues and/or contaminants is difficult. In this sense, extraction techniques based on the enrichment of the analytes in polymeric sorbents is a good alternative to bypass these drawbacks.

2.1.2 Sorbent-Based Techniques

After its introduction in the 1970s, SPE has been the most widely used sorbent-based technique in water analysis. Different solid phases with different absorption properties are commercially available for SPE, and the selection of the most suitable sorbent is carried out regarding its capability to absorb the target compounds. Some important advantages of SPE versus the previous techniques are the possible semiautomation of the extraction process and a high versatility for single and simultaneous extractions of organic residues and/or contaminants with different physicochemical properties belonging to different families, mainly because of the high retention capabilities of certain SPE sorbents [28–45].

However, some drawbacks are associated with SPE, such as the use of relatively high amounts of organic solvent and the impossibility of full automation with the GC–LRMS system. In this sense, new techniques were developed to improve the performance of SPE, such as solid-phase microextraction (SPME) and stir bar sorptive extraction (SBSE). The fundament aspect of both techniques consists in the extraction of the analytes from water samples using a fiber (SPME) or a magnetic stir bar (SBSE) coated by a sorbent, generally polydimethylsiloxane (PDMS) [46]. In the case of SPME, the fiber (∼1-cm long) is placed into the GC injection module after the extraction by the instrument autosampler, and the analytes are then thermally desorbed and analyzed, as has been reported for the analysis of PCBs and OCPs in river waters [47] and PCPs in wastewater and water [48]. In the case of SBSE, the magnetic stir bars have to be manually removed from sample solution and placed in a separate module for thermal desorption. This technique has been applied for the simultaneous extraction and determination of organic residues and/or contaminants in waters [49–52]. Alternatively, stir bars can be desorbed with an appropriate desorption solvent; then an aliquot is injected into the GC system, as it has been reported for the extraction of PAHs in wastewater [53].

Both SPME and SBSE are able to overcome some drawbacks of SPE because they are considered solvent-free techniques, and, in the case of SPME, the extraction can be fully automated with the GC–LRMS system, reducing sample handling. Nevertheless, some disadvantages have been associated with these techniques, such as the lack of quantitative results in recovery experiments, the short lifetime of SPME fibers, and the impossibility of fully automation in SBSE, especially when using solvent desorption. In this sense, the miniaturization of SPE by using the recently reported microextraction in packed sorbents (MEPS) is an interesting choice because it allows a full automation with the GC–LRMS and uses μL-level organic solvent volume. MEPS has been successfully applied for the extraction of PAHs [54] and PCPs [55] in wastewater; and PAHs, PCBs, nonylphenols (NPs), bisphenol A (BPA), and steroids in wastewater and snow melt waters [56].

A summary of the applications and the main performance parameters of the extraction techniques for water samples can be seen in Table 1.

TABLE 1 Summary of the Main Performance Parameters Used in the Extraction of Water Samples

Compounds	Extraction Technique	Extraction Cartridge/ Fiber	Extraction, Desorption or Elution Solvent and Volume Used	References
VOCs	USAEME	–	1-Bromooctane 15 μL	[20]
PAHs	USAEME	–	Chloroform 100 μL	[21]
PCBs	USAEME		Chloroform 100 μL	[22]
PCPs	USAEME	–	1,1,1-Trichloroethane 100 μL	[23]
PCBs	VALLME	–	Chloroform 50 μL	[24]
Pesticides	LDS-SD-DLLME	–	Toluene 50 μL	[25]
OCPs	LDS-SD-DLLME	–	*m*-Xylene	[26]
PAHs	LDS-SD-DLLME	–	*n*-Hexane 50 μL	[27]
EDCs	SPE	C_{18}	Diethyl ether/ MeOH (9:1, v/v) 6 mL	[28]
Pesticides, PAHs, EDCs, and OCs	SPE	Strata-X	MeOH/ isopropanol/AcN (1:1:1, v/v/v) 4 mL	[29]
Pesticides, phthalates, APs, and BPA	SPE		Acetone 10 mL and EtAc 10 mL	[30]
PCBs, PEs, APs, BPA, and hormones	SPE	Oasis-HLB	EtAc 8 mL and *n*-hexane 8 mL	[31]
OPPs and OCPs	SPE	Cigarette filter	EtAc/*n*-hexane (3:7, v/v) 6 mL	[32]
EDCs	SPE	C_{18}	DCM/*n*-hexane (4:1, v/v) 8 mL	[33]
Pharmaceuticals, related compounds, and BPA	SPE	Oasis-HLB	EtAc 8 mL	[34]

TABLE 1 Summary of the Main Performance Parameters Used in the Extraction of Water Samples—Cont'd

Compounds	Extraction Technique	Extraction Cartridge/ Fiber	Extraction, Desorption or Elution Solvent and Volume Used	References
Pharnaceuticals, PCPs, APs, and steroids	SPE	Oasis-HLB	DCM/EtAc/MeOH (2:2:1, v/v/v) 12 mL	[35]
Phenolic compounds	SPE	Oasis-HLB	Acetone 3 mL and DCM 2 mL	[36]
ECs	SPE	Oasis-HLB	EtAc 15 mL	[37]
PAHs	SPE	MWCNTs	*n*-Hexane 15 mL	[38]
ECs	SPE	Oasis-HLB	EtAc 10 mL	[39]
Pesticides	SPE	C_{18}	EtAc 5 mL	[40]
Pharmaceuticals and phenolic compounds	SPE	C_{18}	EtAc 6 mL	[41]
PAHs, PCBs, PBDEs, APs, OCPs, and BPA	SPE	Oasis-HLB	DCM/*n*-hexane (1:1, v/v) 10 mL and DCM/acetone (1:1, v/v) 10 mL	[42]
Pharmaceuticals	SPE	Oasis-HLB and C_{18}	EtAc/acetone (1:1, v/v) 9 mL	[43]
Pharmaceuticals	SPE	C_{18}	MeOH 9 mL	[44]
PAHs	SPE	MIPs	DCM/acetic acid (9:1, v/v) 2 mL	[45]
PCBs and OCPs	HS-SPME	PDMS	–	[47]
PCPs	HS-SPME	DVB/CAR/ PDMS	–	[48]
Phenolic compounds	SBSE	PDMS	–	[49]
Pesticides, PAHs, and PCBs	SBSE	PDMS	–	[50]
PCBs	SBSE	PDMS	–	[51]
PAHs, PCBs, PEs, and NPs	SBSE	PDMS	–	[52]

Continued

TABLE 1 Summary of the Main Performance Parameters Used in the Extraction of Water Samples—Cont'd

Compounds	Extraction Technique	Extraction Cartridge/ Fiber	Extraction, Desorption or Elution Solvent and Volume Used	References
PAHs	SBSE	PDMS	EtAc 1 mL	[53]
PAHs	MEPS	C_{18}	MeOH 50 μL	[54]
PCPs	MEPS	C_{18}	EtAc 50 μL	[55]
PAHs, PCBs, PEs, NPs, BPA, and steroids	MEPS	C_{18}	EtAc/*n*-hexane (1:1, v/v) 75 μL	[56]

AcN, acetonitrile; APs, alkyl phenols; BPA, bisphenol A; DCM, dichloromethane; BFRs, brominated flame retardants; CAR, carboxen; DVB, divinyl benzene; ECs, estrogenic compounds; EDCs, endocrine-disrupting compounds; EtAc, ethyl acetate; FRs, flame retardants; HS-SPME, head space-solid phase microextraction; LDS-SD-DLLME, low-density solvent-based solvent demulsification dispersive liquid–liquid microextraction; MeOH, methanol; MEPS, microextraction by packet sorbents; NPs, nonylphenols; OCs, organic compounds; OCPs, organochlorine pesticides; OPPs, organophosphorous pesticides; PBDEs, polybrominated diphenyl ethers; PAHs, polycyclic aromatic hydrocarbons; PCBs, polychlorinated byphenyls; PCPs, personal care products; PDMS, polydimethylsiloxane; PEs, phthalate esters; SBSE, stir bar sorptive extraction; SPE, solid phase extraction; SPME, solid phase microextraction; USAEME, ultrasound-assisted emulsification microextraction; VOCs, volatile organic compounds.

2.2 Extraction of Solid Samples

2.2.1 Traditional Techniques

The use of traditional extraction techniques, such as Soxhlet and ultrasonic extraction (USE), has been recently reported in some applications. Thus, as mentioned in Section 1, Soxhlet extraction remains a US-EPA reference method for the analysis of pesticides in soils and sediments [19]. On the other hand, USE methodologies have been reported for the extraction of pesticides in agricultural soils [32] and flame retardants (FRs) in agricultural, forested, and industrial soils [57]. In addition, several extraction methodologies have also been reported combining USE with sorbent-based microextraction techniques, such as SBSE for the extraction of phenolic compounds [58] and OCPs, PCBs, PAHs, and PBDEs [59] or SPME for the extraction of PAHs in soil [60]. However, the combination of two consecutive extractions involves an increase in the analysis time and sample handling.

2.2.2 Modern Techniques

In order to minimize the disadvantages of traditional extraction techniques used in solid samples, several modern techniques have been applied. One of the most used alternatives is the PLE technique, which is also known as

accelerated solvent extraction (ASE®). PLE has been applied for the extraction of PAHs, PCBs, PBDEs, and PCPs [61], PAHs [62], PAHs and PCBs [63] and pesticides [64,65] in agricultural soils; the analysis of PBDEs in soil [66]; and the monitoring of BFRs and PBDEs in sediments [67]. This technique allows a high level of automation due to the availability of modern devices equipped with a carrousel that allows the extraction of a high number of samples (extraction cells), as well as the use of different extraction parameters for each sample, increasing sample throughput. In addition, clean-up steps can be performed simultaneously by the dispersion of the sample with a sorbent and packing the mixture in the extraction cell, reducing the need for exhaustive post-cleanup procedures. This extraction is called selective PLE (SPLE) and it has been used for the extraction of estrogenic compounds (ECs) in soils [68].

Despite the wide application of PLE, it presents two important drawbacks: the use of a high amount of extraction solvent and the expensiveness of PLE apparatus in comparison with other extraction devices. Regarding the first drawback, it must be mentioned that organic solvent consumption can be reduced using a modified PLE, pressurized hot water extraction (PHWE), turning PLE into an environmentally friendly technique. PHWE has been applied in combination with SPME for the extraction of PAHs in sediments [69]. Other possibilities are the use of less automated methodologies than PLE but cheaper and more environmental friendly, such as QuEChERS-based methods (quick, easy, cheap, effective, rugged, and safe-based) and MSPD procedures. The first approach is based on the original QuEChERS extraction method, reported in 2003, which was used for the analysis of pesticide residues in vegetables and fruits [70]. This methodology has been applied in environmental analysis with some modifications (QuEChERS-based procedures). The main modifications are the addition of water to dry samples, such as soil, in order to improve the interaction of the extraction solvent with the matrix; the acidification of the extraction solvent; or the modification/removal of the cleanup step by dispersive SPE (D-SPE). In this sense, quick methods have been reported by using QuEChERS-based methodologies for OCPs [71,72] and phenolic compounds [73] in agricultural soils. On the other hand, MSPD extraction methodologies have been reported for the extraction of pesticides in agricultural soils [74] and PCPs and phenolic compounds in agricultural and forested soils [75].

Additionally, it must be mentioned that an interesting alternative has been recently reported for the extraction of PCPs in soil and sediments by means of SBSE avoiding the use of organic solvents and allowing certain automation during the extraction [76]. Briefly, this extraction consisted in the addition of water to the solid sample to allow the use of the SBSE magnetic stir bars.

A summary of the applications and the main performance parameters of the extraction techniques for solid samples can be seen in Table 2.

The majority of the extraction techniques reviewed in this section provides sample extracts containing the analytes of interest. In the case of on-line extraction methodologies, the analytes are also ready to be injected

or desorbed directly into the GC after the extraction. Nevertheless, several families of compounds of environmental interest are not suitable for analysis by GC in their original form, for instance, families of more polar and less volatile compounds. These analytes require the performance of a derivatization step in order to convert them in GC-amenable derivatives.

TABLE 2 Summary of the Main Performance Parameters Used in the Extraction of Solid Samples

Compounds	Extraction Technique	Extraction Solvent	Extraction Sorbent	References
OPPs and OCPs	USE	EtAc 30 mL	–	[32]
FRs	USE	EtAc 5 mL	–	[57]
Phenolic compounds	USE and SBSE	MeOH 15 mL	PDMS	[58]
OCPs, PCBs, PAHs, and PBDEs	USE and SBSE	MeOH 15 mL	PDMS	[59]
PAHs	USE and SPME	*n*-Hexane/ DCM (1:1, v/v)	PDMS	[60]
PAHs, PCBs, PBDEs, and PCPs	PLE	EtAC/MeOH (9:1, v/v)	–	[61]
BFRs and PBDEs	PLE	*n*-Hexane/ acetone (3:1, v/v)	–	[67]
PAHs and PCBs	PLE	*n*-Hexane	–	[63]
Pesticides	PLE	EtAc/MeOH (3:1, v/v)	–	[64]
PAHs	PLE	*n*-Hexane	–	[62]
Pesticides	PLE	Acetone	–	[65]
PBDEs	PLE	DCM	–	[66]
ECs	SPLE	DCM/acetone (3:1, v/v)	–	[68]
PAHs	PHWE and SPME	Water	PDMS/DVB	[69]
OCPs	QuEChERS-based	AcN/water (7:3, v/v) 10 mL	–	[71]

TABLE 2 Summary of the Main Performance Parameters Used in the Extraction of Solid Samples—Cont'd

Compounds	Extraction Technique	Extraction Solvent	Extraction Sorbent	References
Phenolic compounds	QuEChERS-based	AcN (1% acetic acid)	–	[73]
OCPs	QuEChERS-based	AcN (1% acetic acid)	–	[72]
Pesticides	MSPD	EtAc	C_{18}	[74]
PCPs and phenolic compounds	MSPD	AcN 13 mL	C_{18}	[75]
PCPs	SBSE	–	PDMS	[76]

AcN, acetonitrile; DCM, dichloromethane; BFRs, brominated flame retardants; CAR, carboxen; DVB, divinyl benzene; EtAc, ethyl acetate; FRs, flame retardants; MeOH, methanol; MSPD, matrix solid phase dispersion; OCPs, organochlorine pesticides; OPPs, organophosphorous pesticides; PBDEs, polybrominated diphenyl ethers; PAHs, polycyclic aromatic hydrocarbons; PCBs, polychlorinated byphenyls; PCPs, personal care products; PDMS, polydimethylsiloxane; PHWE, pressurized hot water extraction; PLE, pressurized liquid extraction; QuEChERS, quick, easy, cheap, effective, rugged, and safe extraction; SBSE, stir bar sorptive extraction; SPE, solid phase extraction; SPME, solid phase microextraction; USE, ultrasonic extraction.

3 DERIVATIZATION REACTIONS OF COMPOUNDS

Several derivatization reagents are available in the market. For phenolic compounds, the most used derivatization reagents are bis(trimethylsilyl) trifluoroacetamide (BSTFA), pentafluorobenzyl chloride (PFBCl), and pentafluorobenzyl bromide (PFBBr). Nevertheless, certain derivatizing reagents can be prone to degradation in aqueous medium (i.e., BSTFA), show a high cost (e.g., PFBBr, PFBCl), or require long derivatization times (e.g., BSTFA). Simple, cheap, and time-efficient derivatizations can be performed for phenolic compounds [36,58] and certain PCPs, such as parabens, triclosan, and methyl-triclosan [48,76], using acetic acid anhydride and either pyridine, $NaHCO_3$, K_2CO_3, or Na_2PO_4 in order to provide a basic medium. The derivatization reaction consists in the acetylation of the hydroxyl group of phenolic compounds and PCPs, obtaining the correspondent ester derivatives. The basic medium facilitates the deprotonation of the hydroxyl group, helping the reaction to take place. This reaction can be performed at room temperature in a few seconds after the extraction step or *in situ*, namely, simultaneously with the extraction step by adding the derivatization reagents before the extraction. Also, see Chapter 5 in this book for on-column derivatization.

The derivatization of other families, such as pharmaceuticals, steroids, or hormones, is not as easy as in the case of phenolic compounds and PCPs. In this

case, the analyst has to resort to more expensive and toxic derivatization reagents and more time-consuming reactions. The most widespread used reagents for the derivatization of the aforementioned families are BSTFA [33,37] and BSTFA: TCMS (trimethylchlorosilane) 1% [31,39,41,61,68,75,77]. Other reagents less often used are *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA) [43], *N*-*tert*-butyldimethylsilyl-*N*-methyltrifluoroacetamide MTBSTFA [78], and MTBSTFA:TBDMSCl (*tert*-butyldimethylchlorosilane) 1% [35]. The derivatization reaction using these reagents consists in the silylation of the hydroxyl or carboxyl groups of the compounds of interest, obtaining the correspondent silyl derivatives ready to inject in the GC system. This procedure needs higher temperatures and takes longer times in comparison with the acetylation process. For instance, derivatization times and temperatures of the previous referenced works using sylilation ranged from 20 min to 2 h and from 60 to 85 °C, respectively. However, and despite these important drawbacks, silylation reactions provide more weighed derivatives than those provided by acetylation because of the higher weight of sylil groups versus acetyl groups (silyl groups used for this purpose are composed of a silicon atom and methyl or *tert*-buthyl groups). This is an advantage when working with MS detection because this type of derivatization provides ions with a higher *m*/*z* ratio that are more selective than smaller *m*/*z* ions. An interesting alternative has been reported to carry out the derivatization of PCPs in the GC inlet by making multilayer injections of the silylation reagent and the sample [61], avoiding in this way the manipulation of the sample and toxic derivatization reagents in this step.

After derivatization, the new derivative compounds are ready to be determined by GC–LRMS. The most advanced strategies and applications reported with this technique are reviewed in the next section.

4 GC–LRMS ANALYSIS

Different performance parameters can be established for the GC–LRMS analysis, such as type of injection and analytical column used in the GC system; type of ionization and MS acquisition mode; or strategies for a reliable identification and confirmation of the compounds by using LRMS techniques.

4.1 GC Injection and Separation

It is well known that injection is a critical step in GC analysis. Among the different injection techniques, splitless injection has been the most used technique, and a wide range of applications has been described. However, this type of injection can hinder the determination of thermolabile analytes, which can be degraded by the high temperatures applied in the injector. In this sense, the use of large volume injection combined with programmed temperature vaporization (LVI + PTV) allowed the transfer of larger sample volumes, increasing the sensitivity of GC–LRMS detection and the possibility of setting

injector temperature gradients, which facilitated the gradual volatilization of compounds from the injector to the chromatographic column, avoiding the loss of compounds prone to thermal degradation. In this sense, Vallejo *et al.* [77] performed the optimization of the LVI + PTV–GC–LRMS analysis of estrogenic compounds in wastewater and river water. In this study, several LVI + PTV injection parameters were optimized, such as cryo-focusing temperature, vent time, vent flow, vent pressure, injection volume, purge flow in the split vent, splitless time, and injection speed. The obtained limits of detection (LODs) were in the ng/L level, improving the results obtained with splitless inlets. Guitart *et al.* [35] also optimized an LVI + PTV injection for the trace analysis of pharmaceuticals, PCPs, APs, and steroids, in the same matrices, studying the initial inlet temperature, vent time, plunger speed, and overall injection time. Despite the fact that LVI + PTV might seem an injection technique more suited for the analysis of thermolabile analytes, it has also been used for the analysis of nonthermolabile compounds, such as PAHs [21,53,54] and PCBs [63], because of the high sensitivity provided in trace analysis.

With respect to GC separation, the use of capillary columns containing 5% phenyl–95% methylsiloxane as stationary phase is widely applied (typically 30 m × 0.25 mm internal diameter (i.d.) × 0.25 μm film thickness). In fact, these columns are the best choice for the development of multi residue/contaminant separations using GC. However, some drawbacks are present when PAHs are analyzed by GC–LRMS using this analytical column, such as the coelution of three sets of critical groups of PAHs that provide isobaric ions in MS: (i) benz[*a*]anthracene (BaA), cyclopenta[*cd*]pyrene (CPcdP), and chrysene (CHR); (ii) benzo[*b*]fluoranthene (BbFA), benzo[*k*]fluoranthene (BkFA), and benzo[*j*]fluoranthene (BjFA); and (iii) indeno[1,2,3-*cd*]pyrene (IP) and dibenz[*a*,*h*]anthracene (DBahA). Additional difficulties are observed in the analysis of high molecular weight PAHs, such as dibenzopyrenes, which show a strong interaction with the stationary phase providing broad peaks and observing sensitivity problems. In this sense, Barco-Bonilla *et al.* [53] developed a separation method for the analysis of PAHs in wastewater by using a shorter capillary column containing a 50% phenyl–50% methylsiloxane stationary phase (20 m × 0.15 mm i.d. × 0.05-μm film thickness). This column (described as specific for PAHs) provides a better performance for the analysis of dibenzopyrenes and a suitable separation of the coeluted compounds aforementioned, as it can be seen in Figure 2. However, due to its specificity for PAHs, the simultaneous separation of other families of compounds together with PAHs is difficult when using this column.

In the field of flame-retardant analysis, López *et al.* [67] reported a remarkable application for the simultaneous analysis of new BFRs and PBDEs in sediments. A column containing a 5% phenyl–95% methylsiloxane stationary phase (60 m × 0.25 mm i.d. × 0.25-μm film thickness) was used for the simultaneous separation of these compounds. However, some compounds,

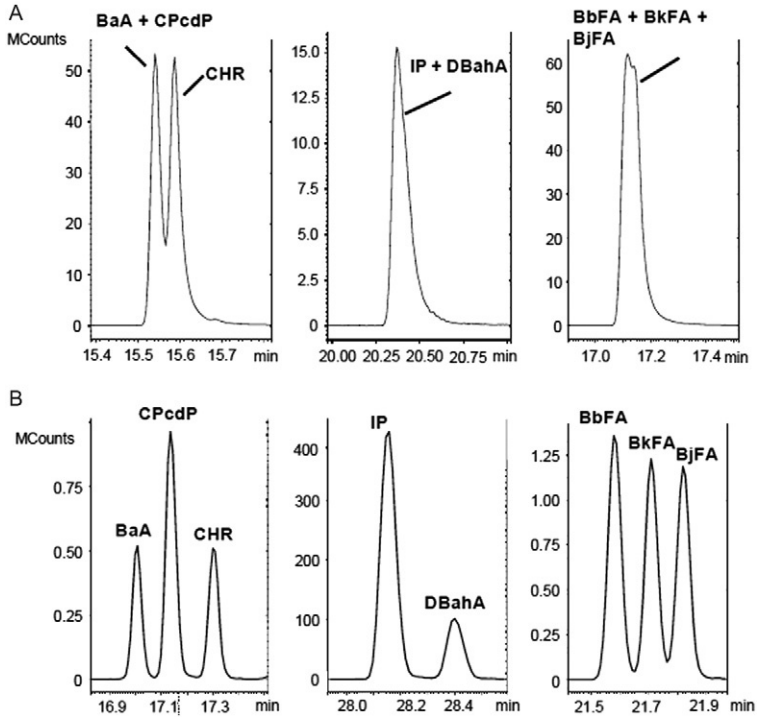

FIGURE 2 Chromatographic separation performance of three groups of PAH isomers provided by (A) a typical 5% phenyl–95% methylsiloxane column at 50 μg/L and (B) a 50% phenyl–50% methylsiloxane column at 1 μg/L. *Reproduced from Ref. [53] with permission of Elsevier.*

such as decabromodyphenylethane (DBDPE), BDE 209, octa-BDEs, and nona-BDEs, showed in-column degradation because of high temperatures. The authors solved this issue by using a shorter column (15-m length) with a similar stationary phase, internal diameter, and film thickness, which diminished the risk of in-column degradation.

4.2 LRMS Detection Strategies

Traditionally, detection has been performed in full scan and selected ion monitoring (SIM) modes. However, these modes can show low selectivity in complex matrices that results in a high background noise as a consequence of the presence of matrix compounds. In addition, low sensitivity is observed due to the high number of ions that have to be measured by the MS instrument. In this sense, the use of MS/MS permits the increase in selectivity and sensitivity, and it has become the main choice in the development of analytical methodologies using GC–LRMS. At this point, it is important to note that the identification and confirmation of compounds using LRMS (working in full scan, SIM, or

MS/MS) are not standardized in environmental applications. In this sense, the SANCO guideline, established for the development and validation of analytical methodologies for the analysis of pesticide residues in food and feed [79], has also become a reference guideline for environmental analysis.

Among the different MS/MS modes, the selected reaction monitoring (SRM) mode is the most used because it provides higher selectivity and sensitivity. However, the determination of some compounds, such as PCBs and PAHs, could be a difficult task. PCBs provide low selective fragments based on Cl losses and, as it is expected, homologue groups (congeners with the same number of chlorine atoms but with different distribution around the aromatic rings) will provide the same MS fragmentation pattern, making difficult their reliable identification when chromatographic resolution is not complete. In this sense, some LRMS strategies based on SRM approaches have been reported to overcome this problem. For instance, the use of three SRM transitions to increase the identification capabilities of PCBs was successfully applied [20,47]. Nevertheless, the establishment of three SRM transitions for PCBs using a single precursor ion is difficult. Martínez-Vidal *et al*. [63] reported an interesting approach by performing the selection and fragmentation of two precursor ions, instead of one, for each PCB in order to obtain three SRM transitions for each compound. Despite this interesting strategy, it must be mentioned that some pairs of PCBs, such as PCBs 28 + 31, 118 + 123, and 128 + 167, could not be individually identified and they have to be determined as a sum. A similar behavior is shown by PAHs. Their main fragmentation pattern is the loss of hydrogen atoms (H) and, as in Cl losses, H losses provide low selective transitions that make the identification of some isomeric compounds difficult; otherwise, a specific column for PAHs is needed as mentioned earlier in Section 4.1 and illustrated in Figure 2.

Another alternative strategy has been recently reported for the analysis of phenolic compounds in agricultural soils after a derivatization step [73] by using the combination of SRM and SIM. As it can be seen in Figure 3, MS spectra of the underivatized phenols were very similar to those of the derivatized analogues, with the only difference being a small peak in the MS spectra of the derivatized phenols corresponding to their molecular ions (ions circled). Because of the low sensitivity shown by these ions, other more sensitive ions were selected as precursors of the derivatized compounds, such as m/z 220 (Figure 3A2) and m/z 195 (Figure 3B2). However, both ions match with ions present in the MS spectra of the underivatized compounds, and this could lead to a nonreliable identification of the derivatized compounds if the derivatization reaction is not totally completed. To fix this issue, SRM was used to quantify and identify the derivatized compounds and an additional point of confirmation was added by SIM monitoring of the derivatized molecular ions to achieve a reliable identification of target analytes.

Finally, some recent applications using GC–LRMS that were focused on the ionization of the compounds are described. Typically, electron ionization

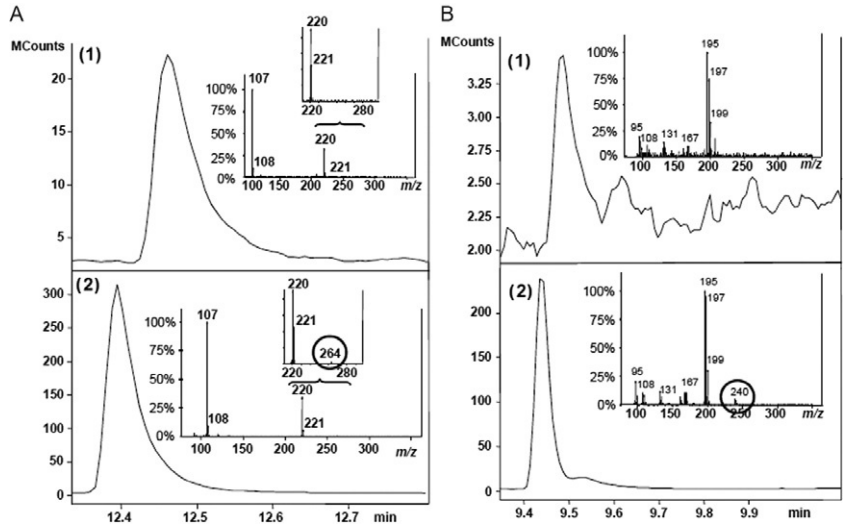

FIGURE 3 Chromatogram and the corresponding spectrum in full scan of (A1) 4-*n*-nonylphenol underivatized and (A2) 4-*n*-nonylphenol derivatized; and (B1) 2,4,6-trichlorophenol underivatized and (B2) 2,4,6-trichlorophenol derivatized. *Reproduced from Ref. [73] with permission of Elsevier.*

(EI) at 70 eV is used as the ionization mode in GC–LRMS because of its high reproducibility. Less energetic ionizations, such as negative chemical ionization and electron capture negative ionization, have been applied for the analysis of organic halogenated residues and contaminants, such as OCPs [80] and BFRs [67], respectively, due to the higher sensitivity observed with chlorine and bromine atom losses versus EI. However, the low energy of these ionization modes provides a mild fragmentation of the molecules, which together with the low selectivity of the Cl and Br fragmentations, makes the development of MS/MS methodologies inadvisable using these ionization techniques.

5 CONCLUSIONS AND FUTURE TRENDS

Despite the wide implementation of LC instruments, GC–LRMS is still a widely used technique with a clear application field, whether in research or routine analysis laboratories. Applications and strategies based on GC–LRMS are characterized by a previous pretreatment of sample before the determination of the compounds. Several extraction strategies have been developed recently for the extraction of water and solid samples, showing a clear future trend toward generic, environmentally friendly, automated, safe and low-cost methodologies. In water analysis, traditional LLE has been replaced by SPE.

A next step forward is the application of miniaturization strategies of LLE and SPE, such as USAEME, VALLME, LDS-SD-DLLME, SPME, SBSE, or microextraction by packet sorbents (MEPS). Similarly, traditional Soxhlet and USE techniques can be substituted by PLE for the determination of solid samples. A trend to the development of methodologies based on the QuEChERS method, MSPD, or SBSE is also observed.

For the final determination of the analytes by GC–LRMS, LVI + PTV is currently the most applied technique versus traditional splitless injection because of its increased sensitivity and its ability to avoid degradation of thermolabile compounds. In GC separations, typical 5% phenyl–95% methylsyloxane (30 m × 0.25 mm i.d. × 0.25-μm film thickness) columns are preferred for chromatographic purposes. In this sense, the development of new stationary phases that achieve successful simultaneous separations of problematic compounds, such as some PAHs and PCBs isomers, together with other less problematic families, will be a future challenge in GC in order to avoid the use of highly specific and less versatile analytical columns.

Finally, LRMS detection and identification of compounds using full scan and SIM have been replaced by MS/MS, which provide a higher selectivity and sensitivity, especially when working in SRM mode. Several strategies have been reported using MS/MS to carry out the reliable detection, identification, and confirmation of the compounds. Nevertheless, the prediction of a future trend in this field is difficult due to the high level of development reached using LRMS instrument.

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Chapter 9

Determination of Pyrethroid Insecticides in Environmental Samples by GC–MS and GC–MS–MS

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1 INTRODUCTION

Insecticides are commonly used in agricultural, domestics, veterinarian, and public health. Since in 1960 Rachel Carson revealed the potential damage of pesticides in the environment, the low persistence and the high specificity of insecticides are important clues to understand which of those are the most used. In that way, it is believed that pyrethroids are one of the best options for

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pest control. Their semisynthetic origin and the mammals' capacity to metabolize them are important factors that seemed to guarantee that pyrethroids could be the *ideal* insecticides.

Nowadays, pyrethroids are highly used in agriculture around the world, for example, in cotton crops and even on stored grain. Moreover, the decision of the US Environmental Protection Agency (EPA) to phase out certain uses of the organophosphate insecticides—because of their potentially toxic effects on humans—has led to their gradual replacement by pyrethroid pesticides. At home, people use pyrethroids to avoid flies, ants, as well as a lot of uses at garden. In the cattle industry, insecticides are often necessary and even some treatments for pet safety also include those pyrethroids. Besides, they also are used directly on humans against lice, scabies, and diseases transmitted by mosquito vectors such as malaria or dengue.

Because of their application on crops, pyrethroids are found in very different foods. Their levels in each kind of commodity product are controlled by organizations like Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) and legislated in each country. However, few studies evaluate their occurrence either in environment or in biota. In humans, pyrethroid exposure is determined by the presence of metabolites in blood and urine, but studies about the direct determination of pyrethroids on human matrices are very rare.

1.1 Structures and Isomerism

Pyrethroids are semisynthetic compounds related to the natural pyrethrin insecticides. Those natural substances are extracted from *Chrysanthemum* spp., usually *C. cinerariifolium*. They are esters of chrysanthemic acid (see

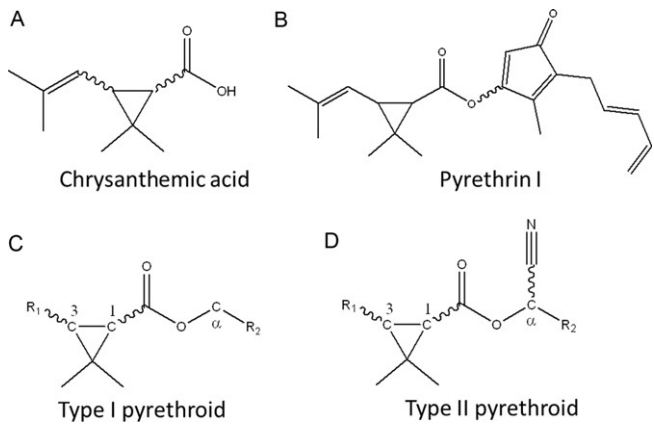


FIGURE 1 Chemical structures of (A) chrysanthemic acid, (B) pyrethrin I, (C) general type I pyrethroid, and (D) general type II pyrethroid. Asymmetric carbons are named as carbon 1, carbon 3, and carbon α, following organic nomenclature.

Figure 1A and B). Nevertheless, pyrethroids are modifications of this structure to increase their stability and, sometimes, change the way of biocide action [1]. Thus, on the basis of their structure, there are two types of pyrethroids. The difference between type I and II is a cyano group bonded to α′ carbon ($C_{\alpha'}$) that is distinctive of type II (see Figure 1C and D).

The $C_{\alpha'}$ gives specific enantiomeric properties to all type II pyrethroids that type I ones do not have. Despite that fact, a lot of pyrethroids of both types preserve the cyclopropane from chrysanthemic acid. In some cases, this rigid structure makes them have a *cis–trans* isomerism and adds two asymmetric carbons. All in all, type I pyrethroids use to have two pairs of enantiomers, that means four isomers, and type II use to have four pairs of enantiomers, so eight different isomers. Though, it is important to remark that some pyrethroids, like fenvalerate, do not keep the cyclopropane, so the geometric isomerism could be lost and also their number of asymmetric carbons would be different.

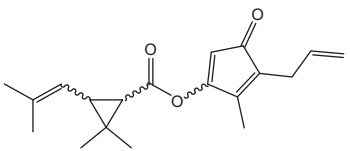
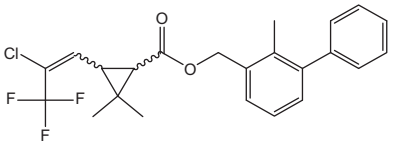
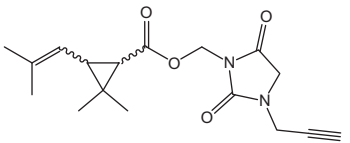
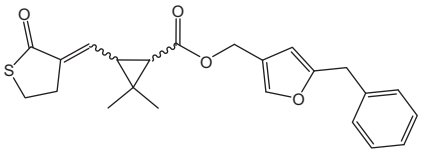
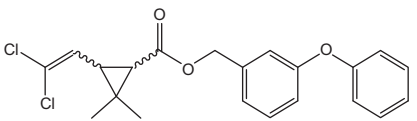
Some pyrethroids have specific names for each isomer or even enantiomer. Thus, esbiol (known as *S*-bioallethrin as well) is the *S* enantiomer of esbiothrin (known as bioallethrin), which is, in turn, the α*RS* mixture of allethrin. Similar to that, there are bioresmethrin, esfenvalerate, and biopermethrin, as a few examples. Other specific pyrethroid isomers are named with Greek characters. For instance, there is λ-cyhalothrin, which is the racemic mixture of 1*R*-3*R*-α*S* and 1*S*-3*S*-α*R* of cyhalothrin or the α-cypermethrin that is a specific mixture of some isomers of cypermethrin.

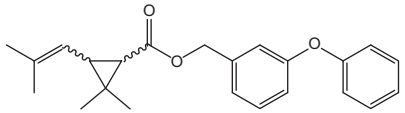
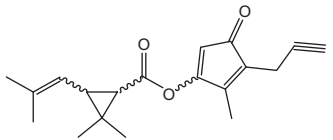
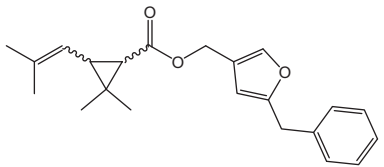
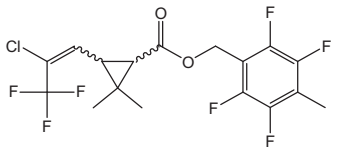
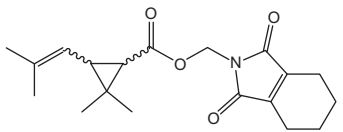
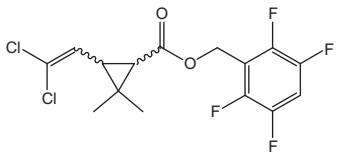
1.2 Physicochemical Properties

All pyrethroids generally have common properties. Table 1 summarizes some properties for the most common pyrethroids. It is important to note that most of these values have been theoretically calculated and that is why it is possible to find in the literature different values depending on the calculation program used. The molecular mass is usually high, ranging from 300 g mol^{-1} (prallethrin) to 665 g mol^{-1} (tralomethrin). Besides, they are lipophilic substances and, with few exceptions, their partition coefficients octanol/water (K_{OW}) are between 10^4 and 10^7 (see Table 1).

Moreover, they are photosensitive molecules and could be hydrolyzed relatively easily. In spite of that, their real lifetime in the environment could vary between some weeks and more than a year, depending on the pyrethroid and different conditions of humidity and oxygen [3]. The University of Hertfordshire database shows a typical degradation time to 50% (DT_{50}) for each pyrethroid [2]. Half-lifetimes are under 90 days, so pyrethroids are not persistent pollutants. However, studies on environment and biota point out the ubiquitous presence of pyrethroid residues [4–6]. That could mean that even when

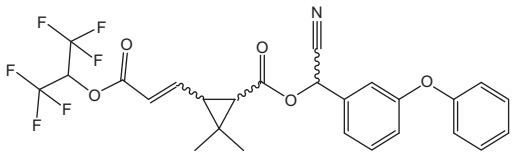
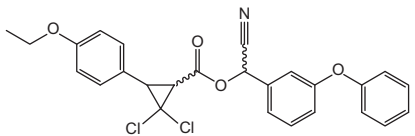
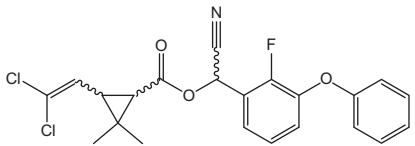
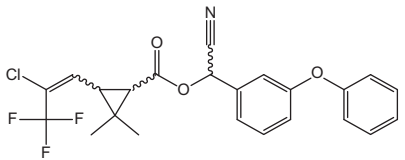
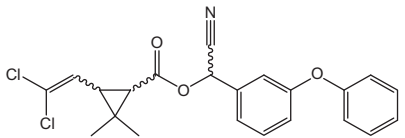
TABLE 1 Pyrethroid Classification, Structure, Properties, and EC_{50} and LC_{50} (mg L^{-1}) for Aquatic Life (Properties by PPDB [2])

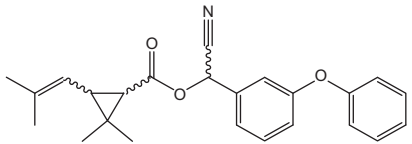
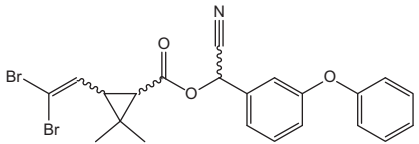
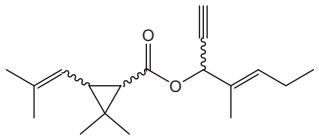
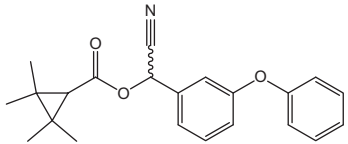
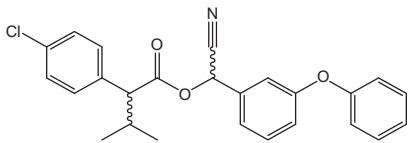
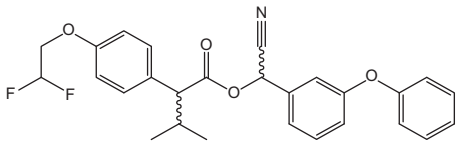
	Structure	Enantiomeric Pairs	Molecular Mass (g mol^{-1})	Log K_{OW}	Water Solubility (mg L^{-1})	DT_{50} (Days)	Invertebrate EC_{50} (*Daphnia magna*)	Crustacean LC_{50} (*Americamysis bahia*)	Trout LC_{50} (*Oncorhynchus mykiss*)
Type I pyrethroids									
Allethrin		4	302.4	4.96	10^{-4}	60	2.1×10^{-2}	–	19
Bifenthrin		2	422.9	6.6	10^{-3}	26	1.1×10^{-4}	4×10^{-3}	0.15
Imiprothrin		2	318.4	2.43	93.5	5	5.1×10^{-2}	–	3.8×10^{-2}
Kadethrin		2	396.5	–	–	–	–	–	–
Permethrin		2	391.3	6.10	0.2	13	–	0.02	0.62

Phenothrin		2	350.5	6.01	9.7×10^{-3}	1	4.3×10^{-3}	2×10^{-5}	2.7×10^{-3}
Prallethrin		4	300.4	4.49	8.03	–	6.2×10^{-3}	–	1.2×10^{-2}
Resmethrin		2	338.4	5.43	10^{-2}	30	3.7×10^{-3}	–	–
Tefluthrin		2	418.7	6.40	1.6×10^{-2}	37	7×10^{-5}	5×10^{-5}	6×10^{-5}
Tetramethrin		2	331.4	4.60	1.83	3	4.5×10^{-2}	–	1.6×10^{-2}
Transfluthrin		2	371.2	5.46	5.7×10^{-2}	–	–	–	–

Continued

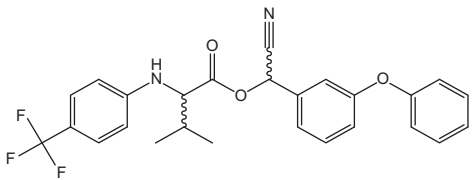
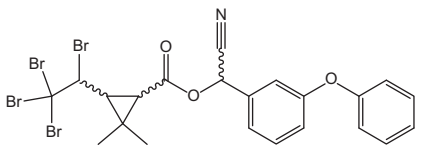
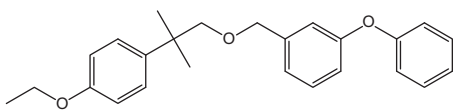
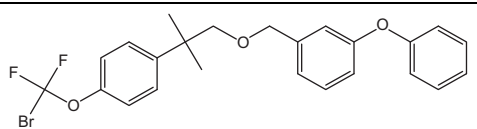
TABLE 1 Pyrethroid Classification, Structure, Properties, and EC_{50} and LC_{50} (mg L^{-1}) for Aquatic Life (Properties by PPDB [2])—Cont'd

	Structure	Enantiomeric Pairs	Molecular Mass (g mol^{-1})	Log K_{OW}	Water Solubility (mg L^{-1})	DT_{50} (Days)	Invertebrate EC_{50} (*Daphnia magna*)	Crustacean LC_{50} (*Americamysis bahia*)	Trout LC_{50} (*Oncorhynchus mykiss*)
Type II pyrethroids									
Acrinathrin		4	541.4	6.30	2×10^{-3}	39	2.2×10^{-5}	–	0.0061
Cycloprothrin		2	482.4	4.19	9.1×10^{-2}	47	10 (3 h)	–	1.6
Cyfluthrin		4	453.3	6.00	6.6×10^{-3}	33	1.4×10^{-4}	0.0024	0.3
Cyhalothrin		4	449.9	6.80	4×10^{-3}	57	0.38	–	0.24
Cypermethrin		4	416.3	5.30	9×10^{-3}	60	3×10^{-4}	0.005	0.39

Cyphenothrin		4	375.5	6.62	10^{-2}	–	4.3×10^{-4}	–	3.4×10^{-4}
Deltamethrin		4	505.2	4.60	2×10^{-4}	13	5.6×10^{-4}	0.0017	0.25
Empenthrin		4	274.4	6.21	0.11	–	2×10^{-2}	–	$>1.7 \times 10^{-3}$
Fenpropathrin		1	349.4	6.04	3.3×10^{-1}	34	5.3×10^{-4}		2.3×10^{-3}
Fenvalerate/ esfenvalerate		2	419.9	5.01	10^{-3}	44	–	3.8×10^{-2}	7×10^{-2}
Flucythrinate		2	541.5	4.70	0.5	60	8.3×10^{-3}	8.3×10^{-2}	0.32

Continued

TABLE 1 Pyrethroid Classification, Structure, Properties, and EC_{50} and LC_{50} (mg L^{-1}) for Aquatic Life (Properties by PPDB [2])—Cont'd

	Structure	Enantiomeric Pairs	Molecular Mass (g mol^{-1})	Log K_{OW}	Water Solubility (mg L^{-1})	DT_{50} (Days)	Invertebrate EC_{50} (*Daphnia magna*)	Crustacean LC_{50} (*Americamysis bahia*)	Trout LC_{50} (*Oncorhynchus mykiss*)
Fluvalinate		2	502.9	3.85	2×10^{-3}	4	7.4×10^{-2}	2.9×10^{-3}	–
Tralomethrin		4	665.0	5.00	8×10^{-2}	3	3.8×10^{-5}	–	1.6×10^{-3}
Other pyrethroids									
Etofenprox		0	376.5	6.90	2.25×10^{-2}	11	1.2×10^{-3}	–	2.7×10^{-3}
Halfenprox		0	477.3	7.70	5×10^{-5}	10	3.1×10^{-5}	–	–

pyrethroids are not persistent, they could remain in the environment because of abusive use and constant dump.

1.3 Toxicity

Pyrethroids are sodium channel modulators. Their effect on insects can be obtained by both contact and ingestion. Even though pyrethroids are specific insecticides, they are very toxic for some nontarget species. For instance, it is known that pyrethroids could be lethal for some aquatic ecosystems at concentrations lower than 1 ppb in water. Nontarget organisms such as aquatic invertebrates and fish are extremely sensitive to the neurotoxic effects of these insecticides [7,8]. Table 1 shows the toxicity of some pyrethroids for different aquatic species indicating their half maximal effective concentration (EC_{50}) or their lethal concentration for the half of the population (LC_{50}). To protect aquatic life, some countries, such as the United Kingdom, have proposed environmental quality standards for some pyrethroids (e.g., 0.001 μg L^{-1} for cyfluthrin and 0.01 μg L^{-1} for permethrin) in the water column [9].

For mammals and other vertebrates, pyrethroids generally are low or moderate toxic, being the oral acute toxicity of, at least, hundreds of mg kg^{-1} body weight [2]. There are some toxic effects described in humans, associated to acute toxicity. They are basically urticarial, respiratory, and gastric diseases, and in very few cases, there can be convulsions and neurologic effects [10].

However, for mammals, there are some known metabolic pathways of pyrethroids, for example, those related to cytochrome P450 systems and some esterases, and finally excreted by urine [11]. Because of these metabolic pathways, the studies of chronic toxicity are few. In fact, a lot of monitoring studies about pyrethroid exposure are based on metabolites found in urine. Recently, some studies about bifenthrin, permethrin, and cypermethrin chronic toxicity are showing that even at doses below the no observable adverse effect level, these pyrethroids could be neurotoxic [12] or have reproductive toxicity [13,14].

Chronic toxicity in humans is not demonstrated and the studies about it are not conclusive. Despite that fact, some authors found relationships between pyrethroid exposure and brain tumors in children [15] or the number of genetic aberrations [16].

Pyrethroids have been included in a list of suspected endocrine-disrupting chemicals by an EU working group [17]. Even though effects on humans are still unclear, the EPA has classified some of them (e.g., cypermethrin, permethrin, and biphenthrin) as possible human carcinogens [18].

Finally, it is important to emphasize that the isomeric properties of pyrethroids have an influence on their toxicity. For example, in mice, it is proved that *cis*- and *trans*-isomers are differently metabolized and could induce dissimilar toxic effects [19]. In that way, legislation on usage has begun to take into account these properties.

2 SAMPLE PREPARATION METHODOLOGIES

The most common analyses of pyrethroids have been carried out on food matrices. The objective of these analyses is to check whether levels in food exceed or not the legislated levels of pyrethroids. Even though the European Regulation establishes "by default" a value of 0.01 mg kg^{-1} as the maximum pesticide residue level in foodstuffs [17], the limits of detection (LODs) of the applied methodologies are usually very high. Since the levels in environmental or biota samples are lower, there is a need for more sensitive methods.

Table 2 summarizes recent analytical techniques used for pyrethroid determinations in environmental matrices. Usually, sample preparation for pyrethroid analysis starts with an extraction process. Typically, that step is a liquid–liquid extraction (LLE) or a solid-phase extraction (SPE). However, other methodologies have been applied including stir-bar sorptive extraction (SBSE), microwave-assisted solvent extraction (MASE), matrix solid-phase dispersion (MSPD), or even supercritical fluid extraction (SFE). QuEChERS (name formed from "quick, easy, cheap, effective, rugged, and safe") methods have been developed, which consist of extracting with lower solvent volumes in order to both decrease the costs of analysis and being environmentally friendly. Some others include solid-phase microextraction (SPME), ultrasound-assisted emulsification extraction (UAEE), and pressured liquid extraction (PLE). Other techniques such as SPME fiber chemically linked with ionic liquid (IL) can be found in the latest studies [33].

2.1 Environmental Samples

Because of the low concentrations of pyrethroids in water, analytical methods should include extraction and preconcentration steps to reach the required LODs. The most common extraction techniques for water samples are generally based on LLE using dichloromethane (DCM) [4] or hexane (Hx) [20] as solvents. Those extractions could reach recoveries from 75%. Similar results were obtained using SPE with Oasis HLB cartridges and methanol (MeOH)/acetonitrile (ACN) (1:1) as eluent [21]. Nonetheless, these methods need large sample volumes (0.5 L). For that reason, methods like SPME and SBSE were developed. Some of them were solventless techniques, thanks to the quantitative introduction of the extracted fraction by thermal desorption into the gas chromatography (GC) system. That is the case of the methods developed by Van Hoeck *et al.* [24] and Ochiai *et al.* [23] who applied SBSE technique to enrich their unfiltered water samples with recoveries from 80% to 115%, respectively. Casas *et al.* [22] optimized some critical parameters of SPME such as temperature, fiber coating, salting-out effect, and sampling mode and found that the best fibers were those of polydimethylsiloxane with recoveries up to 125% for unfiltered tap water, groundwater, and river water. For wastewater and runoff water, they recommended diluting the sample in order

TABLE 2 Some Representative Analysis Methodologies Applied to Pyrethroids in Different Matrices

Matrix	Extraction	Cleanup	Instrumental Analysis	Recoveries	LOD	Observations	References
Environmental matrices							
Water	LLE (DCM)	–	GC-ECD	75–115%	1–3 ng L^{-1}	Large volume sample	[4]
	LLE (Hx)	–	GC-ECD	94–105%	–	Large volume sample	[20]
	SPE (Oasis HLB)	–	GC-μECD	70–103%	5×10^{-4}–2×10^{-2} ng L^{-1}	Large volume sample	[21]
	SPME (polydimethylsiloxane)	–	GC-μECD	81–125%	0.05–2 ng L^{-1}	–	[22]
	SBSE	–	GC–MS	82–113	>10 ng L^{-1}	–	[23]
	SBSE	–	GC–MS	40–80%	0.02–1 ng L^{-1}	–	[24]
	UAEE (chloroform)	–	GC–MS	63–100%	0.03–36 ng L^{-1}	Low volumes of sample and solvent	[25]
Sediment	Sonication (MeOH/ACN)	Florisil	GC-μECD	71–103%	3×10^{-5}–2×10^{-3} ng g^{-1}	–	[21]
	Soxhlet	Florisil	GC–MS	90–135%	0.16–1.5 ng g^{-1}	High-resolution MS	[5]
	SFE (MeOH)	C18	GC-ECD	70–97%	<10 ng g^{-1}	–	[26]

Continued

TABLE 2 Some Representative Analysis Methodologies Applied to Pyrethroids in Different Matrices—Cont'd

Matrix	Extraction	Cleanup	Instrumental Analysis	Recoveries	LOD	Observations	References
	SPME (polyacrylate)	–	GC-μECD	81–122%	4×10^{-3}–1 ng g^{-1}	Headspace	[27]
	PLE	GPC + Florisil	GC-ECD	84–108%	0.5–4 ng g^{-1}	–	[4]
Air	Sonication (acetone)	–	GC–MS	–	0.4–1.7 μg m^{-3}	–	[28]
	SPE (Chromosorb)	Tenax	GC–MS–MS	67–117%	0.5–27 μg m^{-3} (LOQ)	–	[29]
Dust	MASE	Florisil	GC-μECD	84–117%	1–7 ng g^{-1}	–	[30]
	Sonication	C18	GC–MS	51–101%	1–60 ng g^{-1}	–	[31]
	PLE (acidic + neutral silica)	–	GC–MS	85–120%	1–10 ng g^{-1}	–	[32]
Biological matrices							
Fish	PLE	GPC + Florisil	GC-ECD	74–98%	1–4 ng g^{-1}	–	[4]
Vegetables	SPME	–	GC-ECD	67–94%	0.21–0.49 ng g^{-1}	New IL	[33]
	LLE (ACN)	Graphene	GC-ECD	44–92%	2.5–10 ng g^{-1}	–	[34]
Seaweed	MSDP (Graphene)	Florisil	GC–MS	82–109%	0.3–2.4 ng g^{-1}	–	[35]
Oilseed	MSPD (aminopropyl silica)	Florisil	GC × GC-TOF	–	–	–	[36]

Vegetable oil	SPE (alumina)	C18	GC–MS–MS	91–104%	0.3–1.4 ng g^{-1}	–	[37]
Dolphin tissues	LLE (Hx:DCM)	Basic alumina + C18	GC–MS–MS	57–117%	0.02–0.44 ng g^{-1}	–	[6]
Human matrices							
Plasma	SPE (Oasis HLB)	Silica	GC–MS	100–120%	4–8 ng L^{-1}	Acidic pretreatment	[38]
	SPE (polymeric)	–	GC–MS	88–128%	17–93 ng L^{-1}	High resolution	[39]
Whole blood	LLE (Hx:acetone)	–	GC–MS	91–103%	0.2–5 ng L^{-1}	–	[40]
	LLE (Hx)	–	GC–MS	108–124%	$<10^3$ ng L^{-1}	–	[41]
Breast milk	LLE	Florisil	GC-ECD	30–92%	15–30 ng g^{-1} lw	–	[42]
	LLE (ACN)	Silica	GC-ECD	–	0.2–0.6 ng L^{-1} (LOQ)	–	[43]
	LLE (Hx:DCM)	Basic alumina + C18	GC–MS–MS	48–91%	4×10^{-3}–1.1 ng g^{-1} lw	–	[44]

to avoid matrix effects. Feo *et al.* [25] developed an UAEE method where the aqueous phase is emulsified with a small volume of chloroform (1 mL). Recoveries ranged from 63% to 100% for 13 different pyrethroids in river water. This last method presents good recoveries and needs small volumes of sample and few sample manipulation steps.

In solid samples, the hydrophobicity of pyrethroids requires that the extractions be more exhaustive. Unfortunately, the more extensive the extraction procedure used, the more coextracted interferences can be expected [45]. To solve this problem, some authors included an additional step of cleanup. Sonication with MeOH/ACN (1:1) [21] or Soxhlet extraction [5] followed both with a cleanup step using Florisil was used for surface sediment analyses, obtaining recoveries from 103% to 135%, respectively. SFE was also used with MeOH at 400 bar and 60 °C. The cleanup was performed with C18 stationary phase and DCM/Hx (1:1) as eluent. Recoveries were up to 97% [26]. For these solid samples, Fernández-Alvarez *et al.* [27] developed a solvent-free method using headspace SPME. The optimal conditions were obtained extracting at 100 °C with ultrapure water and employing a polyacrylate coating fiber. Their recoveries were from 81% to 122%. Also a PLE method was improved with gel permeation chromatography (GPC) using DCM as eluent and an extra cleanup with Florisil. Recoveries were up to 108% [4].

Yoshida [28] developed a simple method for pyrethroid determination in air samples. Samples were collected with a small pump with two filters as adsorbents. The filters were then extracted together by sonication using acetone as solvent. The extract was concentrated and analyzed. Recoveries were up to 109%. Other methods including SPE extraction with sorbents as Chromosorb and Tenax and ethyl acetate as eluent led to recoveries from 67% to 117% [29].

Different methodologies have been developed to determine pyrethroids in dust samples, reaching good recoveries. One method applied MASE followed by a Florisil cleanup. Recoveries ranged from 84% to 101% [30]. Sichilongo [31] obtained recoveries from 51% to 101% extracting by sonication and cleaning with C18 cartridges. On the other hand, Van Emon and Chuang [32] developed a method consisting of PLE with acidic and neutral silica. They demonstrated that extra cleanup after that extraction was not required. Recoveries ranged from 85% to 120%.

2.2 Biological Samples

A few methods have been developed for biological samples. Besides, most of them are only applied with spiked tissues, and they do not reflect the situation of the analysis of real samples in which levels must be lower.

Different methodologies were developed in the field of food analysis. However, we will focus on the most recently published. The same PLE + GPC + SPE method used for soils was applied to fish tissues with

recoveries from 74% to 98% [4]. Zhang *et al*. [33] developed a methodology based on the use of SPME. First of all, they synthesized a novel IL, 1-vinyl-3-hexadecylimidazolium hexafluorophosphate, in order to use it as SPME fiber. With the optimal conditions, recoveries were up to 94% for spiked vegetables. Recently, a new method extracting cucumber and spinach directly with ACN has been published. In order to eliminate pigmentation, graphene was added to the solution. Generally, recoveries were up to 117% [34].

Garcia-Rodriguez *et al*. [35] developed a method for pyrethroid determination in seaweed consisting of an MSPD with graphite carbon black and Florisil using Hx/ethyl acetate as eluent. They obtained recoveries up to 109%. Wang *et al*. [36] developed an extraction method for oil seeds. The method used aminopropyl silica as dispersion sorbent, Florisil as cleanup sorbent, and ACN as eluent. Estimated recoveries were around 100%. Esteve-Turrillas *et al*. [37] developed another SPE methodology for the pyrethroid determination in different vegetable oils. The method consisted of the use of two cartridges in tandem (alumina and C18) and elution with ACN. Recoveries ranged from 91% to 104%.

Recently, Alonso *et al*. [6] developed a method for the determination of 11 different pyrethroids in dolphin tissues. Briefly, it consisted of an LLE with hx:DCM (1:1) followed by a double SPE in tandem being basic alumina and C18 adsorbents using ACN as eluent. Their recoveries in liver samples ranged from 57% to 117%.

Some authors have evaluated the presence of pyrethroids in human fluids as breast milk, serum, blood, and meconium. Channa *et al*. [38] developed a method working with plasma samples. It consisted of a previous formic acid treatment and two sequential SPE steps, the first one with an Oasis HLB cartridge and the second one with deactivated silica. The eluents were Hx and DCM at different proportions. Their recoveries ranged from 100% to 120%. Pérez *et al*. [39] preferred a direct SPE of 2 mL of plasma with a polymeric cartridge (ABS elut NEXUS) eluting with water and MeOH. A sample concentration of 200-fold was obtained, with recoveries ranging from 88% to 128%. Ramesh and Ravi [40] observed that the best recoveries for pyrethroid analysis in blood samples were reached analyzing the hemolyzed blood compared with the serum or the nonhemolyzed blood. That demonstrated the high affinity of pyrethroids for the erythrocytes. These authors applied 20 min of ultrasounds to the whole blood in order to lyse the erythrocytes. After that, an LLE with Hx:acetone (8:1) was carried out. Their recoveries on spiked samples reached 103%. Corrion *et al*. [41] developed a method in maternal and umbilical cord blood that consisted of diluting the sample with a buffered MeOH and an LLE with Hx. The recoveries ranged from 108% to 124%. These studies point out the determination of pyrethroids in the whole hemolyzed blood as a better choice than in plasma.

Because milk is a very complex matrix, the sample preparation for that matrix is a laborious process that can include four consecutive extractions.

For human breast milk samples, three main methods have been developed. The first one consisted of two LLE and two SPE extracts of the sample with acetone/petroleum ether (1:1), evaporating and redissolving in Hx, extracting with ACN, and finally, a SPE step with Florisil cartridges. Most of the recoveries were up to 92%, but for deltamethrin and fluvalinate, they were only 30% and 40%, respectively [42]. The second method needs an acidification with HCl. After that, the sample was extracted with ACN and with a cleanup of silica gel and Hx as eluent [43]. However, in this study, recoveries were not reported. For the third method, the sample extraction was done with Hx:DCM (2:1) and needed a further cleanup with a basic alumina cartridge in tandem with a C18 one. ACN was the eluent [44]. Recoveries were up to 91%, being the lowest recovery for deltamethrin with a value of 48%. These methods seem to be useful for most pyrethroids, but perhaps, they have lower recoveries for pyrethroids with low K_{OW}, such as deltamethrin and fluvalinate.

3 GAS CHROMATOGRAPHY SEPARATION

Different analytical separation techniques have been tested in order to determine pyrethroid levels. GC is undoubtedly the most widely used technique, reaching LODs able to determine real environmental occurrence. Liquid chromatography (LC) techniques have also been tested for the determination of pyrethroid residues in different fields [29,46,47] due to some advantages. For example, LC does not require an extensive cleanup of many real samples because of the strong retention of the pyrethroids on a reversed-phase column, while polar interferences contained in the matrix are only slightly retained. However, the relatively low sensitivity of LC compared to GC adversely affects analysis of pyrethroid residues at very low levels, at which they are usually present in environmental samples. Alternatively, McCoy *et al.* [48] developed methodologies based on immunoassays or LC in which the pyrethroid is previously converted to 3-phenoxybenzoic acid. Even when LODs could be good enough, these methodologies are unspecific, given that a lot of different pyrethroids could be converted to the same acid. On the other hand, some pyrethroids have structures not related to that acid. All in all, those methods evaluate neither specific pyrethroids nor the whole of them.

3.1 Achiral Columns

GC methods use different chromatographic columns. The nonpolar columns are preferred, DB5 (5% phenyl and 95% methylpolysiloxane) being the most commonly used. Different lengths such as 60, 30, or 15 m were used [21,45,49]. However, in order to reduce the analysis time, Ochiai *et al.* [23] used a shorter DB5 column (10 m). The retention time can be significantly decreased using shorter columns. For example, Mekebri *et al.* [4] described

the retention time of permethrin and fenvalerate in a DB5 of 60 m as 18 and 36 min, respectively. For the same compounds, Sichilongo [31] used a 30-m column and the retention times were around 18 and 22 min. In a 15-m column, Feo *et al*. [49] found the same products at 9.1 and 10.3 min, while Ochiai *et al*. [23] found them at 5.65 and 6.00 min. Nevertheless, other authors used semipolar columns such as SPB-608 (35% diphenyl and 65% dimethylpolysiloxane) [20], HP-608 (methyl 50% phenylpolysiloxane) [26], or even DB1701 (7% methyl, 7% cyanopropyl, and 7% phenylpolysiloxane) [50]. These columns did not separate all the isomers with sufficient resolution [20] or they overly retained the compounds [26].

Few authors have tried to apply multidimensional GC (GC × GC) for pyrethroids. Despite the complexity of this technique, instrumental LODs could be improved by 2–12 times because of the narrower and sharper peaks [51]. Recently, Wang *et al*. [36] developed a methodology based on the use of GC × GC. In this work, the first column was fixed as a DB5 of 30 m, and then the second one was optimized. Their results point out the interest of choosing a polar second column, and they recommended a DB-17ht (50% phenyl and 50% methylpolysiloxane). Both authors applied the methodology for a multiresidue goal for which a simple GC is not able to avoid coelution of analytes.

All these GC studies conclude that the best choice for pyrethroid determinations could be a DB5 column, the length of which would depend on the quantity of analytes and the resolution needed. GC × GC is not necessary for the common analysis of pyrethroids but it could help in case of multiresidue determinations.

As explained above, pyrethroids have several isomers, so most of them presented more than one peak in a GC chromatogram. Table 1 indicates the number of enantiomeric pairs each pyrethroid has. Each of these pairs corresponds to one peak in a nonchiral GC column. For instance, permethrin has two enantiomeric pairs leading to two chromatographic peaks in a DB5 column of 15 m. The two peaks corresponded to the *cis*- and *trans*-permethrin (Figure 2A). The same situation corresponded to pyrethroids such as resmethrin, phenothrin, tetramethrin, fluvalinate, and fenvalerate. Besides, Figure 2B shows the four peaks obtained for cypermethrin, where two peaks are assigned to *cis*-configuration and two others to *trans*-configuration. The same situation is observed for cyfluthrin. The *cis*- and *trans*-assignation could be made with the labeled compounds, in which only the *trans*-compounds are present (Figure 2C and D), taking into account that deuterated compounds have a little bit shorter retention time (differences of 0.02 min, approximately).

Tralomethrin and deltamethrin are structurally very similar (see Table 1). With relatively high temperature, tralomethrin suffers an elimination reaction. As a result of losing a bromine molecule, its structure is the same as deltamethrin, making impossible to differentiate these two pyrethroids. Under the GC conditions commonly used in pesticide residue analysis, tralomethrin breaks down to deltamethrin. It was found that this transformation occurs in the

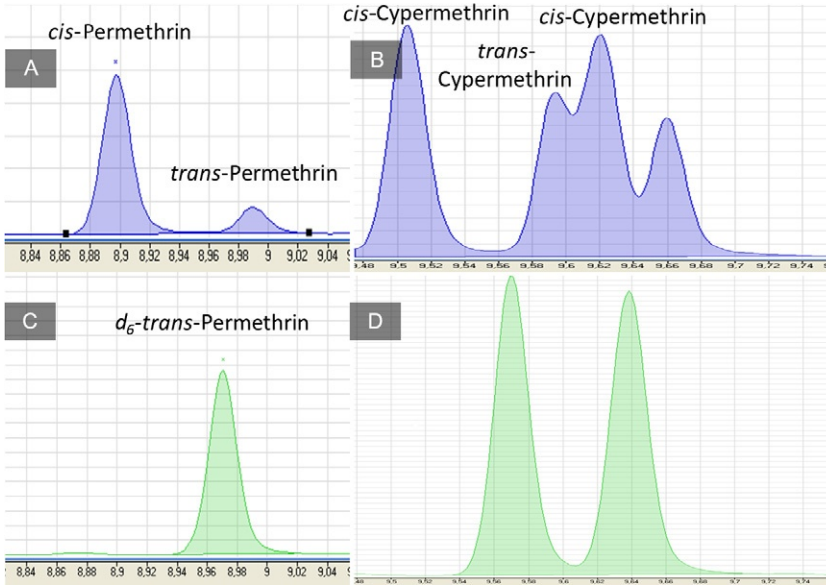


FIGURE 2 Chromatograms of (A) permethrin, (B) cypermethrin, (C) d_6-*trans*-permethrin, and (D) d_6-*trans*-cypermethrin. Isomeric *cis–trans* separation with a DB-5 column.

injector port of the GC system. In order to avoid this problem, Valverde *et al.* [52] used an LC method.

3.2 Chiral Columns

A few authors have developed enantiomeric methods to analyze separately each isomer of pyrethroids. In that sense, Kutter and Class [53] tested some columns to resolve the enantiomers of cypermethrin and allethrin. They used a Lipodex C, a CDX-8, and a DB1701. Their best methodology let them separate qualitatively the four *trans*-enantiomers of allethrin with a tandem of CDX-8 and DB1701 columns, but the *cis*-isomers were not resolved. None of the columns could resolve well those *cis*-enantiomers either. They could not resolve cypermethrin enantiomers. Liu and Gan [54,55] developed different chromatographic methods to separate cyfluthrin and cypermethrin enantiomers (Type II pyrethroids) and bifenthrin and permethrin ones (Type I pyrethroids). They tested some different chiral columns concluding that the best separation was reached with a β-cyclodextrin-coated column (BGB-172).

It is important to point out that for each pyrethroid, 2^n enantiomers (where n is the number of chiral centers) exist. Ideally, chiral chromatography is able to

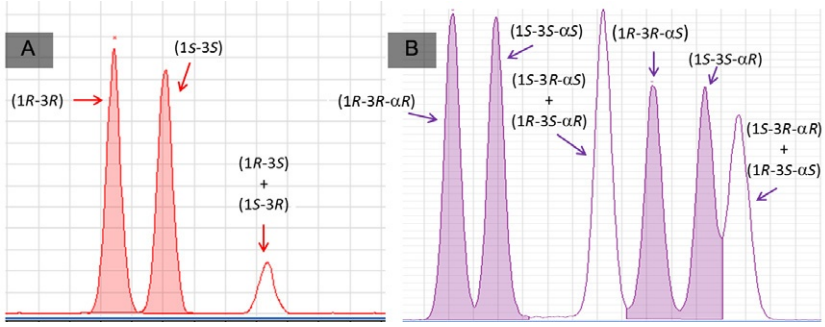


FIGURE 3 Chiral separation of (A) permethrin and (B) cypermethrin. Shaded peaks are *cis*-isomers and white ones correspond to the *trans*.

separate all the enantiomers showing 2^n peaks. However, till date, none of the authors have been able to separate quantitatively *trans*-enantiomers for any of the pyrethroids studied. This means that pyrethroids like permethrin will appear as three peaks, two peaks corresponding to *cis*-enantiomers and one peak for the two *trans*-ones. Pyrethroids like cypermethrin, that presented four peaks with achiral chromatography, showed six peaks on enantiomeric studies because of the low resolution between *trans*-enantiomers. Figure 3 shows the assignment of permethrin (A) and cypermethrin (B) enantiomers obtained with a BGB-172 column following the assignment made by Liu and Gan [54,55].

Some authors reported isomerization of pyrethroids as a result of factors such as light, head space, or polar solvents [56,57], even during GC analysis. Some of them proposed reducing the residence time in the GC inlet as a solution for that problem [58]. Enantiomers due to the chirality provided by the cyclopropyl ring are stable, but those ones due to the αC could suffer some isomer conversion at high temperatures or in water. At an inlet temperature of 260 °C, that conversion could reach up to 9%. For cypermethrin and cyfluthrin, the isomer conversion in water occurs at a slow rate [57].

Because of the difficulty of resolving pyrethroid enantiomers by GC and the potential chiral conversion, a large number of authors prefer to work with LC–MS or even with micellar electrokinetic chromatography despite their lower LODs [59].

4 MASS SPECTROMETRY DETECTION

Authors generally prefer GC-ECD for analysis of compounds which possess halogenated atoms because of high sensitivity. However, it was demonstrated that, for pyrethroids, GC–MS selectivity could get LODs at least one order of magnitude lower by reducing matrix background signal [50]. Other authors preferred increasing the sensitivity using microelectron-capture detector

(μECD) but they needed good GC resolutions, which usually means increasing the column length and the analysis time. The selectivity of MS allows the use of shorter columns and reduces substantially the time of analysis. Altogether, MS and MS–MS can achieve high sensitivities, due to their selectivity, and allow a simpler GC method.

4.1 Mass Spectrometry

Typically, analysis of pyrethroids by GC–MS uses negative chemical ionization (NCI) instead of electron ionization (EI) because of the softer fragmentation conditions. EI produces low-mass ions with the same *m/z* ratios for different pyrethroids [45]. That leads to a loss of selectivity and the requirement of improving the chromatographic separation. NCI mode can be performed with both methane and ammonia as reagent gases. Fragmentation of pyrethroids in NCI mode occurs at the ester bond and forms stabilized carboxylate ions [60].

Some authors like Feo *et al*. had compared the sensitivity of EI and NCI modes for pyrethroid analysis. For most of the pyrethroids studied, NCI (with ammonia) mode obtains instrumental LODs as far as one order of magnitude lower. This was the case of cypermethrin and λ-cyhalothrin [49]. Table 3 shows the comparison for both modes, EI and NCI. In spite of low levels of detection, a lot of authors have continued to select EI mode in recent works.

In NCI-MS mode, the *m/z* selected for monitoring the pyrethroids was the corresponding carboxylate ion. As most pyrethroids are structurally similar, the same fragment could correspond to different pyrethroids. This is the case of cypermethrin, cyfluthrin, and cypermethrin ($m/z = 207$); phenothrin, allethrin, imiprothrin, and tetramethrin ($m/z = 167$); or bifenthrin and cyhalothrin ($m/z = 205$) whose fragments correspond to the carboxylic acid moiety in all cases. For tralomethrin and deltamethrin, the selected *m/z* could be 79, corresponding to bromine ion.

Most authors used quadrupole mass spectrometers. A few used a time-of-flight (TOF) mass spectrometer [36,51]. In these cases, the authors have developed multiresidue GC × GC methods, and the speed of TOF was a requirement.

4.2 Tandem Mass Spectrometry

Some works tried to improve selectivity of pyrethroid analysis using tandem mass spectrometry techniques. As background noise is reduced, sometimes the MS^2 mode allows improvement of sensitivity as well.

Different authors selected the chemical ionization (CI) mode to work with tandem MS. The reagent gas, however, could vary. For instance, Sichilongo [31] preferred using MeOH on the quadrupole ion trap (QIT) system with the upper instrumental limit of detection (iLOD) of 0.4 ng μL^{-1} for cyfluthrin, while Bauerle *et al*. [61] used isobutene on the same QIT, getting their best iLOD in 0.05 ng μL^{-1} for allethrin.

TABLE 3 Comparison of EI and NCI (Ammonia) Modes with MS (Quadrupole)

		EI			NCI		
Compound	Rt[a] (min)	*1st MS*	*2nd MS*	*MS iLOD[b] (fg-Injected)*	*1st MS*	*2nd MS*	*MS iLOD[b] (fg-Injected)*
cis-Bifenthrin	7.99	181	166	46	205	141	24
λ-Cyhalothrin	8.87	197	141	225	205	141	17
Cyfluthrin	9.43	226	206	283	207	171	62
Cypermethrin	9.65	163	127	528	207	171	17
Deltamethrin (tralomethrin)	10.88	253	79	450	79	137	375
Fenvalerate	10.28	167	125	375	211	167	13
Fluvalinate	10.43	250	208	190	294	250	79
Permethrin	9.03	183	165	45	207	171	1122
Resmethrin	7.68	123	81	324	337	149	50
Tetramethrin	7.92	164	77	285	331	167	104

[a]*Retention time of first isomer.*
[b]*Instrumental limit of detection, calculated as average of single LODs of each isomer.*
Adapted from Feo *et al.* [49].

Esteve-Turrillas *et al.* [37] chose EI mode in QIT as well. Their LODs for vegetable oil samples ranged from 1 to 5 ng mL^{-1}, improving previous works of other authors with CI. This improvement could be due to selecting the negative ion mode, in spite of the extended use of positive mode.

Feo *et al.* [49] used triple quadrupole (QqQ). In their work, they compared EI and NCI modes and finally chose NCI with ammonia as reagent gas. They proved good iLODs and tested their method with different matrices such as water, sediment, and milk with LODs ranging from 0.07 to 2.31 ng L^{-1}, from 0.0001 to 0.081 ng g^{-1}, and from 0.003 to 0.7 ng g^{-1}, respectively.

It is important to note that the work of Feo *et al.* is much more recent (2011) than the others (2005). And because of this, probably they used newer instrumentation, which was more sensitive. Despite that, those studies confirm the selection of negative ion mode as the best one. Moreover, NCI seems to be more sensitive than EI, as well for MS–MS, as it is shown in Table 4.

TABLE 4 Comparison of Different MS–MS Modes with QIT (CI Mode (MeOH) MS–MS (QIT) (Adapted from Sichilongo [31]) and EI Mode MS–MS (QIT) (Adapted from Esteve-Turrillas *et al.* [37])) and with QqQ (EI and NCI with Ammonia (Adapted from Feo *et al.* [49])).

	CI (QIT)			EI (QIT)			EI (QqQ)			NCI (QqQ)		
Compound	*1st MS→MS*[a]	*2nd MS→MS*[a]	*iLOD (pg-Injected)*	*1st MS→MS*[a]	*2nd MS→MS*[a]	*iLOD (pg-Injected)*	*1st MS→MS*	*2nd MS→MS*	*iLOD (fg-Injected)*	*1st MS→MS*	*2nd MS→MS*	*iLOD (fg-Injected)*
Bifenthrin	361→344		340	181→166		1	181→166	181→165	87	205→141	205→121	0.2
Cyfluthrin	226→206		400	226→199		3	197→141	197→161	187	205→141	205→121	0.2
λ-Cyhalothrin	–		–	197→141		3	226→206	226→199	229	207→35	209→35	2
Cypermethrin	208→181		160	163→127		4	163→127	163→91	64	207→35	209→35	2
Deltamethrin	–		–	172→77		5	253→172	253→174	300	297→79	297→81	450
Esbiol (allethrin)	303→285		160	–		–	–	–	–	–	–	–
Esfenvalerate	167[b]	–	180	225→147		2	167→125	167→139	27	211→167	213→169	0.4
Flucythrinate	412→413		340	225→147		2	–	–	–	–	–	–
Fluvalinate	503→457		170	250→200		2	250→200	250→208	190	294→250	294→194	64
Permethrin	365→183		140	183→165		2	183→168	183→165	349	207→35	209→35	6
D-*t*-phenothrin	351→333		140	183→165		4	–	–	–	–	–	–
Resmethrin	–	–	–	–	–	–	123→81	123→93	617	337→149	337→187	7
Tetramethrin	314→164		110	164→77		5	164→77	164→107	106	331→167	–	45

[a]*Transition selection based on the given relative abundance of fragments.*
[b]*No transition found.*

5 LEVELS OF REAL SAMPLES

5.1 Environmental Samples

In river water samples, cypermethrin, deltamethrin, and cyhalothrin were found in Portugal at levels of 553, 276, and 282, respectively [62]. Feo *et al.* [63] found levels of cypermethrin up to 30 ng L^{-1} and deltamethrin up to 59 ng L^{-1} in a Spanish river. They suggest the relationship between levels found and the pesticide application period. In sediments of the same Spanish river, they found the same pyrethroids as well. Previous reports of river waters in Beijing showed the presence of the same pyrethroids and fenvalerate as well, reaching levels of 4.30 ng L^{-1} [21]. In California, Weston and Lydy reported levels of bifenthrin and cyhalothrin up to 17.5 ng L^{-1} [64]. In sediments, You *et al.* [65] found levels between 1.48 and 365.5 ng g^{-1} in California; Xue *et al.* [21], in Beijing, found levels up to 0.30 ng g^{-1}, and in Spain, levels from 8.27 to 71.9 ng g^{-1} were reported [63].

In indoor air samples, pyrethroids were detected after known use of domestic insecticides, so levels found depended on the specific use and it was usual to find them at different levels [28].

5.2 Biological Samples

In seaweeds, levels of tetramethrin, empenthrin, and cypermethrin were described. However, all levels were very close to limits of quantification (LOQs) and below the maximum residue levels established by the European Union [35].

Alonso *et al.* [6] studied the presence of pyrethroids in dolphins. They found an interesting relationship between levels and the length of the animal, directly related to the age. Figure 4 shows graphically the relation they found. In calves, they reported a maximum concentration of 68.4 ng g^{-1} lipid weight

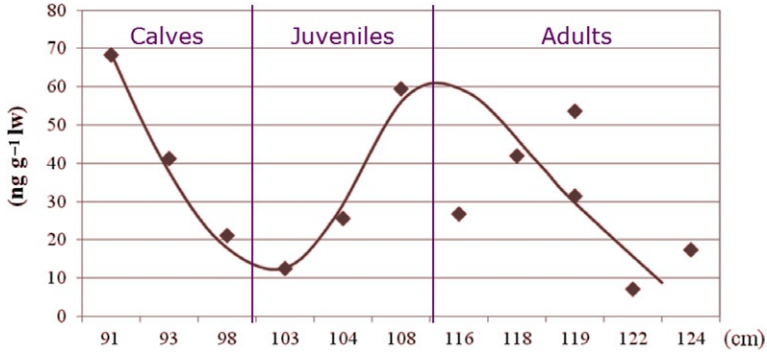


FIGURE 4 Levels of pyrethroids in dolphins versus length. Classification of calves, juvenile, and adult dolphins. *Adapted from Alonso* et al. *[6].*

(lw) corresponding to the youngest calf, and the minimum value was 7.04 ng g^{-1} lw for the oldest adults. Their explanation for this behavior implies the mother transference of pyrethroid contamination via placenta and breast milk and metabolic changes with age. Their assumption seems to be supported by the levels found in placenta and breast milk (up to 1812 and 4.77 ng g^{-1}, respectively).

In human samples, Channa *et al*. [38] reported levels of permethrin, cyfluthrin, cypermethrin, and deltamethrin up to 86, 78, 18, and 102 pg mL^{-1} in women plasma from rural regions of South Africa. Ostrea *et al*. [66] did not find levels of cyfluthrin and cypermethrin above the LOQ neither in hair nor in blood. They only found levels up to 3.98 μg mL^{-1} in meconium with only 2.8% of samples being positive.

Zehringer and Herrmann [42] were the first authors to report levels of pyrethroids in human breast milk. They found levels of allethrin, bifenthrin, cyfluthrin, cyhalothrin, cypermethrin, fenpropathrin, flucythrinate, fluvalinate, permethrin, and tetramethrin, permethrin being the most prominent and reaching a maximum value of 152 μg kg^{-1} of lw. Their study was carried out in Switzerland. Bouwmann *et al*. [43] studied South African women, finding permethrin, cyfluthrin, deltamethrin, and cypermethrin in decreasing order of prevalence. Levels ranged from nondetected to 13.9 μg g^{-1} lw. Feo *et al*. [44] analyzed breast milk of mothers of Mozambique and found levels of cyhalothrin, permethrin, fenvalerate, cypermethrin, tetramethrin, bifenthrin, and cyfluthrin in that order of importance. Levels were up to 695 ng g^{-1} lw. These studies in Africa point out the presence of pyrethroids in human breast milk due to the intensive use of these insecticides in agriculture, domestic uses, and malaria control.

Finally, Corcellas *et al*. [67] studied the presence of 13 pyrethroids in breast milk from Brazil, Colombia, and Spain in nontarget populations exposed to pyrethroids. They found the presence of pyrethroids in all samples. Cypermethrin, permethrin, cyhalothrin, and fenvalerate were the pyrethroids present in most of the samples. However, resmethrin, fluvalinate, cyfluthrin, and phenothrin were not found in any sample. The levels ranged from 1.45 to 24.2 ng g^{-1} lw. Even when they did not find statistical differences between levels in each country, they showed a different pattern of pyrethroid mixture depending on the sample origin. Their study pointed out the potential relationship between parity and pyrethroid levels, as well as a slight tendency to decrease levels over time. Moreover, these authors related the isomeric specificity to accumulation. As a conclusion, all these authors agreed that levels of pyrethroids in human breast milk were under maximum safe values.

5.3 Enantiomeric Results

GC–MS enantiomeric studies on real samples are scarce. Liu and Gan [54] analyzed water samples from a runoff discharge channel at a nursery site in

California. They found that the concentration of 1*R*-3*R* enantiomers of *cis*-bifenthrin and *cis*-permethrin were consistently higher than the 1*S*-3*S* enantiomer.

Kuang *et al*. [68] determined the enantiomeric fractions of cypermethrin and *cis*-bifenthrin in Chinese teas. For *cis*-bifenthrin, they found enantiomeric factors (EF, calculated as the area of one enantiomer divided by the sum of all enantiomers) around 0.5, meaning that there is no enantiomeric preference for one enantiomer. However, for cypermethrin, they found deviations of the EF values from the standard values for specific samples, even when the mean values were not so different. They suggest that the different fermentations and temperatures of each tea treatment could affect the chirality of these pyrethroids, but more studies are needed to understand that phenomenon.

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ABBREVIATIONS

ACN	acetonitrile
CI	chemical ionization
DCM	dichloromethane
DT_{50}	typical degradation time to 50%
EC_{50}	half-maximal effective concentration
ECD	electron-capture detector
EF	enantiomeric factors
EI	electron impact
EPA	Environmental Protection Agency
FAO	Food and Agriculture Organization
GC	gas chromatography
GC × GC	multidimensional GC
GPC	gel permeation chromatography
Hx	hexane
IL	ionic liquid
iLOD	instrumental limit of detection
K_{OW}	partition coefficients octanol/water
LC_{50}	lethal concentration for half of the population
LLE	liquid–liquid extraction
LOD	limit of detection
LOQ	limit of quantification

MASE	microwave-assisted solvent extraction
MeOH	methanol
MS	mass spectrometry
MSPD	matrix solid-phase dispersion
NCI	negative chemical ionization
PLE	pressured liquid extraction
QIT	quadrupole ion trap
QqQ	triple quadrupole
QuEChERS	quick, easy, cheap, effective, rugged, and safe
SBSE	stir-bar sorptive extraction
SFE	supercritical fluid extraction
SPE	solid-phase extraction
SPME	solid-phase microextraction
TOF	time of flight
UAEE	ultrasound-assisted emulsification extraction
WHO	World Health Organization
μECD	microelectron-capture detector

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Chapter 10

GC–MS–MS for the Analysis of Phytoestrogens in the Environment

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1 INTRODUCTION

Phytoestrogens are a group of nonsteroidal polyphenolic compounds that occur naturally in a wide range of plants and induce biological responses based on their ability to bind to estrogen receptors. Usually, they are present at high concentrations in legumes such as soy, clover, alfalfa, beans, and peas [1,2]. Beneficial effects of flavonoids (water-soluble plant pigments derived from the 2-phenyl-1,4-benzopyrone structure) on humans have been reported

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in recent reviews [3–6] and include their ability to scavenge free radicals [5,7] and to have immunomodulatory [4] effects. However, plant phytoestrogens have been also cited as possible endocrine disruptors in fish along with synthetic hormones and human estrogens from wastewater effluents [8]. Additionally, phytoestrogens, especially daidzein, genistein, and glycitein, have been correlated with low sperm counts in North American men with high and frequent soy food intake, more than twice per week [9]. Because phytoestrogens are excreted, not only by plants but also by humans and livestock via food consumption, it is important to evaluate their sources in food and their concentration in surface water and wastewater. Previous studies have found concentrations of biochanin A, daidzein, formononetin, and coumestrol ranging from 1 to 10 ng/L in rivers in Australia, Germany, and Italy [10–13], whereas 43 μg/L daidzein and 143 μg/L genistein were detected in a Japanese river [8], perhaps because of higher soy impact.

Several analytical methodologies have been described for the identification and quantitation of flavonoids, including gas chromatography [14–16] and liquid chromatography [17–19]. In fact, an extensive recent review by de Rijke *et al*. [20] reports the advantages and disadvantages of these methodologies for the analysis of flavonoid compounds. Using LC–MS-based methods, matrix interferences have been reported [19], which make quantitation of phytoestrogens difficult, especially in those cases where the matrix is complex, such as wastewater samples. Because of potential matrix interferences the use of selective ion-trap or tandem mass spectrometric methodologies (either LC/MS–MS or GC/MS–MS) is crucial to identify and confirm the presence of these analytes in complex samples. In general, GC-based methods provide high chromatography resolution and low-detection limits for the phytoestrogens, although they require derivatization to form the trimethylsilyl ether derivatives that increases volatility and improves thermal stability. Usually, MS–MS techniques that look at two or more characteristic fragments are required to fully characterize the silanized phytoestrogens since they provide higher selectivity than single ion monitoring techniques [21].

In this study, two GC–MS–MS methodologies using triple quadrupole and ion-trap mass spectrometry were developed for the identification and confirmation of eight phytoestrogens in soy products and water samples from a wastewater treatment plant in Boulder, CO. The wastewater from this plant has been under study for endocrine disrupting effects on fish downstream of the plant [22]. The concentration of natural plant phytoestrogens in wastewater in this study area is currently unknown and is a valuable data set for future biological studies of plant phytoestrogenic effects on native fish, especially given the wide occurrence of plant indicators in wastewater, such as beta sitosterols [23].

The phytoestrogens were analyzed both in soy milk and wastewater using the characteristic fragmentation of the molecular ions for each of the analytes studied. An extensive study on the fragmentation patterns of each analyte was

carried out in this study and it is described here in detail. Finally, the principal phytoestrogens occurring in commercial soy-milk and the influent and effluent wastewater from a soy-milk plant are reported and discussed.

2 EXPERIMENTAL

2.1 Chemicals and Reagents

Biochanin A, coumestrol, daidzein, equol, formononetin, genistein, glycitein, and prunetin were purchased from Sigma–Aldrich (St. Louis, MO, USA). Isotopic labeled compounds daidzein-d_4 and genistein-d_4 were obtained from Cambridge Isotopes (Cambridge, MA, USA). The derivatization reagents: *N*, *O-bis*(trimethylsilyl)trifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS) were obtained from Sigma–Aldrich (St. Louis, MO, USA) and 500 μL of TMCS was added to 5 mL of BSTFA in order to have a 10% TMCS derivatization reagent. In the same way, 2 mL of pyridine were mixed with 8 mL of BSTFA to form a BSTFA/Pyridine (5:1; v:v) solvent mixture that is added to the dry extracts before injection onto the GC. Fresh derivatizating reagents were prepared every 2 weeks. Methanol, ethyl acetate, and dichloromethane were obtained from Burdick and Jackson (Muskegon, MI, USA). All materials and reagents were of analytical purity.

Standards of individual phytoestrogens were prepared in methanol at concentrations of 500 mg/L. From these solutions, diluted working solutions were prepared to be derivatized and analyzed by GC–MS–MS. All standard solutions were stored at −20 °C and allowed to equilibrate at ambient temperature for at least 2 h before use. Calibration samples were prepared by mixing different volumes of stock mixture (4–20 μL) and internal-standard mixture (20 μL).

2.2 Derivatization Procedure and Sample Preparation

A modified version of a previously reported methodology for the derivatization of estrogen compounds [22] was used here. The analytes were derivatized to their trimethylsilyl ethers with BSTFA and TMCS. The calibration standards were evaporated to dryness under a stream of nitrogen in silanized 5-mL reaction vials. Then, 200 μL of 10% TMCS/BSTFA derivatizing reagent was added to the vials and vortexed for 15 s. The vials were placed on a heating block at 60 °C for 4 h. After this time, the vials were removed from the heating block, set aside 15 min to cool, and the reagents were evaporated to dryness under nitrogen. Finally, the dry residue was dissolved in 200 μL of 5:1 BSTFA/pyridine injection solvent and vortexed for 30 s. Daidzein-d_4 and genistein-d_4 were used as internal standards before extraction and derivatization procedure and they were added together with the

calibration standards. The derivatized extracts were transferred into autosampler vials using a clean silanized glass pipette and were analyzed by GC–MS–MS.

A simple and rapid procedure for the isolation of the phytoestrogens from soy milk and water was carried out using ethyl acetate as an extracting solvent. The soy-milk sample (1 mL) and the water and wastewater samples (5 mL) were liquid–liquid extracted with 15 mL of ethyl acetate (two times with 7.5 mL each) and evaporated to dryness under a stream of nitrogen. The internal standards, daidzein-d_4 and genistein-d_4 were added before extraction to account for any loses during the extraction process. The residues were derivatized with TMCS and BSTFA/pyridine, at 60 °C for 4 h.

2.3 GC–MS–MS Instrumentation

Two different tandem GC–MS types of instrumentation were used for the identification of phytoestrogens: a triple quadrupole and an ion trap. For the soy-milk analyses, the triple quadrupole instrument was used and for the analyses of wastewater samples the ion-trap instrument was used.

The GC–MS–MS analyses were performed using an Agilent Model 7890 gas chromatograph coupled to a triple quadrupole mass spectrometer, Agilent Model 7000A (Agilent, Santa Clara, CA, USA). The chromatographic separation was performed using a HP-5 (5% phenyl, 95% methylpolysiloxane), 30 m × 0.25 mm i.d., fused-silica capillary column (Agilent Technologies, Santa Clara, CA, USA) of 0.25-μm film thickness. The carrier gas was helium at a constant flow rate of 1.2 mL/min held by electronic pressure control. Injector temperature was 280 °C, and splitless injection mode was used. The oven temperature program was 100 °C (held for 1 min) to 240 °C at 40 °C/min (held for 1 min), to 300 at 10 °C/min (held for 4 min). The MS operating conditions were the following: positive electron ionization mode (EI+) using automatic gain control (AGC) with an electron energy of −70 eV. The ion source temperature was 300 °C. Gain voltage was set to 30. A dwell time of 50 ms was used for every multiple reaction monitoring (MRM) transition. One microliter of the extracts was injected on the system. MassHunter software was used for control, general operation, and data acquisition of the results.

The GC ion-trap MS analyses were carried out with a Trace GC 2000 gas chromatograph coupled to a GCQ/Polaris ion-trap mass spectrometer (Thermo-Finnigan, Austin, TX, USA) equipped with an AS2000 autosampler (ThermoElectron, Milan, Italy). The chromatographic separation was performed using a DB-5MS (5% phenyl, 95% methylpolysiloxane), 25 m × 0.25 mm i.d., fused-silica capillary column (J&W Scientific, Folsom, CA, USA) of 0.25-μm film thickness. The carrier gas was helium at a constant flow rate of 1.2 mL/min held by electronic pressure control. Injector

temperature was 280 °C, and splitless injection mode was used. The oven temperature program was 80 °C (held for 2 min) to 230 °C at 40 °C/min (held for 1 min), to 300 at 4 °C/min (held for 3 min) and finally to 310 °C at 10 °C/min (held for 1 min). The MS operating conditions were the following: EI+ using AGC with an electron energy of 70 eV. The ion source and transfer line temperatures were 200 °C and 280 °C, respectively. The instrument was tuned using perfluorotributylamine (FC-43) according to manufacturer's recommendations in order to achieve the best sensitivity. Electron multiplier voltage was set to 1500 V by automatic tuning. EI full-scan data acquisition was registered over the range *m/z* 50–500 at 0.66 s per scan. One microliter of the extracts was injected on the system. Xcalibur version 1.2 software was used for control, general operation, and data acquisition of the results.

3 GC–MS–MS ANALYSES

3.1 Optimization of Derivatization and GC–MS Detection

Phytoestrogens are polar, nonvolatile compounds and therefore not well suited to GC analysis in their native form. In order to make these compounds more amenable to GC–MS analysis, they must first be derivatized to more volatile, less polar species. This is usually done by derivatizing the hydroxyl groups with suitable protecting groups. The derivatization procedure described in Section 2 and used in this work has been already reported for hormone compounds by Vajda *et al*. [22], and it was successfully applied here to the phytoestrogens. However, a slightly modification was included in this work, which was the removal of *O*-methoxyamine hydrochloride that was not necessary because the chemical structure of the phytoestrogens does not contain an adjacent keto group to a double bond in the ring, so no blocking of the carboxyl groups is necessary for the derivatization of these compounds. The derivatization time needed for the complete formation of the trimethylsilyl derivatives also was optimized in this study. Several reaction times were studied, and it was concluded that a step of 4-h derivatization yielded best efficiency (data not shown) in the least time for the formation of the trimethylsilyl derivatives for all the phytoestrogens.

Table 1 shows the chemical structures for each of the trimethylsilyl derivatives of the phytoestrogens studied along with the *m/z* values for the molecular ions or main precursor ions detected by GC–MS scan. The deuterated standards, daidzein-d_4 and genistein-d_4, are also shown. All the compounds presented the molecular ion as a base peak in the spectrum except for biochanin A, genistein, and prunetin. In these cases, the $[M-CH_3]^{+\bullet}$ ion was observed, probably due to the closer position of the trimethylsilyl to the keto group in the chemical moiety, making this methyl group more susceptible to be fragmented in the EI source. The rest of compounds exhibited the

TABLE 1 Formula, Precursor Ion Chosen, and Chemical Structures of the BSTFA Derivatives of the Phytoestrogens Studied

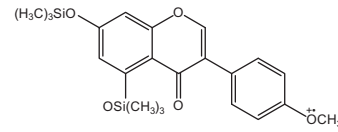
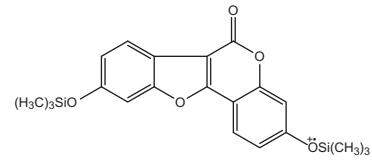
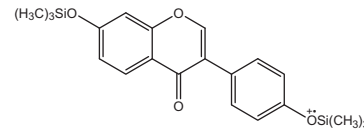
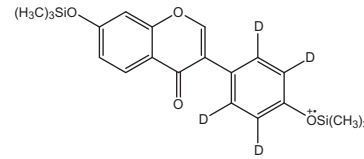
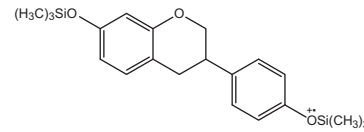
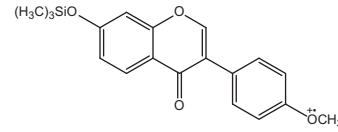
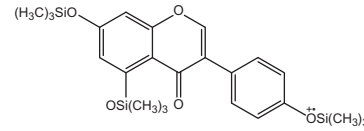
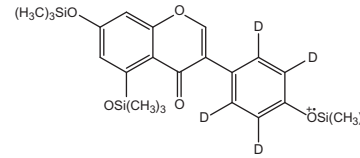
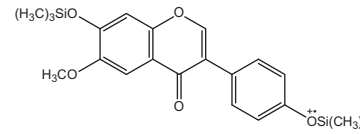
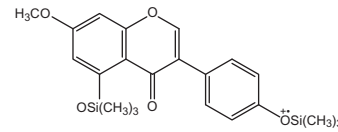
Name	Formula	M^+ or $M^{+\cdot}$	Chemical Structures
Biochanin A	$C_{22}H_{28}O_5Si_2^{+\cdot}$ *m/z* 428	413	
Coumestrol	$C_{21}H_{24}O_5Si_2^{+\cdot}$ *m/z* 412	412	
Daidzein	$C_{21}H_{26}O_4Si_2^{+\cdot}$ *m/z* 398	398	
Daidzein-d_4	$C_{21}H_{22}D_4O_4Si_2^{+\cdot}$ *m/z* 402	402	
Equol	$C_{21}H_{30}O_3Si_2^{+\cdot}$ *m/z* 386	386	
Formononetin	$C_{19}H_{20}O_4Si^{+\cdot}$ *m/z* 340	340	
Genistein	$C_{24}H_{34}O_5Si_3^{+\cdot}$ *m/z* 486	471	

TABLE 1 Formula, Precursor Ion Chosen, and Chemical Structures of the BSTFA Derivatives of the Phytoestrogens Studied—Cont'd

Name	Formula	M^+ or $M^{+\cdot}$	Chemical Structures
Genistein-d_4	$C_{24}H_{30}D_4O_5Si_3^{+\cdot}$ *m/z* 490	475	
Glycitein	$C_{22}H_{28}O_5Si_2^{+\cdot}$ *m/z* 428	428	
Prunetin	$C_{22}H_{28}O_5Si_2^{+\cdot}$ *m/z* 428	413	

molecular ion as a major or base peak ion in the mass spectrum. The molecular ions shown in this table were the ones chosen for the subsequent selective MS–MS experiments, as detailed in the section below.

3.2 Optimization of MS–MS Conditions

3.2.1 Triple Quadrupole GC–MS–MS

For GC–MS–MS analyses using the triple quadrupole, each compound was optimized for MS–MS analysis to determine collision energies for both the quantifying and qualifying MRM ion transitions. Collision energies varied between 10 and 40 V. The optimized MRM transitions used for this study are shown in Table 2. This table shows the precursor ions and the main fragment ions for all the analytes studied. In some cases, the fragmentation of the trimethylsilyl group is involved such as for genistein and prunetin. In other cases, either a methyl or a methyl and a carbonyl group are lost and rearrangement of the structure occurs. From an analytical point of view, it is of primary importance to select those fragment ions that can be used for quantitative and

TABLE 2 MRM Transitions and MS Operating Parameters Selected for the Analysis of the Phytoestrogens Studied Using a Triple Quadrupole GC–MS–MS

Compound	MRM Transitions (m/z)	Collision Energy (eV)
Biochanin A	413 > 370	30
	413 > 341	30
Coumestrol	412 > 397	20
	412 > 369	20
Daidzein	398 > 383	20
	398 > 355	30
Daidzein-d_4	402 > 387	20
	402 > 359	30
Equol	386 > 207	10
	386 > 192	10
Formononetin	340 > 325	10
	340 > 297	20
Genistein	471 > 399	30
	471 > 327	40
Genistein-d_4	475 > 403	30
	475 > 331	40
Glycitein	428 > 413	10
	428 > 398	20
Prunetin	413 > 370	30
	413 > 341	30

confirmatory purposes and typically do not involve the loss of the TMS (trimethylsilyl) group. For this reason and based on the relative abundance, when possible two selective fragment ions were chosen for each analyte. Only genistein incurred two losses of 72 mass units consistent with two losses of the TMS group and did not show the characteristic loss of a methyl radical and a CO group. Because of the noncharacteristic double loss of 72 mass units (other derivatized compounds could exhibit this same loss), care was taken in the exact assignment of retention time for a correct assignment of genistein.

TABLE 3 Precursor Ions and Main Product Ions Obtained by Ion-Trap GC–MS–MS for the Phytoestrogens Studied

Name	Formula	M^+ or $M^{+\cdot}$	Fragment Ions
Biochanin A	$C_{22}H_{28}O_5Si_2^{+\cdot}$ *m/z* 428	413	398 $[M^+ - (^{\cdot}CH_3)]^{+\cdot}$ 370[a] $[M^+ - (^{\cdot}CH_3)—CO]^{+\cdot}$
Coumestrol	$C_{21}H_{24}O_5Si_2^{+\cdot}$ *m/z* 412	412	397[a] $[M^{+\cdot} - (^{\cdot}CH_3)]^+$ 369 $[M^{+\cdot} - (^{\cdot}CH_3)—CO]^+$
Daidzein	$C_{21}H_{26}O_4Si_2^{+\cdot}$ *m/z* 398	398	383 $[M^{+\cdot} - (^{\cdot}CH_3)]^+$ 355[a] $[M^{+\cdot} - (^{\cdot}CH_3)—CO]^+$
Daidzein-d_4	$C_{21}H_{22}D_4O_4Si_2^{+\cdot}$ *m/z* 402	402	387 $[M^{+\cdot} - (^{\cdot}CH_3)]^+$ 359[a] $[M^{+\cdot} - (^{\cdot}CH_3)—CO]^+$
Equol	$C_{21}H_{30}O_3Si_2^{+\cdot}$ *m/z* 386	386[a]	No fragment ions detected in MS–MS, only $M^{+\cdot}$
Formononetin	$C_{19}H_{20}O_4Si^{+\cdot}$ *m/z* 340	340	325 $[M^{+\cdot} - (^{\cdot}CH_3)]^+$ 296[a] $[M^{+\cdot} - (^{\cdot}CH_3)—^{\cdot}HCO]^{+\cdot}$
Genistein	$C_{24}H_{34}O_5Si_3^{+\cdot}$ *m/z* 486	471	399[a] $[M^+ - Si(CH_3CH_3CH_2)]^+$ 327 $[M^+ - (Si(CH_3CH_3CH_2))_2]^+$
Genistein-d_4	$C_{24}H_{30}D_4O_5Si_3^{+\cdot}$ *m/z* 490	475	403[a] $[M^+ - Si(CH_3CH_3CH_2)]^+$ 331 $[M^+ - (Si(CH_3CH_3CH_2))_2]^+$
Glycitein	$C_{22}H_{28}O_5Si_2^{+\cdot}$ *m/z* 428	428	413 $[M^{+\cdot} - (^{\cdot}CH_3)]^+$ 398[a] $[M^{+\cdot} - (^{\cdot}CH_3)_2]^{+\cdot}$
Prunetin	$C_{22}H_{28}O_5Si_2^{+\cdot}$ *m/z* 428	413	370[a] $[M^+ - (^{\cdot}CH_3)—CO]^{+\cdot}$ 341 $[M^+ - Si(CH_3CH_3CH_2)]^+$

See Figure 1 for complete fragmentation pathways from the molecular ion.
[a]*Ion used for quantitation.*

3.2.2 *Ion-Trap GC–MS–MS*

Fragmentation patterns were studied for all the phytoestrogens under different voltages using the ion-trap GC–MS system. An optimization of the collision voltage in the trap was carried out ranging from 3 to 10 V. The majority of compounds fragmented easily yielding at least two characteristic fragment ions, at a voltage of 6 V. However, daidzein, genistein, and prunetin required a higher fragmentation voltage of 9 V to achieve good sensitivity for the fragment ions. Only one compound, equol, did not fragment under any conditions, due to the high stability of this molecule related to the symmetry of this phytoestrogen. Table 3 shows the precursor ions and the main fragment ions for all the analytes studied. In some cases, the fragmentation of the trimethylsilyl group is involved such as for genistein and prunetin. In other cases, either a

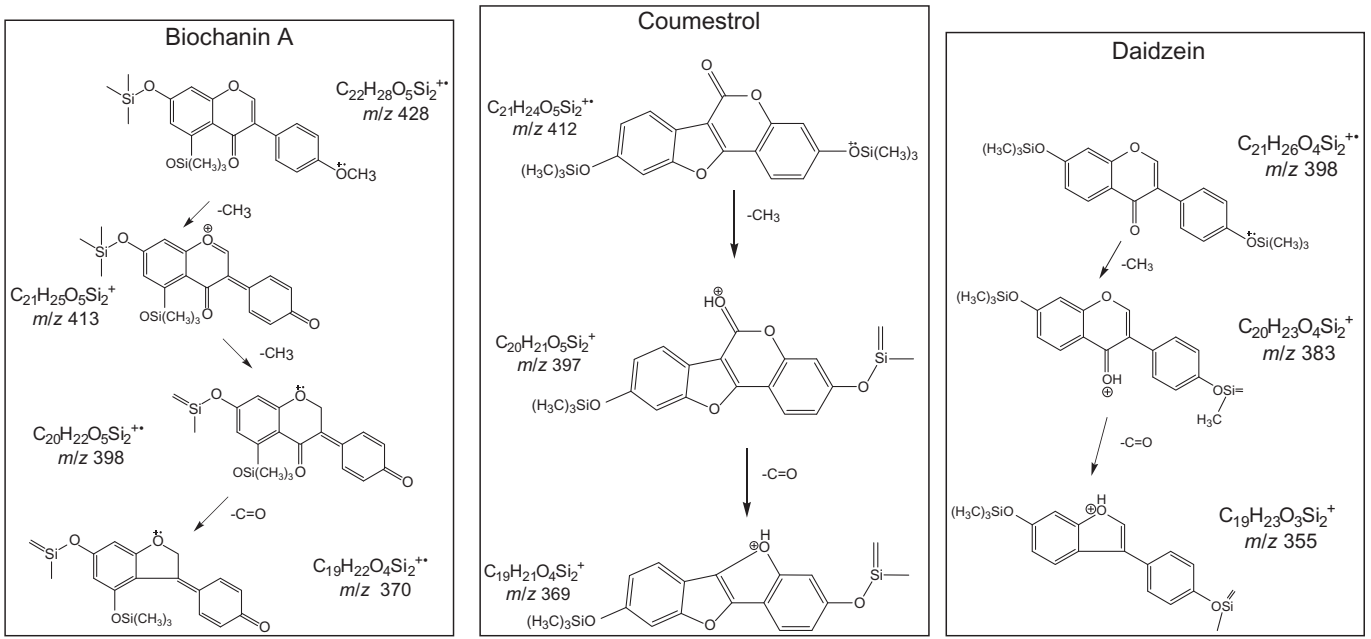


FIGURE 1 Fragmentation pattern of the phytoestrogens studied by GC–MS–MS.

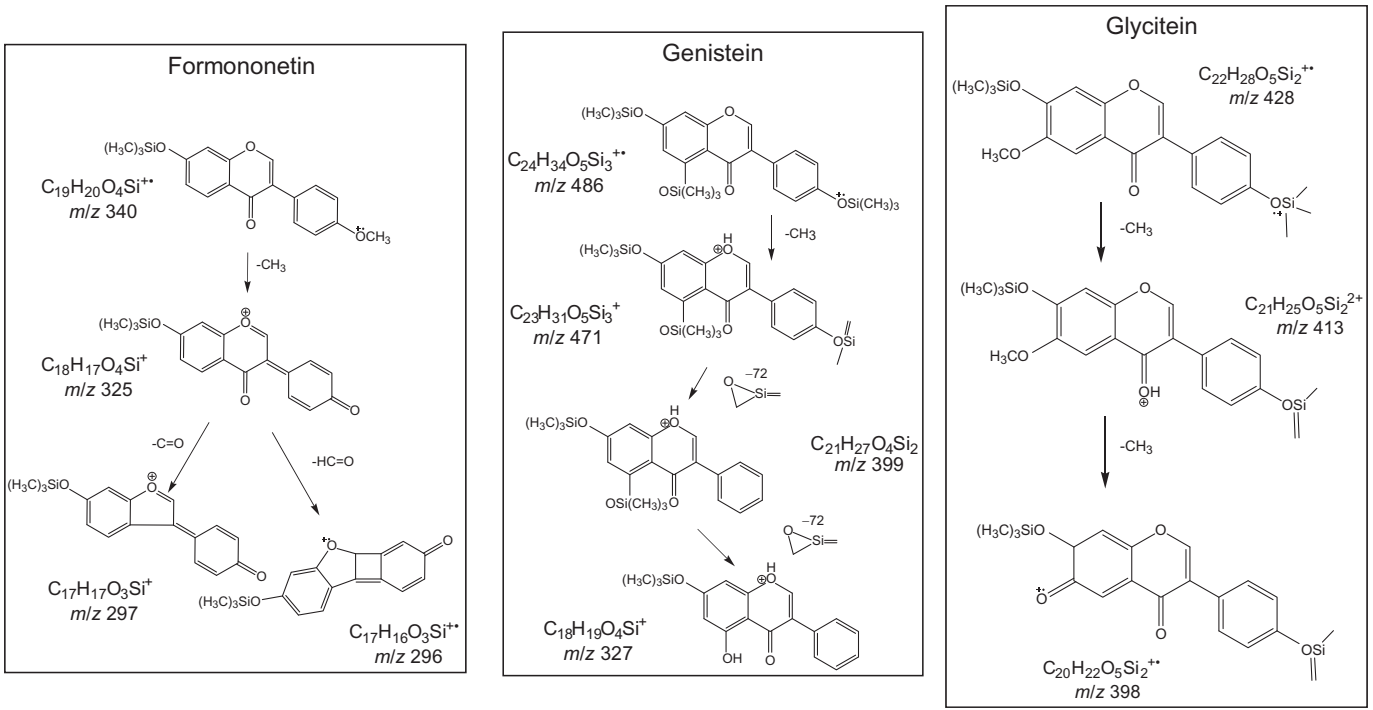


FIGURE 1—Cont'd

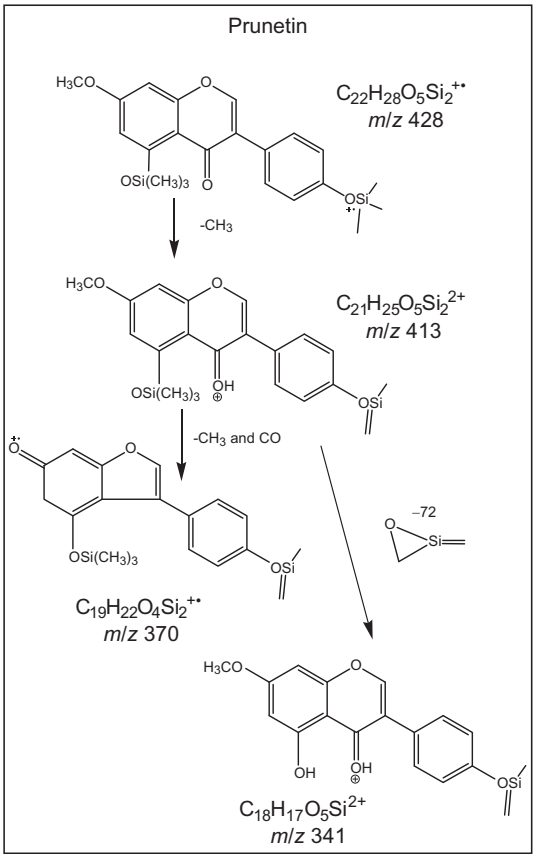


FIGURE 1—Cont'd

methyl or a methyl and a carbonyl group are lost and rearrangement of the structure occurs, similar to the fragmentation observed using a triple quadrupole instrument.

Figure 1 shows the detailed fragmentation pattern of the group of phytoestrogens. Based on their relative abundance, two selective fragment ions were chosen for each analyte for ion-trap GC–MS analyses. One of them (having the highest abundance) was used for quantitation purposes (shown with an "a" in Table 3). And the second one was used as a qualifier ion for identification and confirmatory purposes. Equol was the only compound that would not fragment and no fragments could be obtained, so in this case only the molecular ion was used for both quantitation and confirmation. Thus, caution must be used in interpretation of the analysis for this compound more than any of the other phytoestrogens when using an ion trap.

3.3 Chromatographic Separation of the Phytoestrogens

3.3.1 Triple Quadrupole GC–MS–MS

GC–MS chromatographic conditions were optimized such that baseline resolution was achieved between all analytes while also keeping an adequate run time (16 min) so as to maximize the throughput of samples. Because of the similar polarity exhibited by all of the derivatized phytoestrogens, the mix of compounds was separated using a slow temperature program (4 °C/min) in the GC–MS oven. Figure 2 shows an MRM chromatograms corresponding to 50-ppb standard mix of all the phytoestrogens studied. Extracted ion chromatograms are overlaid for each one of the target analytes according to their respective quantifying MRM transition. Good chromatographic separation was obtained with the gradient reported in Section 2.

As can be observed in this figure, the BSTFA derivatives eluted in an order depending on the number of trimethylsilyl groups substituted, and the position of other groups in the molecule such as methoxy and carbonyl groups. In general, the compounds eluted in the order of one, two, and three trimethylsilyl groups substituted with the exception of coumestrol, which has a different chemical structure with four rings and thus presents a later retention time than the other phytoestrogens. Glycitein was the other exception being the last eluting compound of the mix since the ortho/meta position of the trimethylsilyl and the methoxy groups gives this molecule a highly hydrophobic nature. Equol eluted first because it was the only compound lacking the keto group in the middle aromatic ring. Formononetin, with only one trimethylsilyl group eluted second, followed by biochanin A, prunetin, and daidzein with two trimethylsilyl groups, and finally, genistein with three trimethylsilyl groups, which was the last one of this group of three member ring phytoestrogens.

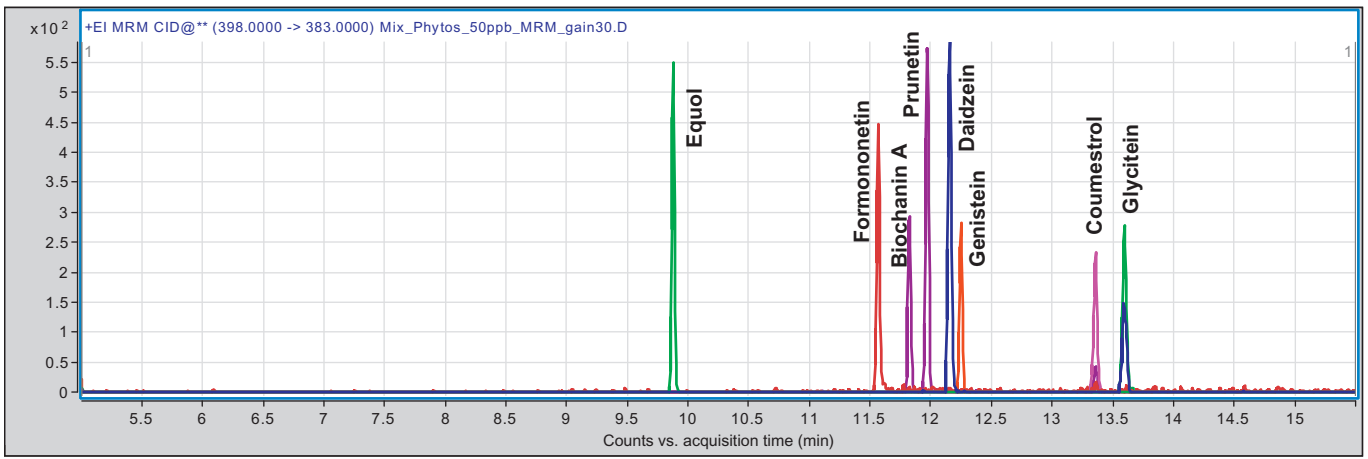


FIGURE 2 MRM extracted chromatogram for the eight phytoestrogens at 50 ppb concentration, using a triple quadrupole GC–MS–MS. Extracted ion chromatograms for the quantifier transition are shown.

3.3.2 Ion-Trap GC–MS–MS

A similar temperature program was used for ion-trap GC analyses. A comparable separation of all the phytoestrogens was achieved (results not shown here) [24].

3.4 Analytical Performance

3.4.1 Triple Quadrupole GC–MS–MS

Calibration charts were plotted for concentrations of 0.1, 0.5, 1, 5, 10, 50, and 100 µg/L of standard solutions with constant amounts of internal standards. The charts were found to be linear between 1 and 100 µg/L for all the analytes. An example is shown in Figure 3 for glycitein. Values for the coefficient of determination, R^2, were >0.99. Calculated instrumental limits of detection (LOD's) for all analytes are shown in Table 4. The lowest LOD's were for daidzein and prunetin. Formononetin showed the lowest sensitivity (i.e., signal strength/concentration) and consequently produced a higher LOD. Intra- and interassay coefficients of variation (CV, $n = 3$) for the standards ranged from 2% to 8%, showing good reproducibility of the methodology.

3.4.2 Ion-Trap GC–MS–MS

Calibration charts were plotted for concentrations of 0.05, 0.1, 0.25, 0.5, 1, and 2.5 µg/mL of standards with constant amounts of internal standards. The charts were found to be linear between 0.1 and 2.5 µg/mL for all the analytes. Values

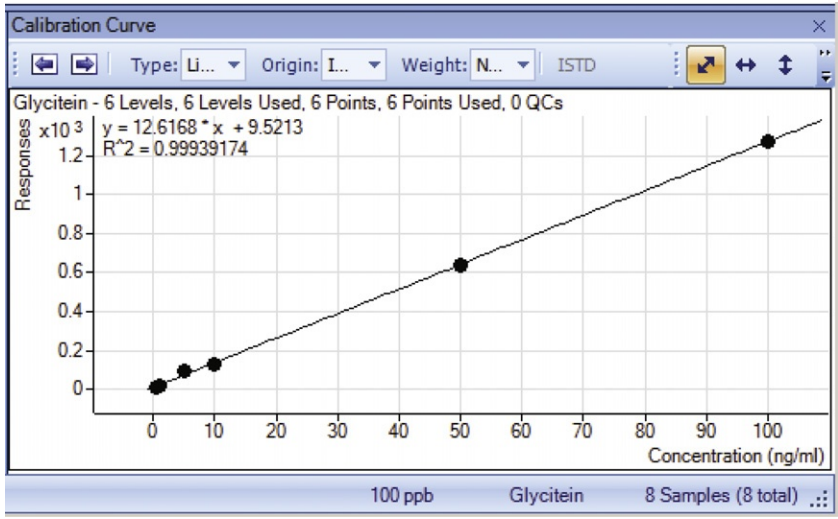


FIGURE 3 Calibration curve for glycitein using a six point curve from 0.1 to 100 µg/L (ppb) using a linear fit with no origin treatment.

TABLE 4 Instrumental Limits of Detection (LOD's) for the Phytoestrogens Studied

	Triple Quadrupole GC–MS–MS			Ion-Trap GC–MS–MS		
Name	*Calibration Curve*	R^2	*LOD's (μg/L)*	*Calibration Curve*	R^2	*LOD's (μg/L)*
Biochanin A	21.8×-25.1	0.992	2	0.751×-0.1705	0.992	15
Coumestrol	$9.4\times+3.9$	0.999	3	0.425×-0.0322	0.993	25
Daidzein	$20\times+8.8$	0.999	1	0.317×-0.0338	0.994	20
Equol	14.3×-3.8	0.999	3	0.856×-0.0566	0.993	100[a]
Formononetin	$13.3\times+18.6$	0.999	5	0.310×-0.0519	0.992	20
Genistein	11.4×-12.5	0.994	2	1.107×-0.3071	0.998	10
Glycitein	$12.6\times+9.5$	0.999	2	0.545×-0.5433	0.995	15
Prunetin	41.7×-59.3	0.992	1	0.721×-0.2342	0.997	30

[a]*LOD of compound in full scan.*

for the coefficient of determination, R^2, were >0.99. Calculated instrumental LOD's for all analytes are shown in Table 4. The lowest LOD's were for genistein and biochanin A. Prunetin showed the lowest sensitivity (i.e., signal strength/concentration) and consequently produced a higher LOD. Equol had to be quantitated in full-scan conditions (as explained in an earlier section) and for this reason a high LOD value of 100 μg/L was achieved. Intra- and inter-assay coefficients of variation (CV, $n=3$) for the standards ranged from 5% to 15%, showing again good reproducibility of the methodology. In terms of sensitivity, the triple quadrupole methodology was an order of magnitude more sensitive that the ion-trap methodology.

4 APPLICATION TO FOOD AND ENVIRONMENTAL SAMPLES

4.1 Application to Soy-Milk Analysis

Figure 4 shows the chromatographic analysis of commercially available soy milk purchased from a local grocery store, using triple quadrupole GC–MS–MS. Genistein (50,000 μg/L) and daidzein (15,000 μg/L) were the most common phytoestrogens identified in the soy-milk sample (see Table 5). Also, the presence of glycitein at much lower concentration (200 μg/L) was confirmed by this method. Dilution of the extract had to be performed due to the high concentration of these two analytes in soy milk to avoid overloading of the

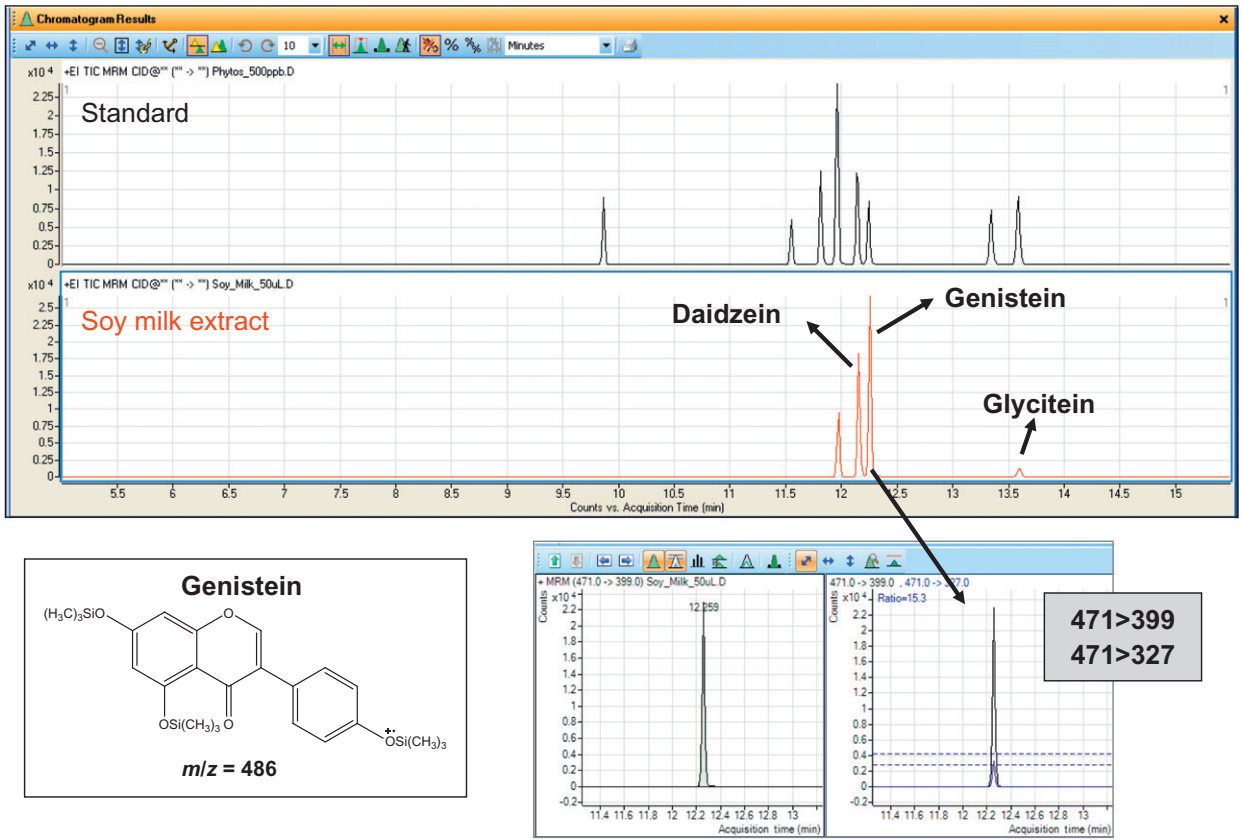


FIGURE 4 MRM chromatograms of a soy-milk sample showing the identification of daidzein, genistein, and glycitein. Ion ratios for the two MRM transitions for genistein are also shown.

TABLE 5 Concentrations of Phytoestrogens (in μg/L) in Various Samples

Compound	Soy Milk	Soy Milk Processing Plant Effluent	Wastewater Treatment Plant Influent	Wastewater Treatment Plant Effluent
Genistein	50,000	2000	20	<1
Daidzein	15,000	500	20	<1
Glycitein	200	50	<1	<1

signal in the detector. Figure 4 also shows the presence of the two MRM transitions for genistein and the respective ion ratios. As shown in this figure, phytoestrogens were easily identified in this complex matrix due to the selectivity of the MRM transitions and instrument sensitivity. When running these types of complex samples on a GC–MS instrument, it is very important to preserve the integrity of the system, especially the ion source and the chromatographic column. For this reason, a backflush procedure is recommended in these cases where the sample is complex and contains lots of interferents [8]. After determining the major phytoestrogens in the soy milk, we next examined waste and wastewater that was receiving influent from a soy-milk plant.

4.2 Application to Wastewater Analysis

Figure 5 shows the GC–MS ion-trap analysis of wastewater (WW) from a soy-product plant. Genistein and daidzein were identified at concentrations of 2000 and 500 μg/L, respectively. In this case, 5 mL of wastewater sample were extracted and preconcentrated to a final volume of 200 μL. Glycitein was below the limit of quantitation, but it was present in this sample as shown in the chromatogram at an estimated concentration of ~50 μg/L. This experiment showed the applicability of the technique for the analysis of phytoestrogens in wastewater samples that are directly discharged from a food processing plant. This is a specific location in Colorado and should not be extrapolated beyond other untreated wastewater locations.

Figure 6A shows the GC–MS ion trap chromatogram for an influent to the Boulder wastewater treatment plant. Genistein and daidzein were about the same concentration of ~20 μg/L, which is 100× less than the effluent (Figure 5) from the soy wastewater. In Boulder wastewater treated effluent, phytoestrogens were less than 1 μg/L (see Figure 6B). The fact that the influent of the Boulder plant was 20 μg/L suggests that the soy wastewater is not the only source for the genistein and daidzein in the influent. The volume of wastewater is estimated at 10 millions gallons per day and the soy wastewater plant makes a small contribution to this total, approximately 0.1% of

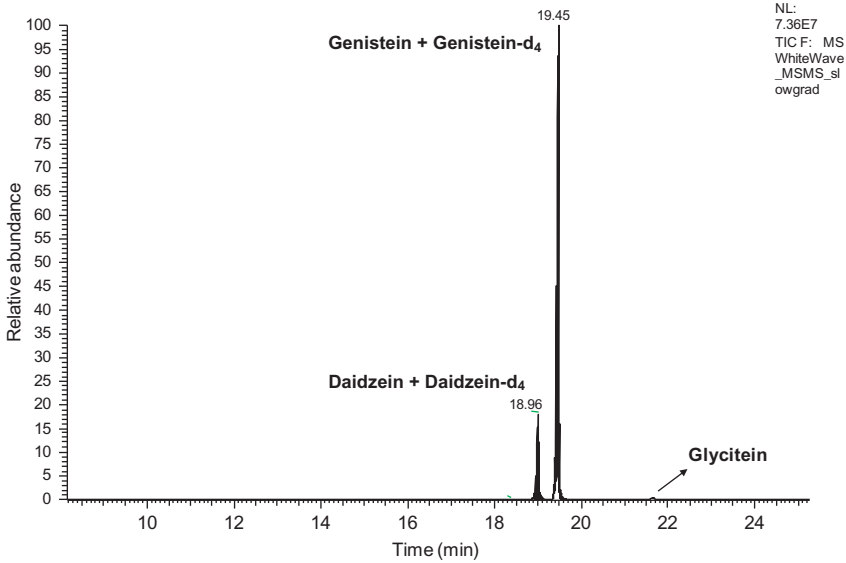


FIGURE 5 Ion-trap GC–MS–MS chromatogram corresponding to the analysis of the effluent of a soy product making plant in Boulder, CO.

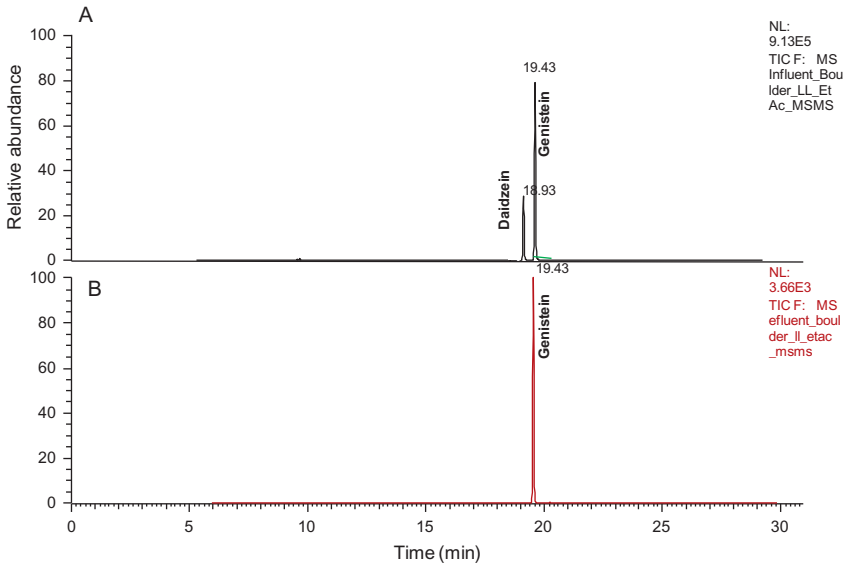


FIGURE 6 Ion-trap GC–MS–MS chromatogram corresponding to the analysis of an (A) influent and an (B) effluent of the Boulder wastewater treatment plant.

the flow. Thus, at maximum there is no more than 5% of the total phytoestrogens that are coming from the soy milk wastewater and it makes a small contribution to the total phytoestrogen pool. Thus, there must be a considerable input from other sources to the Boulder wastewater-plant influent. Obviously, there is the input from human consumption of soy products, this would include not only soy milk, but many other products that use soy, including many soy product additives and beverages. It is interesting to note that the other phytoestrogens (biochanin A, formononetin, coumestrol, prunetin, and equol) were not detected. These results suggest that these phytoestrogens are not sufficiently present in the wastewater influent or in the soy milk effluent to reach the LODs of the method. Furthermore, they are apparently not part of the diet of the city of Boulder; whereas, genistein, daidzein, and glycitein are. This is also an important negative result for future studies on the impact of phytoestrogens on fish studies of Boulder Creek.

5 CONCLUSIONS

An analytical method for the identification of eight plant phytoestrogens (biochanin A, coumestrol, daidzein, equol, formononetin, glycitein, genistein, and prunetin) was developed using gas chromatography with triple quadrupole (tandem in space) and ion trap (tandem in time) using trimethylsilyl derivatives. Furthermore, the fragmentation patterns of all the phytoestrogens were investigated by fragmenting the precursor ions in the collision cell and a typical fragmentation involving the loss of a methyl and a carbonyl group was discovered. There was no difference in fragmentation from the two methods developed. Two characteristic fragment ions for each analyte were chosen for identification and confirmation. Finally, the developed methodology was applied to the identification and confirmation of phytoestrogens in soy milk, in wastewater effluent from a soy-milk processing plant, and in wastewater (influent and effluent) from a treatment plant. Detected concentrations of genistein ranged from 50,000 and 2000 μg/L in soy milk and in wastewater from a soy plant, respectively, to 20 and <1 μg/L for influent and effluent from a wastewater treatment plant, respectively. Finally, future endocrine disruption studies on fish will focus on the effects of genistein and daidzein, as they are the main compounds occurring in the influent to this wastewater plant.

ACKNOWLEDGMENTS

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Chapter 11

Principles and Applications of Gas Chromatography Quadrupole Time-of-Flight Mass Spectrometry

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Chapter Outline

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1 INTRODUCTION

Mass spectrometry is widely used for the measurement of ions in a variety of workflows, from screening of known contaminants to the discovery of novel compounds. For many environmental applications, the compounds of interest are known, and the goal is to confirm whether they are present in a sample and at what concentration level. The single quadrupole (SQ) and triple quadrupole (QqQ) mass spectrometers (MSs) are predominately used for target screening and quantitation. Quadrupole-based MSs are excellent tools for detecting contaminants in the environment. These MSs provide very good sensitivity, selectivity, and quantitation when the quadrupole analyzer is set to filter specific ions, such as when operating in selected ion monitoring mode or multiple reaction monitoring mode. This excellent selectivity and sensitivity are required when performing targeted analyses. In recent years,

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environmental applications have expanded to include nontargeted approaches and the pursuit of unknown identification. This often requires the collection of data in an untargeted manner and necessitates the MS to acquire full spectrum (full scan) data. When quadrupoles are operated in full scan mode, the sensitivity is considerably reduced due to the discarding of ions as the quadrupole sequentially scans the mass range at a relatively slow rate. Additionally, in the absence of reference mass spectra or standards, identification of unknowns can be challenging with unit mass instruments. In contrast, time-of-flight (TOF) mass analyzers are capable of acquiring a full mass spectrum many times per second with significantly higher sensitivity than a quadrupole. They are also capable of acquiring high-resolution accurate mass data that can greatly facilitate the identification of an unknown component. These attributes have led to the increase of TOF-MSs being adopted into environmental laboratories. Quadrupole time-of-flight (QTOF) MSs are particularly attractive for emerging contaminants or emergency response because of the combination of high sensitivity, mass accuracy, acquisition speed, and high resolution, for both precursor (MS) and product ion (MS/MS) spectra. High selectivity can be achieved in a QTOF by extracting ion chromatograms with narrow mass windows, as well as through the use of MS/MS. Section 2 reviews the various aspects of GC/QTOF technology.

2 OVERVIEW OF GC/QTOF INSTRUMENTATION

The QTOF is a hybrid instrument that merges quadrupole and TOF technology. These instruments are often used to analyze complex samples, and thus it is important that they are coupled to effective chromatographic techniques. Schematics of a gas chromatograph and a QTOF are shown in Figure 1. The schematic in Figure 1A shows an inlet, gas chromatography (GC) oven, column, and transfer line of the gas chromatograph. Figure 1B shows the ion source, quadrupole mass filter, hexapole collision cell, and orthogonal axis flight tube with ion pulser, ion mirror (reflectron), and detector for the QTOF-MS. This section reviews some of the key aspects of GC/QTOF.

2.1 Gas Chromatography

The coupling of a chromatograph to a mass spectrometer results in a very powerful analytical tool. The chromatograph is used to separate components within a sample. This is particularly important in complex samples that contain hundreds to thousands of compounds. In simple terms, the chromatographic separation spreads out the compounds in the sample over time, thus reducing the complexity of the sample and the number of compounds the MS detects at any given period of time. This gives the MS more time to acquire data on the individual components in the samples. The chromatograph

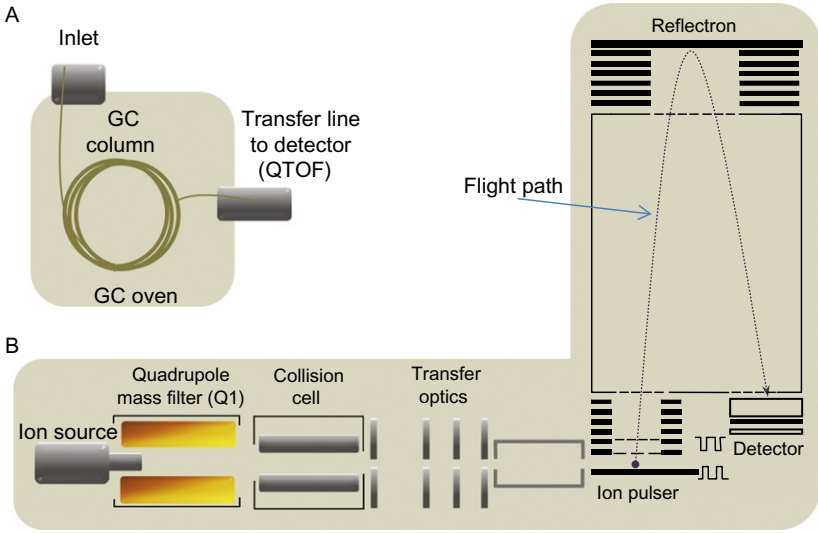

FIGURE 1 (A) Simplified schematic of a gas chromatograph, with inlet, GC column, and transfer line. (B) Simplified schematic of a quadrupole time-of-flight MS, with ion source, quadrupole mass filter, collision cell, transfer optics, flight tube, reflectron, and detector.

also provides selectivity and resolution, which can vary depending on the chromatographic method and the type of column used. Chromatographic separation is often required in order to resolve isomers or compounds with similar masses. Thus it is highly desirable to combine chromatography with mass spectrometry.

Fortunately, there are many types of chromatographic separation techniques that can be coupled to MSs: GC, liquid chromatography (LC), capillary electrophoresis, ion chromatography, and supercritical fluid chromatography are among some of the techniques used. The two most prominent techniques are GC and LC. The choice of chromatography should be based on the types of samples being analyzed and the type of ionization required. Generally speaking, volatile and semivolatile compounds are analyzed by GC techniques.

The process of GC involves the injection of a sample (e.g., via an inlet or headspace), sample vaporization, and introduction of the vaporized sample onto a capillary column with the concomitant mixing of the sample with carrier gas (e.g., helium). As the sample flows through the capillary column, it interacts with the stationary phase. Different compounds interact distinctly with the stationary phase, thus producing unique retention times. This leads to the chromatographic separation of the sample. The type of gas that is selected, the velocity of the flow, the stationary phase, and the temperature

will affect chromatographic efficiency and retention times. GC capillary columns provide excellent separation efficiency, with hundreds of thousands of theoretical plates [1]. This is particularly useful for high complexity samples or for the resolution of isomers. As the chromatographic separation progresses, the components exit the column and are introduced into the ion source. It is in the ion source where ionization of the components occurs. The ionization is an essential step required for detection by mass spectrometry and it will be discussed in Section 2.2.

2.2 Ionization

Fundamentally, MS instruments measure ions. Thus effective production of ions is critical to this technique. The two primary modes of ionization for GC/MS are electron ionization (EI) and chemical ionization (CI). EI was one of the early modes of ionization to be described [2], and it is the most widely adopted ionization technique for GC/MS. CI is another type of ionization used in GC/MS [2–5]. CI is a softer ionization technique and can provide complimentary information to EI. There are several other types of ionization techniques that can be coupled to GC/MS instruments; however, this section focuses solely on EI and CI.

2.2.1 Electron Ionization

In EI, a metal filament (e.g., tungsten) is used to generate a beam of electrons that is introduced into the ion source with the intent of ionizing the components that are coming off the GC column. EI forms a radical cation ($M^{+\bullet}$) via the removal of an electron. It can be represented by the Equation (1), that depicts a reaction of an electron with a neutral mass (M) in the gas phase.

$$e^- + M \rightarrow 2e^- + M^{+\bullet} \tag{1}$$

However, this mode of ionization can lead to extensive fragmentation, where the radical cation ($M^{+\bullet}$) subsequently reacts with another electron to form another radical cation, as shown in Equations (2) and (3). This leads to the fragmentation rich nature of the EI spectra (Figure 2).

$$e^- + M^{+\bullet} \rightarrow 2e^- + [M-n]^{+\bullet} + n \tag{2}$$

$$e^- + M^{+\bullet} \rightarrow 2e^- + [n]^{+\bullet} + [M-n] \tag{3}$$

The mass spectra from EI can provide useful information for verifying the identity of a compound. The standardized use of 70 eV for EI has permitted the production of reproducible fragmentation spectra. This has enabled the creation of spectral libraries, which are widely used for identification and confirmation of compounds in GC/MS techniques. Figure 2 shows an

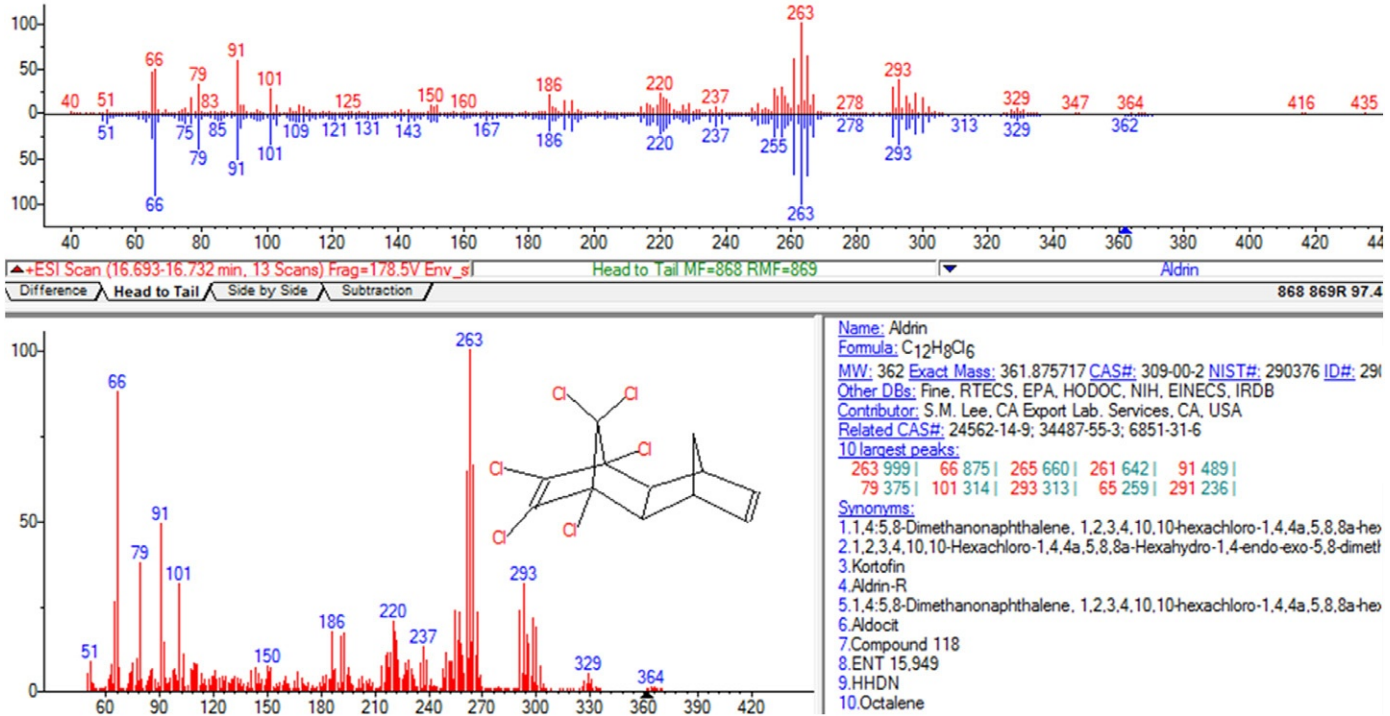

FIGURE 2 EI Spectral Library search of data collected on a GC/QTOF. The top panel is a mirror plot of the GC/QTOF spectrum (top, red) and the reference spectrum (bottom, blue) from a spectral library. Electron ionization can produce reproducible fragmentation spectra. This allows for the creation of libraries that can be subsequently used for spectral matching. *Data courtesy of Sofia Aronova, Agilent Technologies, Inc.*

example of a 70 eV spectrum that was obtained from a GC/QTOF and its corresponding library match of aldrin, a banned insecticide. One can see the similarity (match) in the library spectrum (bottom, blue) compared to the QTOF spectrum (top, red). It is the reproducible fragmentation that allows the wide-spread use of library matching in GC/MS, even across all (Q)TOF and quadrupole-based MS platforms.

EI is a desirable mode of fragmentation because of its universality for ionizing compounds, its high ionization efficiency (which provides better sensitivity), and the reproducible fragmentation patterns that enable standardize spectral library searching. These attributes have made EI one of the most widely used modes of ionization. However, when analyzing the samples that have many components with similar structures, the extensive fragmentation generated by EI can lead to all of these components having similar or near identical spectra (Figure 3). In cases where the molecular ion of the compound is not present in the mass spectrum, it may not be possible to distinguish the different components, and thus impossible to identify the specific compounds that are in the sample. Figure 3 shows the EI spectra of octadecanoic acid methyl ester and nonanoic acid methyl ester. Although these two compounds are similar in structure, they are different in molecular weight and size and could readily be distinguished if their molecular ions were present in the spectra. However, when the compounds are ionized by EI, they produce very similar fragmentation spectra with no detectable molecular ion, which can make it difficult to distinguish the two compounds.

In addition to this, extensive fragmentation in complex samples (with many components coeluting) can generate very complex spectra that can increase the difficulty of data analysis. For these reasons, it is sometimes important to use soft ionization techniques, which provide less fragmentation and generate mass spectra that include the molecular or pseudo-molecular ion peak. CI is one of the techniques used to achieve this.

2.2.2 Chemical Ionization

In CI, a filament is again used to generate electrons; however, in this case, the reagent gas is first ionized. In Equation (4), an electron is reacting with the reagent gas (methane, CH_4), to generate a radical cation ($CH_4^{+\bullet}$), which can undergo further reactions with methane to produce CH_5^+ (Equation 5). Subsequently, a charge transfer between the ionized gas and a neutral molecule (M) can occur (Equation 6), resulting in the ionization of that molecule (Equations 6–10).

$$e^- + CH_4 \rightarrow 2e^- + {CH_4}^{+\bullet} \quad (4)$$

$${CH_4}^{+\bullet} + CH_4 \rightarrow {CH_5}^+ + {CH_3}^\bullet \quad (5)$$

$${CH_5}^+ + M \rightarrow CH_4 + [M+H]^+ \quad (6)$$

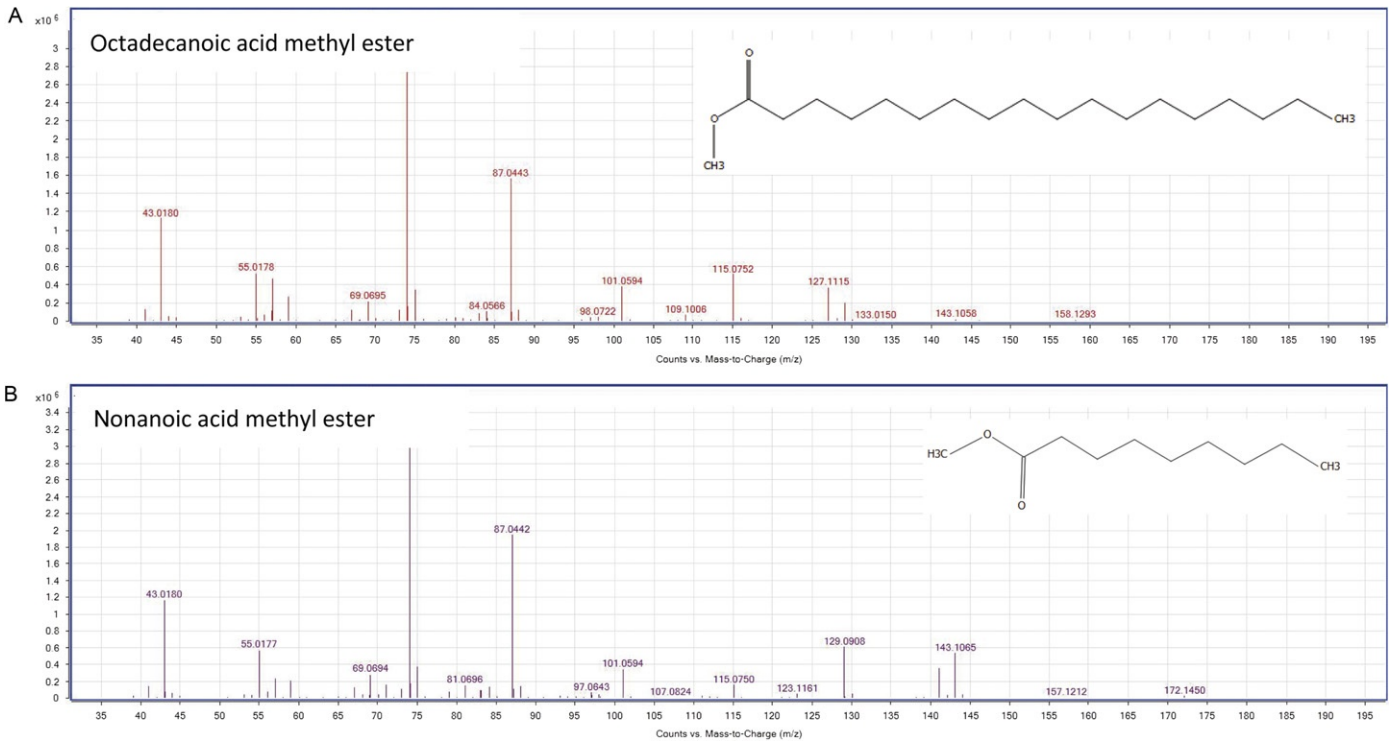

FIGURE 3 EI spectra generated by a GC/QTOF. (A) EI spectrum of octadecanoic acid methyl ester. (B) EI spectrum of nonanoic acid methyl ester. *Data courtesy of Sofia Aronova, Agilent Technologies, Inc.*

$$CH_4^{+\bullet} \rightarrow H_2 + CH_3^+ \quad (7)$$

$$CH_3^+ + M \rightarrow CH_4 + [M-H]^+ \quad (8)$$

$$CH_5^+ + CH_4 \rightarrow C_2H_5^+ + 2H_2 \quad (9)$$

$$C_2H_5^+ + M \rightarrow [M + C_2H_5]^+ \quad (10)$$

This ionization technique is less energetic than EI and produces less fragmentation. This can be useful when a molecular ion or pseudo-molecular ion $(M+H)^+$ is desired. However, it should be noted that CI ionization is less efficient than EI ionization, and will thus result in lower sensitivity. With CI, one must also carefully select the reagent gas that is appropriate to the types of samples being analyzed. These can include gasses such as methane, ammonia, or isobutane, to name a few. CI may also lead to the formation of adducts (Equations 4–10), and thus it is important to understand the ionization process in order to be able to predict the types of adducts that will be formed as a result of the interaction of the ionized gas with the compound molecules. Equations (5)–(10) show how subsequent reactions can lead to the ionization of a neutral mass (M) to form a protonated ion $[M+H]^+$, a deprotonated ion $[M-H]^+$, or an adduct formation $[M+C_2H_5]^+$.

Ultimately, the choice of the ionization that will be utilized may depend on the class of compounds being studied, the type of analytical questions that need to be answered, and the type of data analysis that will be performed. Fortunately, many of the commercially available GC/MS instruments can be coupled with both types of ionization methods, thus providing greater flexibility.

2.3 Quadrupole Analyzers

Quadrupoles are excellent mass filters and can be used in mass spectrometry to select for a narrow range of mass-to-charge (m/z) values. This allows for a more selective analysis and can help filter out unwanted ions (noise). Quadrupoles can also be operated in RF-only mode, thus allowing a broad range of m/z values to pass through the MS. In a QTOF, the quadrupole is set to RF-only when operating in TOF-only mode. When running MS/MS experiments, the quadrupole is set to selectively filter a particular m/z value.

Over the years, there have been significant changes in quadrupole technology. For example, hyperbolic quadrupole designs have been favored over round rods since they provide lower field errors and better transmission efficiency. There have also been improvements made specifically for GC/MS instruments. Quadrupoles have been typically manufactured with metal; however, due to the fact that GC/MS systems are operated at higher temperatures, metal rods are not ideal since the metal can expand and distort

with the temperature changes that occur during operation of the MS. One of the materials that has been successfully employed for GC/MS quadrupoles is gold-plated quartz. Quartz has an extremely low temperature coefficient of thermal expansion; thus even when subjected to wide temperature changes, it will maintain excellent dimensional stability. This is important because it will lead to better quadrupole resolution and mass axis stability. Gold has been employed as the conductive coating for quartz structure because it has excellent electrical properties for high-voltage fields, which are required for transmitting higher m/z ions.

Quadrupoles provide a mass filtering capability in QTOF instruments. This is particularly important when performing MS/MS experiments, where the quadrupole will filter ions before they enter the collision cell. For GC/QTOF, using quadrupoles that are specifically designed for high-temperature operation will provide optimal GC/MS performance.

2.4 Collision Cell

Collision cell designs have also undergone changes throughout the years. There are a variety of multipole designs (e.g., quadrupole, hexapole, octapole, tapered, hyperbolic, high pressure). The basic function of the collision cell is to fragment precursor ions, which were selectively filtered by the first quadrupole, into product ions. Fragmentation occurs when the precursor ions are accelerated via an electric potential and collide with gas molecules in the collision cell. The kinetic energy from the collision is converted to internal energy, which can result in the breakage of a bond in the ionized molecule. The resulting fragment ions will produce an MS/MS spectrum. This process is referred to as collision-induced dissociation (CID). Examples of collision gas include nitrogen, argon, and helium. CID is a nonselective process that may occur in multiple steps. The fragmentation process can be controlled by increasing or decreasing the applied voltage or the gas pressure of the collision cell.

Another key function of the collision cell is to efficiently transmit ions out of the cell. If any ions linger within the cell, it can lead to crosstalk, which is a phenomenon where fragment ions from one round of fragmentation are seen in the mass spectrum of the subsequent round of fragmentation. In order to avoid this, analyzers with higher transmission efficiencies (e.g., hexapoles employing axial fields) can be used.

CID fragmentation can provide structural information for the identification or confirmation of a compound. CID can also be used to distinguish isomers, assuming that they have different MS/MS fragmentation spectra. In addition to this, MS/MS can provide additional selectivity, which may be essential when a compound is present at relatively low level in a high matrix

background. In a QTOF, both MS and MS/MS spectra have high resolution and high mass accuracy. This is particularly important when analyzing unknowns.

2.5 Time-of-Flight

TOF analyzers quite literally measure the ion flight time: ions are pulsed through a drift tube by utilizing a high-voltage potential and a measurement of the time it takes the ions to traverse the length of the drift tube is recorded. This measurement is recorded in microseconds (μs), and can be correlated to the mass (m) to charge (z) ratio of the detected ion. Thus, fundamentally, TOF instruments record the temporal information of the ions being measured. Although there are various approaches to TOF mass analysis (e.g., linear, orthogonal, reflective), this section focuses primarily on orthogonal axis TOFs with reflectron technology.

When operating a QTOF in TOF-only mode, the quadrupole mass filter is operated in RF-only mode to allow transmission of ions over a broad mass-to-charge ratio range. Ions are launched orthogonally into the flight tube via a pulser that applies an electric field at a rate of approximately 7–10 kHz. The ions traverse a field-free region before passing through the ion mirror or reflectron. The reflectron changes the trajectory of the ions and reflects them back through the field-free regions. The ions then hit the detector and the spectral data are recorded. Some of the key benefits of TOF analyzers include mass resolution and mass accuracy. The next two sections will review these topics.

2.5.1 Mass Accuracy

The high mass accuracy of TOF instruments is in part due to the resolving power of the instrument. TOFs can produce spectra with narrow mass peaks enabling high mass resolution. This provides some degree of precision. However, one also needs to properly calibrate the TOF instrument in order to convert the ion flight time measurement into an accurate m/z value. This is done by analyzing compounds with ions for which the exact mass is known (calibrant ions). The known or theoretical m/z values of these ions are used to correct the m/z values that are measured by the TOF, and thus calibrate the system. This is typically done with a higher-order polynomial correction factor. Once a TOF system is properly calibrated, the mass measurements should provide accuracy in the low parts per million (ppm) range. Mass accuracy is generally reported as a ppm error and is calculated by taking the difference between the theoretical mass (m_t) and the measured experimental mass (m_e), dividing by the theoretical mass, and then multiplying by 10^6 (Equation 11). The magnitude of the result is highly dependent on the mass that is being measured. As shown in Equation (12), an absolute difference of

0.001 Da in a measurement will equate to a 5 ppm difference at m/z 200, but it is a $1.\overline{6}$ ppm difference at m/z 600 (Equation 13). Thus, it is important to keep in mind the m/z value when considering absolute mass accuracy errors.

$$\text{mass error}\,(\text{ppm}) = \frac{m_e - m_t}{m_t} \times 10^6 \tag{11}$$

$$\text{mass error}\,(\text{ppm}) = \frac{200.001 - 200.000}{200.000} \times 10^6 = 5\,\text{ppm} \tag{12}$$

$$\text{mass error}\,(\text{ppm}) = \frac{600.001 - 600.000}{600.000} \times 10^6 = 1.\overline{6}\,\text{ppm} \tag{13}$$

The combination of resolution and accurate mass provides analysts with information-rich spectra. For GC/QTOF, this allows one to propose empirical formulas for the majority of the mass peaks in the mass spectrum. This is critically important when trying to identify unknown compounds.

2.5.2 Mass Resolution

The mass resolution of an instrument can be calculated by dividing a mass (m) by the delta mass (Δm), with the delta mass being defined as the full width of the mass peak at half maximum (FWHM) [6]. The resolution on a TOF instrument is affected by many factors. One of the key factors is the initial velocity spread of the ions as they are entering the TOF pulser. The transmitted ions will arrive into the TOF with a certain degree of spatial spreading: with some ions being deeper in the pulser, while others are shallower. There will also be differences in the magnitudes and directions ions energies; some ions will be traveling away from the TOF flight path, while others will be traveling in the direction of the TOF flight path. Any ions that had initial velocities opposite to the TOF flight path direction must be decelerated and then reaccelerated in the opposite direction. In other words, the ions will have to "turn around." This delay time is referred to as *turn around time* and contributes to the spatial spread of ions. This leads to wider mass peaks (or Δm), and thus decreases the resolution. One must also consider that if the ions are distributed at different depths in the pulser field when accelerated, they will exit the pulser with different velocities, increasing the energy spread. Most modern TOF instruments include multistage electrostatic ion mirrors that can help correct for the spread of the ion energy and spatial distributions. This correction will compress or refocus the ion packages, leading to improved resolution. Other factors that affect resolution include ion beam cooling and conditioning prior to entering the TOF pulser, the length of the flight path, stability of high-voltage power supplies, alignment of key instrument components, and the detector configuration.

TOF MSs have been described since the mid-1940s [7], and are widely used in many application spaces. It was approximately a decade later, in 1953, when

quadrupole analyzers were described by Paul and Steinwedel [8]. Since then, quadrupole-based MSs have grown into the most dominant type of MS instrument type, being primarily used for target quantitation and screening. It took some 40 years before a hybrid quadrupole-TOF instrument was commercially available [9]. If we consider that J.J. Thomson's first descriptions of MSs were at the turn of the twentieth century [10,11], QTOF MS is a relatively recent development. Although initially used for life science applications (e.g., proteomics), QTOFs are now widely used to meet many analytical challenges, including those in food safety, petrochemistry, and environmental sciences.

3 ENVIRONMENTAL APPLICATIONS

Environmental applications span a wide range of analyses, including contaminants in water, flora, fauna, soil, and air. The contaminants can range from a variety of known compounds to unknown or emerging contaminants. Additionally, harmful metabolites and degradation products of these compounds may also be present in the environment. The complexity of analysis can be compounded by new policies that require lower levels of measurement and more severe consequences when regulations are violated. Thus, analyses must be more sensitive and have a higher degree of accuracy than in the past.

Mass spectrometry can provide an effective tool for these types of analyses. SQ and QqQ-MSs can be used for routine-targeted analyses, while QTOF-MSs are emerging as tools for more complex problems, where mass resolution and accuracy are required.

3.1 Water Analysis

The protection of our water resources is critically important to sustaining, protecting, and improving the quality of our water. Environmental agencies are responsible for monitoring various sources of water, including rivers, lakes, waste water, etc. Testing of these sources has become increasingly rigorous. This has been coupled with the increase in hazardous compounds detected in the environment. In December 2000, the European Water Framework Directive (WFD) was put into place. The aim of the directive is to protect, defend, and improve the quality of water in the European Union states, with an ultimate goal of eliminating key hazardous substances from the environment. The directive recognizes the growth in the demand for good quality water and highlights improvements in ecological quality of surface water and river beds, the protection of ground water against pollutants, and the conservation of wetlands. These measures should positively impact the aquatic ecosystems (e.g., fish populations), the aquatic environment, as well as terrestrial ecosystems that are dependent on water sources. It will also involve the reduction of hazardous compounds that are being released into the environment.

Thus water resources need to be evaluated in both qualitative and quantitative terms. As stated above, SQ and QqQ MSs are excellent for target analysis of known compounds. They can provide very good quantitative information on the amount of a target compound that is present in the sample. However, when one wants to have a more broad evaluation of all compounds in the sample, a full spectrum analysis is required. The QTOF provides superior detection limits in full spectrum mode and can provide invaluable information through high mass accuracy and resolution.

One of the priority substances for the WFD is hexachlorobenzene (C_6Cl_6). This compound is a fungicide that was used primarily to treat seeds (e.g., wheat) against *Tilletia indica*, the fungus that causes bunt disease. Hexachlorobenzene has been banned globally due to the harmful effects of this compound. Additionally, it is has been classified as a Group 2B carcinogen by the International Agency for Research on Cancer (IARC). Figure 4A shows a total ion chromatogram of the water sample, generated by a GC/QTOF. It demonstrates the high complexity of this sample, showing a multitude of peaks across the chromatogram. In Figure 4B, we mimic the resolution of a quadrupole instrument by applying an extraction window of ± 0.5 amu. Here, we can see that there are still many ions that fall within that range, thus making it difficult to confirm the presence of hexachlorobenzene. By using the high selectivity of the QTOF and reducing the extraction window to ± 2 ppm, the mass peak of hexachlorobenzene is revealed and the matrix interferences are eliminated (Figure 4C). We can see that in a highly complex sample, the resolving power of the QTOF allows a tight window of selection around a particular m/z value, resulting in the selective extraction of a particular m/z value associated with a compound of interest, in this case: hexachlorobenzene. This is particularly useful when the retention time of the compound is unknown. The QTOF provides the clear advantage when looking for compounds without *a priori* knowledge of chromatographic retention. The QTOF can also provide excellent quantitation, as shown in the calibration curve inset in Figure 4C.

Another concern for environmental agencies is the increase in personal care and pharmaceutical products in the environment. One such class of emerging compounds is polycyclic musks. In Figure 5, we see an example of one of these synthetic musks: cashmeran. In this example, we highlight the importance of mass resolving power. Cashmeran has an m/z value of 191.1430; however, there is an interference at m/z 191.1785 (a 0.035-Da difference). This interference is a compound that is present in the water matrix. With insufficient resolving power, a MS would not be able to separate these two masses and the two ions would be seen as one peak. This can cause errors in both quantitation and identification. However, the QTOF has sufficient resolving power to separate these two ions and correctly assign an identity, as well as provide a more accurate measure of the response associated with each individual compound.

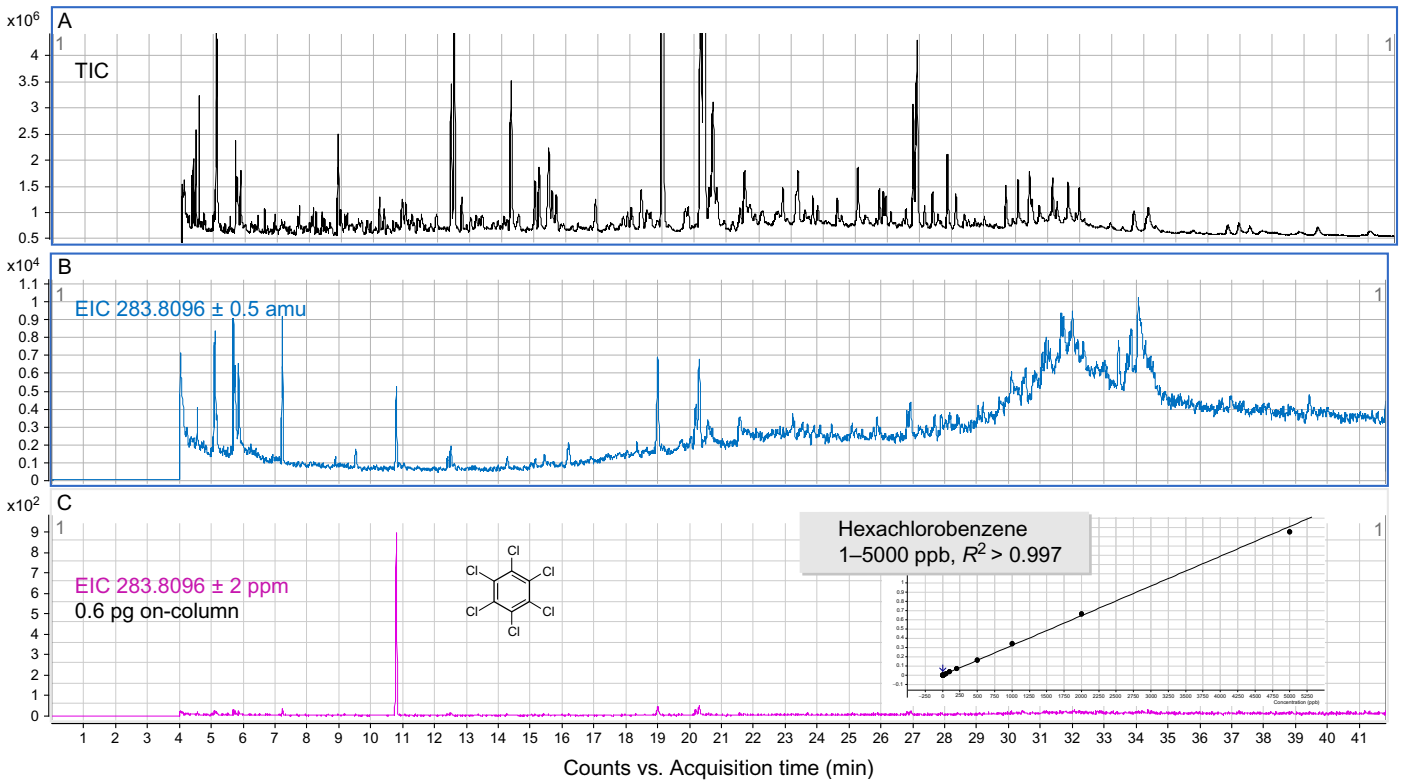

FIGURE 4 (A) Total ion chromatogram (TIC) of a river water sample analyzed by GC/QTOF. (B) Extracted ion chromatogram of *m*/*z* 283.8096 corresponding to hexachlorobenzene, with an extraction window of ±0.5 amu. This extraction window would be similar to what can be achieved on a quadrupole mass spectrometer. (C) Extracted ion chromatogram with an extraction window of ±2 ppm. Here, we can see the selectivity of the QTOF, which can readily extract the ion for hexachlorobenzene. *Data courtesy of Sofia Aronova, Agilent Technologies, Inc. and Anthony Gravel, Natural Resources Wales – Llanelli Laboratory.*

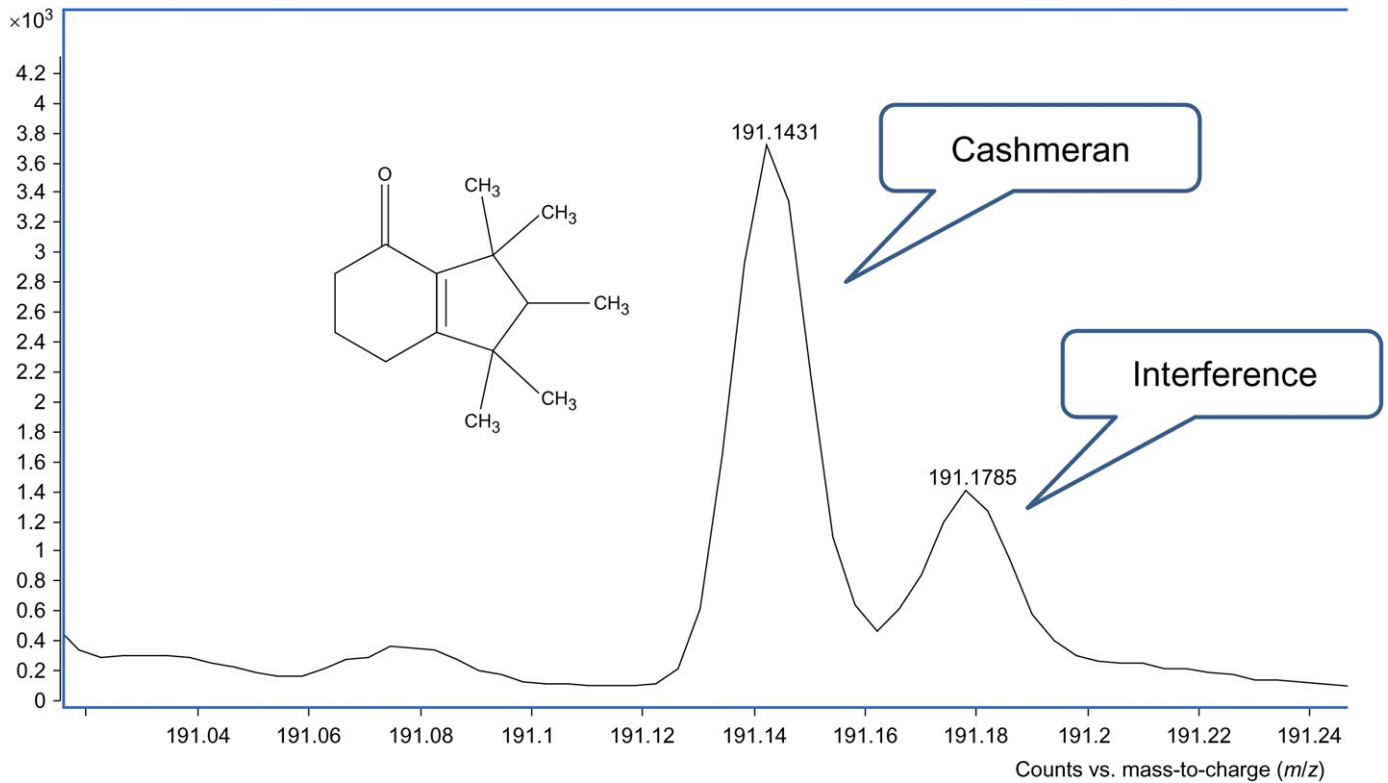

FIGURE 5 GC/QTOF mass spectrum showing the fragment ion for cashmeran (6,7-dihydro-1,1,2,3,3-pentamethyl-4(5*H*)-indanon) and another ion that is 0.035 Da higher. Without the resolving power of the QTOF, these compounds would be seen as one mass peak.

4 CONCLUSIONS

The marriage of quadrupole analyzers with TOF analyzers produces a hybrid instrument with powerful functionality. The extension of this technology into GC-based ionization techniques (EI and CI) further expands the versatility of QTOF instruments. For environmental applications, this will enable the use of higher performance MSs to be used for the analysis of GC-amenable compounds. The ability of the GC/QTOF to deliver full spectrum sensitivity in MS and MS/MS mode, while simultaneously delivering high mass accuracy and resolution, is invaluable to discovery based workflows and characterization of emerging contaminants. GC/QTOFs can also be used successfully for the methododologies that search for a targeted list of compounds without the requirement of the knowledge of the retention time information beforehand. Additional examples of work done on GC/QTOF can be found in Chapter 20 of this book.

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Chapter 12

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

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Chapter Outline

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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].

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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 1 Characteristics of Current GC–TOF-MS Systems

Instrument (Company)	Upper Mass Limit (Da)	Mass Resolving Power	Maximal Acquisition Rate (spectra/s)	Mass Accuracy (ppm)	Linearity (Orders of Magnitude)	Acquisition System	Ionization Mode	
							EI	*CI*
Pegasus GC-HRT (Leco)	1:4 from the heaviest acquired mass (e.g., *m/z* 100–400, *m/z* 250–1000)	50,000 FWHM	200	1	4	ADC	+	PCI
	1000	25,000 FWHM						
	1000	Unit (equiv. 2500 FWHM)	200	—				
TruTOF HT TOF-MS (Leco)	1000	Unit (equiv. 2500 FWHM)	80	—	4	ADC	+	PCI
Pegasus 4D GC × GC-TOF-MS (Leco)	1000	Unit (equiv. 1000 FWHM)	500	—	4	ADC	+	–
GCT Premier (Waters)	1500	7000 FWHM	20	5	4	TDC	+	PCI, NCI
7200 GC/Q-TOF (Agilent)	1700 (TOF only) 1050 (Q upper limit)	12,500 FWHM	50	5	5	ADC	+	PCI, NCI
Kronus (Scientific Analysis Instruments)	2000	Unit (equiv. 1000 FWHM)	100	—	5	ADC	+	PCI, NCI

AccuTOF GCv 4G (Jeol)	5000	8000 FWHM	50	4	4	ADC	+	PCI, NCI
Master TOF GC/MS (DANI Instrument)	1024	Unit (equiv. 1500 FWHM)	1000	—	5	ADC	+	–
GCT (Micromass)	1500	7000 FWHM	10	5	3	TDC	+	PCI, NCI
JMS-T100GC (JEOL)	2000	5000 FWHM	25	5	4	ADC	+	PCI, NCI
Tempus (Thermo)	1000	Unit (equiv. 1400 FWHM)	100	—	4	ADC	+	PCI, NCI

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.

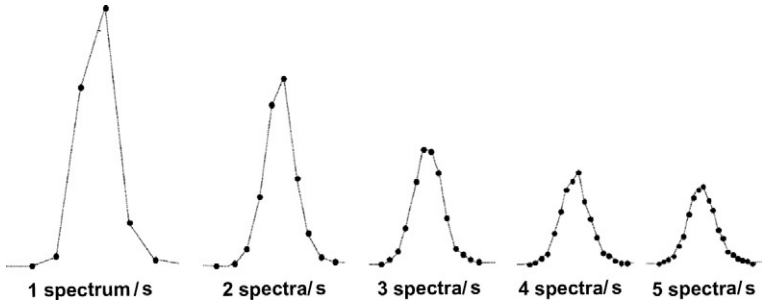

FIGURE 1 The influence of acquisition rate on peak shapes. *Reprinted with permission from Ref. [10].*

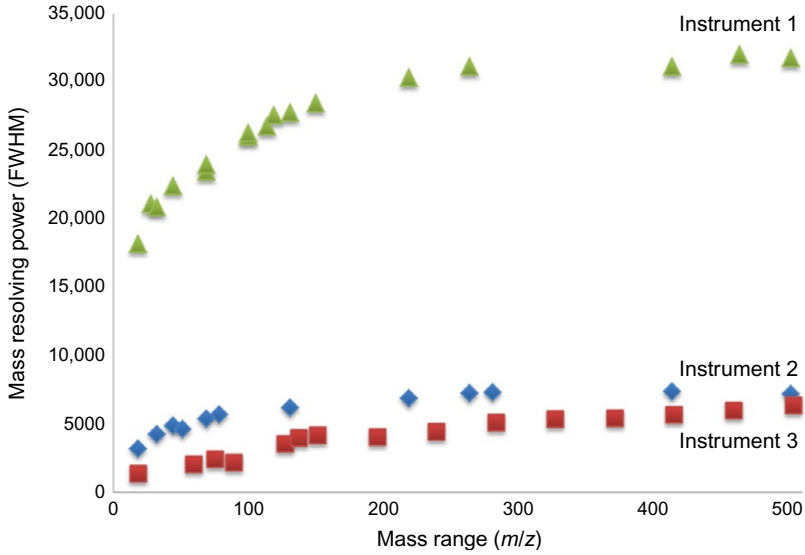

FIGURE 2 Mass resolving power (FWHM) versus mass range (m/z) for different HR-TOF-MS instruments.

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

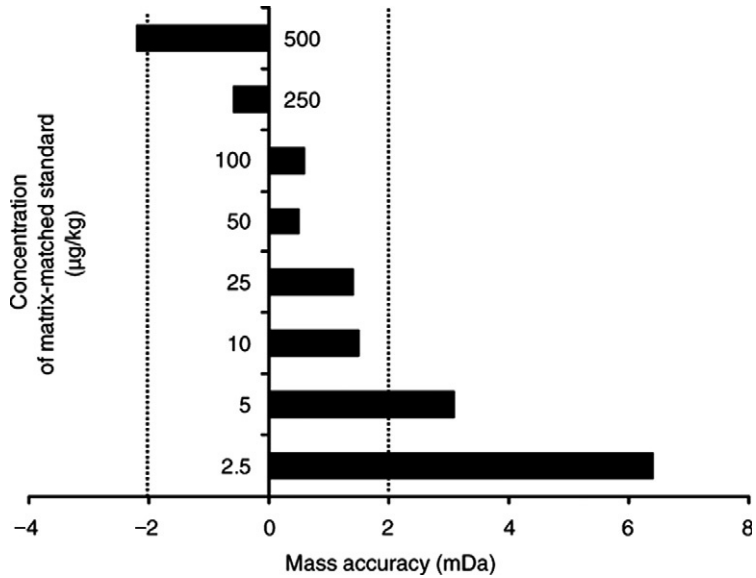

FIGURE 3 Mass accuracy in mDa (*x*-axis) of fragmentation ion of dimethoate (theoretical mass 124.9826 Da) in dependence on concentration of analyte in matrix-matched standards (*y*-axis). *Reprinted with permission from Ref. [10].*

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 (1*S*)-1,7,7-trimethylnorbornan-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.

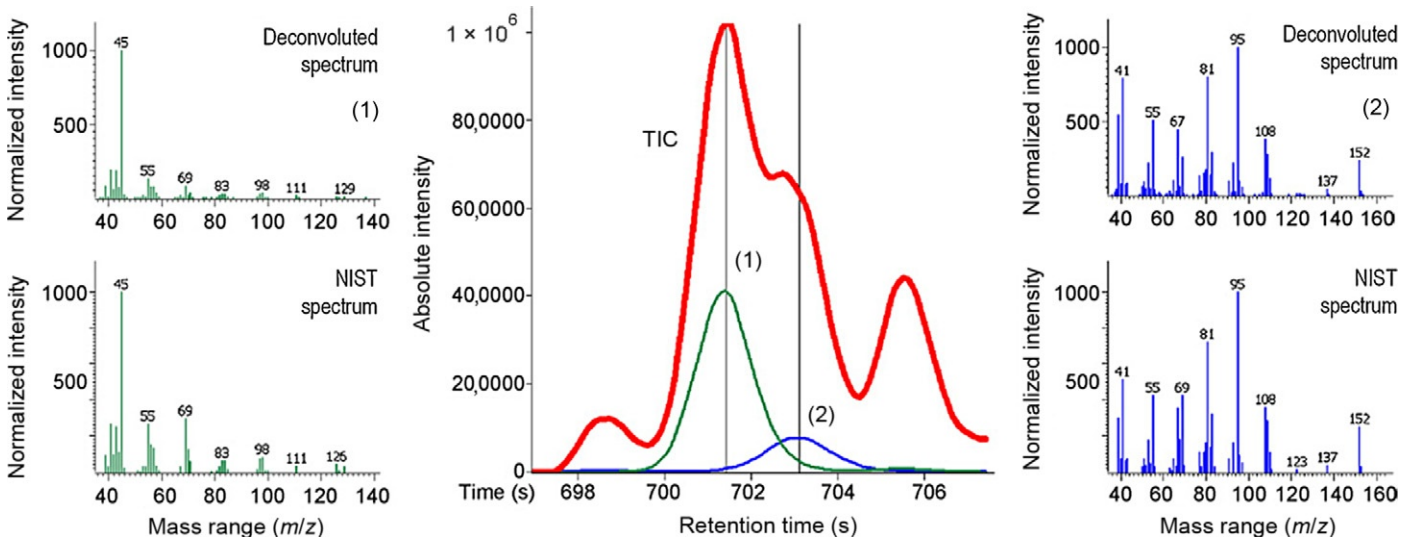

FIGURE 4 Spectral deconvolution of two closely eluted beer markers of the GC–HS-TOF-MS fingerprint. (1) Nonan-2-ol, *m/z* 45 displayed; (2) (1*S*)-1,7,7-trimethylnorbornan-2-one (camphor), *m/z* 95 displayed. *Reprinted with permission from Ref. [15].*

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 $\leq$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

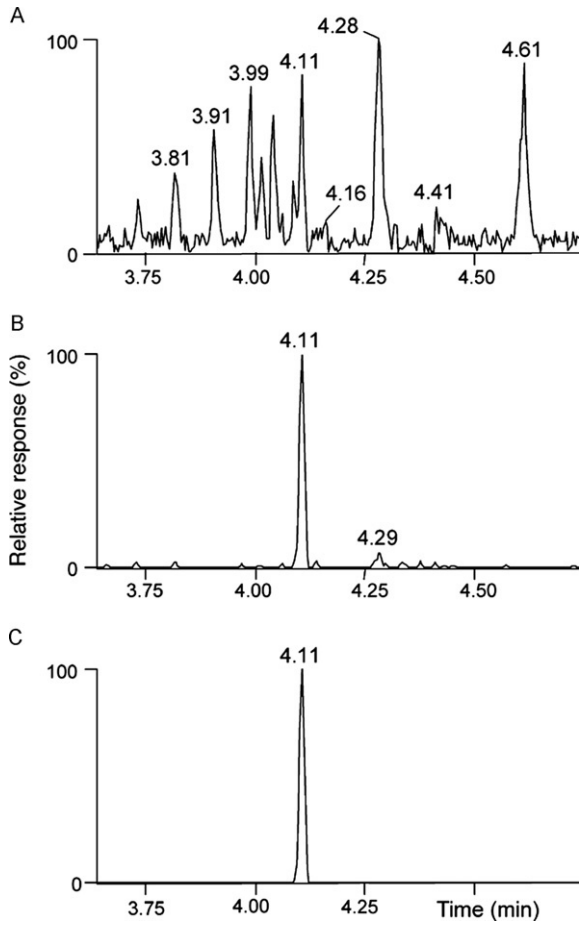

FIGURE 5 Influence of mass window setting for detection of 0.01 mg/kg phosalone ($t_R = 4.11$) in apple baby food extract using HR-TOF-MS. Target ion *m/z* 182.001 extracted using a mass window of (A) 1 Da, (B) 0.1 Da, and (C) 0.02 Da. *Reprinted with permission from Ref. [17].*

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.

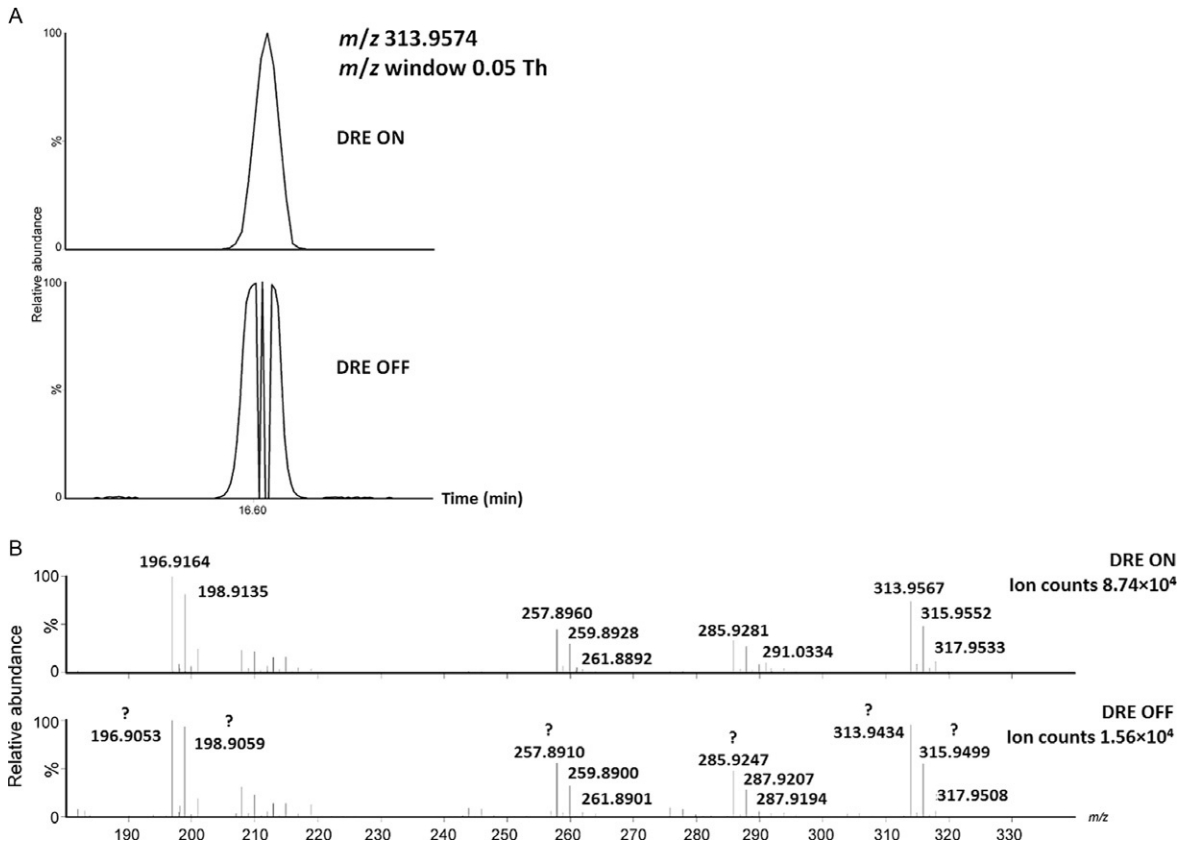

FIGURE 6 (A) Extracted ion chromatograms of chlorpyrifos (*m/z* 313.9574) with DRE ON and OFF (HR-TOF-MS instrument with the TDC) and (B) its spectra, in a lettuce matrix-matched standard at 1.0 μg/mL. The saturated ions are marked by question-marks. *Reprinted with permission from Ref. [19].*

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 non-targeted. 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

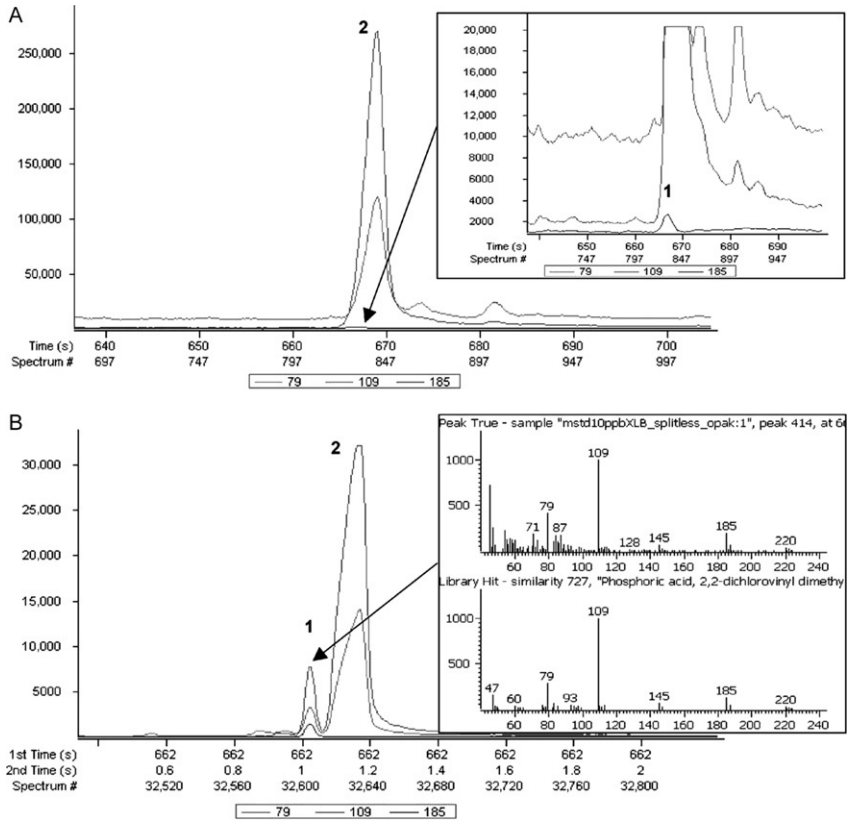

FIGURE 7 Separation of dichlorvos (1) in apple extract at 0.01 mg/kg from matrix coextract 5-(hydroxymethyl)-5-furancarboxaldehyde (2). Plotted are three most abundant ions in the mass spectrum of dichlorvos (79, 109, and 185). Chromatogram of (A) 1D-GC analysis of zoomed section shows the peak of dichlorvos (*m/z* 185) and matrix interference (*m/z* 79 and 109); and (B) GC × GC analysis (DB-XLB × DB-17 columns); matrix interference resolved on medium polar DB-17 column. Data acquired by HS-TOF-MS at acquisition rates of 5 spectra/s and 250 spectra/s, respectively. *Reprinted with permission from Ref. [18].*

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

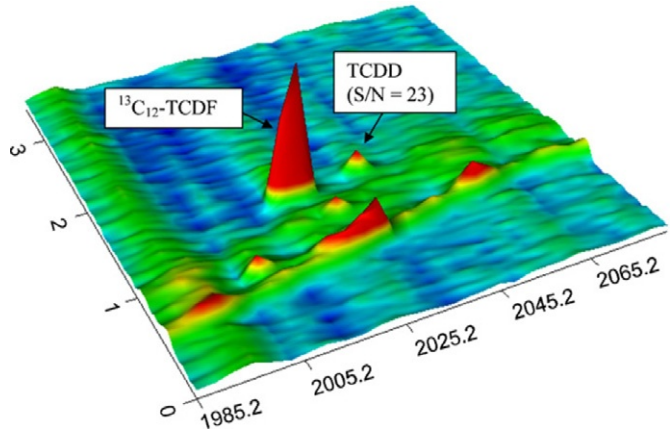

FIGURE 8 DSI–GC × GC–HS-TOF-MS chromatogram of 1 pg TCDD (*m/z* 322) injected at 0.1 pg/μL (5 pg/g cod liver oil equivalent) in a matrix-matched solution. *Reprinted with permission from Ref. [29].*

between DSI–GC × 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

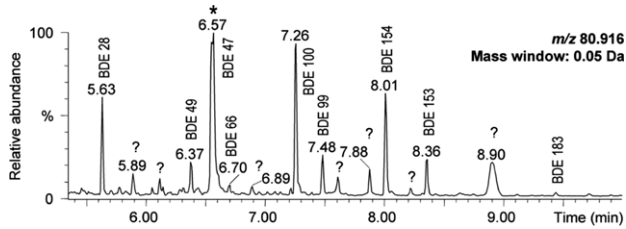

FIGURE 9 GC–HR-TOF-MS chromatograms of fish extract in NCI mode (sample equivalent of 3.2 mg injected). The saturated peak is marked by an asterisk and "unknown" compounds are marked by question marks. The target ion (*m/z* 80.916) was extracted using a 0.05 Da mass window. *Reprinted with permission from Ref. [31].*

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

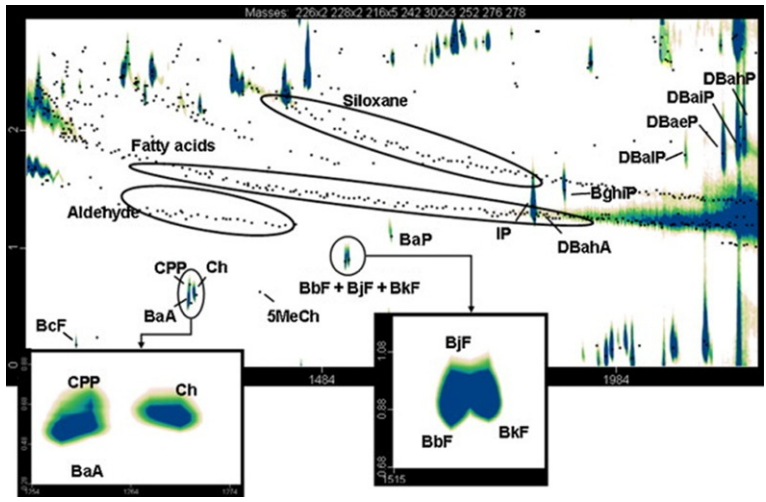

FIGURE 10 Contour plot example of an oil fortified with PAHs. Others class of compounds are present in the chromatogram as identified (aldehydes, fatty acids, and siloxanes). (Note: benz[*a*]anthracene (BaA), benzo[*b*]fluoranthene (BbF), benzo[*c*]fluorene (BcF), benzo[*j*]fluorene (BjF), benzo[*k*]fluorene (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].*

represented by (i) benz[*a*]anthracene, cyclopenta[*cd*]pyrene, and chrysene; (ii) benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, and benzo[*k*]fluoranthene; and (iii) dibenz[*ah*]anthracene, indeno[1,2,3-*cd*]pyrene, and benzo[*ghi*]perylene. Using GC × GC–HS-TOF-MS, the LOQs of the optimized sample preparation method were between 0.05 and 0.2 mg/kg. The linearity of the calibration curves were in the range of 0.05–100 ng/mL for most of the target PAHs.

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 ^{13}C-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 μg/kg, PBDEs 0.5 μg/kg, and PAHs 0.05–0.25 μg/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 μg/kg; PBDEs, 0.025–5 μg/kg; PAHs, 0.025–0.5 μg/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

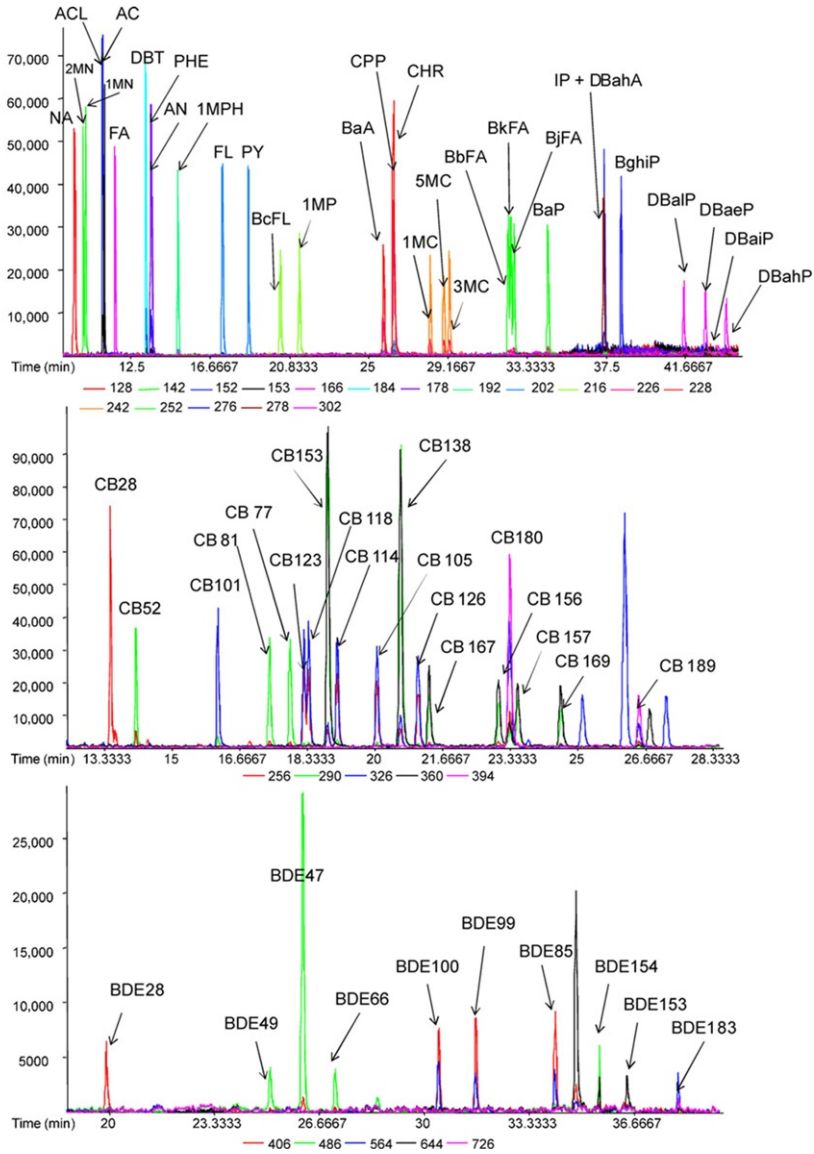

FIGURE 11 An example of a GC–HS-TOF-MS chromatogram of fish muscle tissue spiked with PCBs, PBDEs, and PAHs at 5 µg/kg (major PCBs 138, 153, and 180 and PBDE 47 at 25 µg/kg). (*Note:* acenaphthene (AC), acenaphthylene (ACL), anthracene (AN), benz[*a*]anthracene (BaA), benzo[*a*]pyrene (BaP), benzo[*b*]fluoranthene (BbFA), benzo[*c*]fluorene (BcFL), benzo[*j*]fluoranthene (BjFA), benzo[*k*]fluoranthene (BkFA), benzo[*ghi*]perylene (BghiP), chrysene (CHR), cyclopenta[*cd*]pyrene (CPP), dibenz[*ah*]anthracene (DBahA), dibenzo[*ae*]pyrene (DBaeP), dibenzo[*ah*]pyrene (DBahP), dibenzo[*ai*]pyrene (DBaiP), dibenzo[*al*]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].*

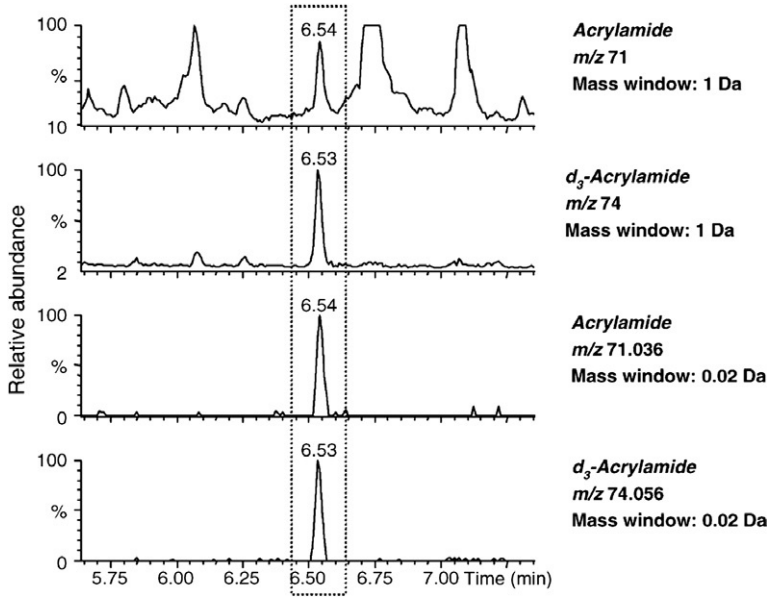

FIGURE 12 GC–HR-TOF-MS analysis of acrylamide in crisp bread samples (450 µg/kg) under conditions of different mass window settings (1 and 0.02 Da) for extraction of target ions. *Reprinted with permission from Ref. [42].*

(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 µg/kg) compared to those attainable by GC–MS, which uses a laborious and time-consuming bromination step (2–25 µg/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

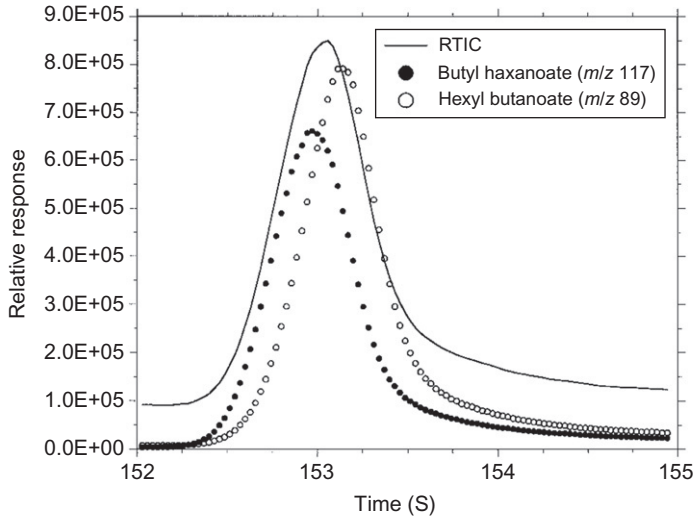

FIGURE 13 Demonstration of high-speed spectral generation (40 spectra/s) enabling the detection and quantification of coeluting compounds by using GC–HS-TOF-MS. The solid line represents the reconstructed total ion current (RTIC). Retention times differ by approximately 0.2 s. *Reprinted with permission 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, acetals, 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.

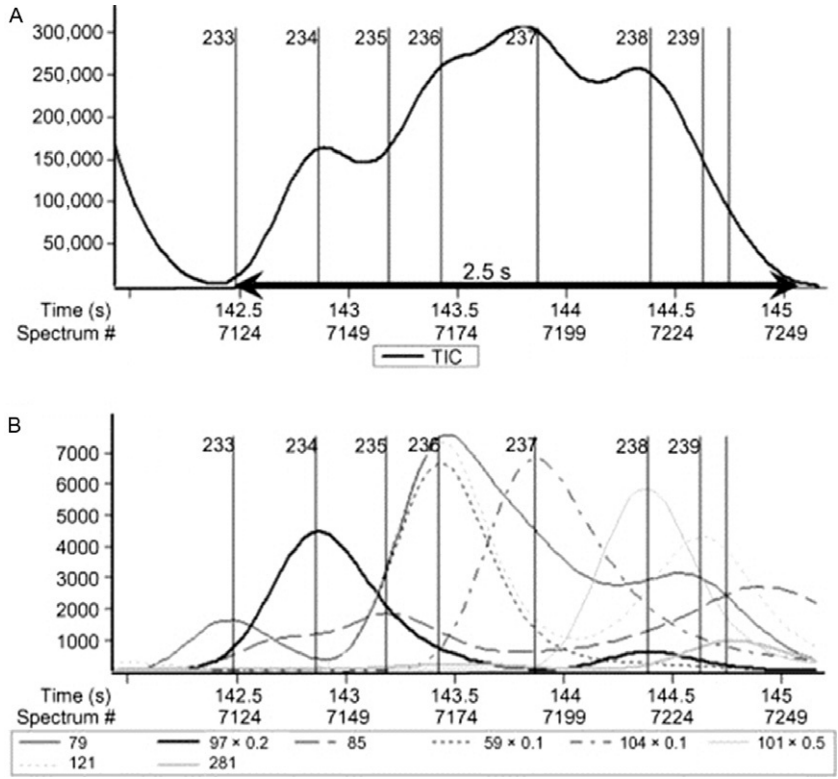

FIGURE 14 Automated peak find algorithm in the ChromaTOF software (HS-TOF-MS instrument); (A) analytical ion chromatogram (total ion current after the baseline correction and spectral deconvolution), (B) seven different compounds assigned under the analytical ion chromatogram. *Reprinted with permission from Ref. [51].*

3.2.2 Food Authenticity and Origin

Risticevic *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

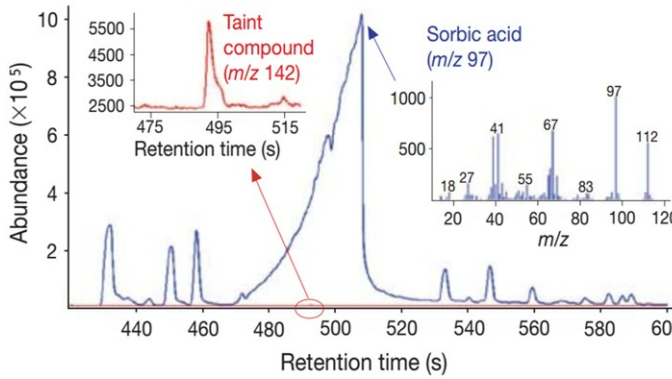

FIGURE 15 The overlay of SPME–GC–HS-TOF-MS chromatograms of sorbic acid (*m/z* 97) and taint compound (*m/z* 142) illustrating the extreme difference in respective ion intensities. *Reprinted with permission from Ref. [60].*

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,

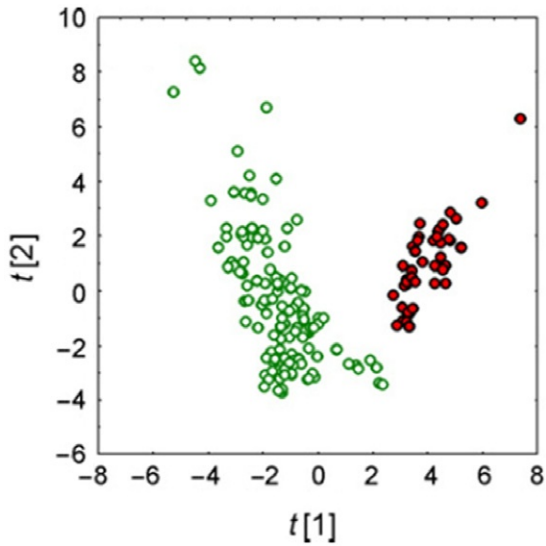

FIGURE 16 First and second PLS scores for Rochefort 6, 8, and 10 (red) versus the rest (green) of beer samples (SPME–GC–HS-TOF-MS analysis of volatiles). Graph constructed using calibration data set ($n = 166$). *Reprinted with permission from Ref. [15].*

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.

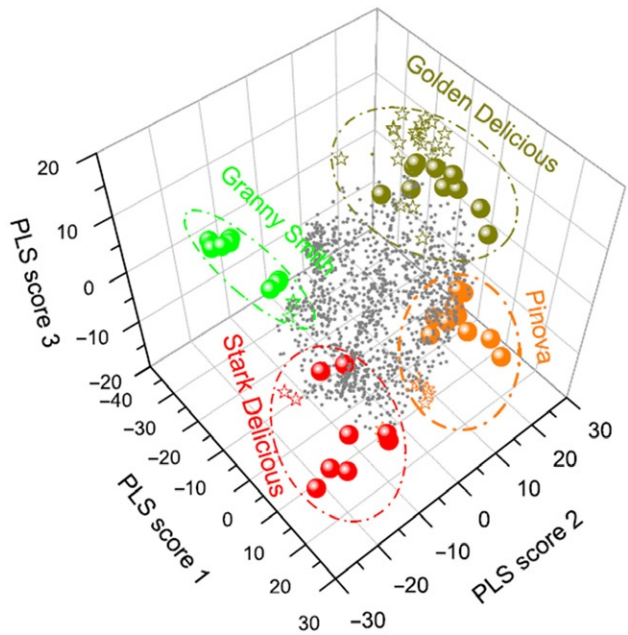

FIGURE 17 PLS-DA plot showing grouping of samples according to apple variety (SPME–GC–HR-TOF-MS analysis of volatiles). Loadings with variable importance values (VIP) >1 are also shown (in gray). Significant characteristic loadings (VIP > 1.7) for each variety are shown as stars. *Reprinted with permission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

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Chapter 13

Ultra-High Mass Resolution Miniaturized Time-of-Flight Mass Spectrometer "infiTOF" for Rapid Analysis of Polychlorinated Biphenyls

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1 INTRODUCTION

There is no doubt that mass spectrometry (MS) is a widely applied technique in several research fields. The initiative to develop miniaturized mass spectrometers is an active subject area in MS today. We believe cutting-edge analytical instruments have the ability to create novel applications in individual research fields and vice versa. Numerous miniaturized mass spectrometers are reported and commercialized from a lot of institutes and industries. The instruments have widespread applications, for example, detection and identification of chemical and biological hazards for homeland security [1,2], food safety [3], and so on. Due to their small size and lightweight, miniaturized mass spectrometers have a potential for field use. These features are absolutely suitable for on-site environmental analyses; however, there are few reports on application of miniaturized mass spectrometers in this research field. This chapter describes instrumentation and application feasibility of miniaturized mass spectrometers for polychlorinated biphenyls (PCBs) analysis. This chapter consists of the following four sections:

1. new analytical concept of "On-site mass spectrometry" and field usable mass spectrometers,
2. introduction of our technology: multiturn time-of-flight (TOF) mass spectrometer (MULTUM),
3. miniaturized ultra-high mass resolution multiturn TOF mass spectrometer system "infiTOF,"
4. PCBs analysis using infiTOF.

2 "ON-SITE MASS SPECTROMETRY" USING MINIATURIZED MASS SPECTROMETERS

In this section, a novel analytical concept "Precision On-site mass spectrometry," overview of several reported miniaturized mass spectrometers, and issues for the field use are mentioned.

2.1 On-Site Mass Spectrometry

The basis of science is to measure phenomena of nature in real time, and with strict accuracy, however, we know that it is most difficult to perform such

measurements using high-performance analyzers. Often, a multitude of instruments is required in order to detect specific targets. In this sense, the features of mass spectrometers, for example, variations of detectable targets, sensitivity, and throughput, have advantages over other analytical instruments. Based on this point, "on-site mass spectrometry" could be highly beneficial by bringing high specification mass spectrometers into various field sites to provide high-quality mass spectrometric data in real time. This concept is challenging, but on-site mass spectrometry attracts rising attention.

Considering conventional analytical methodologies using mass spectrometers, samples are usually taken from field sites, brought into laboratories, and sufficiently purified before being introduced into high-performance mass spectrometers. High-performance mass spectrometers equipped in the laboratory generally have a large footprint. The ability to bring mass spectrometers on-site is required in several research fields; however, bringing such instruments on-site is almost impossible? It is essential to miniaturize mass spectrometers while keeping performances ideal or equivalent to their full size counterparts. Furthermore, the requirement is not only simple, high-performance miniaturized mass spectrometers that are able to measure poorly purified samples, but there is also a need for simple sampling methods and sample preparations.

2.2 Overview of Miniaturized Mass Spectrometers

Methods to reduce weight and size have been attempted by various research groups. According to the reported papers on miniaturized mass spectrometers, a wide variety of instruments type including ion traps, quadrupole mass filters (QMFs) [4], magnetic sector mass spectrometers [5,6], and TOF mass spectrometers [7–10] were described. It is considered that ion traps or QMFs are more favorable than other instruments for miniaturization. In fact, almost all commercialized miniature mass spectrometers, for example, *Guardion*-7 [11] and *Griffin Analytical* 600, have adopted ion traps and QMFs. In addition, it can be noted that there is a large variety of ion traps: (1) three-dimensional hyperbolic ion traps, (2) rectilinear ion traps [12–15], (3) toroidal ion traps [11], (4) planar electrode ion traps [16–18], and (5) cylindrical ion traps [19–21].

Why were so many miniaturized mass spectrometers developed using ion trap techniques? The main reasons for choosing ion traps for portable instruments are that ion traps provide a relaxed vacuum condition and simple structures for easily miniaturized geometry with weight savings. A portable ion trap mass spectrometer system "Mini 11" was reported in 2009 [22], whose total weight with batteries was 5.0 kg, power consumption was 35 W, and dimensions were 22 cm × 12 cm × 18 cm. In addition to the miniaturized characteristics, the instrument has been coupled with wide varieties of ambient ionization sources, for example, desorption electrospray ionization, electrospray ionization, and paper spray ionization.

2.3 Issues with Miniaturized Mass Spectrometers

Miniaturized MS instruments, especially ion traps or QMS described above, appear to have usable performance for field use; however, sensitivity and mass resolution in these physically smaller devices are lower compared to laboratory instruments. To overcome the loss of sensitivity due to lower transmission of ions into and out of the analyzer, ion traps using array geometry were proposed. On the other hand, high mass resolution cannot in principle be obtained in QMFs or in ion traps. The typical mass resolution in miniaturized mass spectrometers is less than a few hundreds $R = m/\Delta m$. Here, m and Δm are mass of interest and the difference in mass. This lower mass resolution is one of the drawbacks in ion trap-based instrumentation. We believe that miniaturized instruments will be more commonly utilized in the future. In particular, high mass resolution instruments will be critically important when difficulties to perform sufficient sample preparations, "as described above," are encountered. In this case, high mass resolution is important to avoid false positives and false negatives from contaminant peaks.

How can miniaturized high mass resolution mass spectrometers become a realization? In general, mass spectrometers for high mass resolution mass spectrometry are magnetic sector mass analyzers, Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometers [23,24], and TOF mass spectrometers. However, if high mass resolution is to be achieved in these instruments, the size of instruments become restrictively large and heavy when considered for field use. This fact becomes more apparent when examining magnetic sector mass analyzers and FT-ICR mass spectrometers, which require large electrical magnets and superconductive magnets, respectively. Therefore, it is difficult to simply miniaturize these instruments and still retain their mass resolution. This point is supported by specifications of IonCam [25].

According to the structural simplicity and weight, TOF mass spectrometers are more favorable for reduction in size. Here, the mass resolution ($m/\Delta m$) of TOF can be written as

$$\frac{m}{\Delta m} = \frac{T}{2\Delta T} \tag{1}$$

where T and ΔT are flight time of interested ion and peak width (FWHM: full width at half maximum), respectively. We can easily find that mass resolution is directly proportional to TOF (i.e., the size of the instrument). Therefore, simply shortening the flight length to miniaturize the instrumentation size results in decreased mass resolution.

To overcome this fundamental problem, the flight length can be extended by various methods. The proposed systems are listed as follows:

1. Electrostatic multipass mirror systems [26,27].
2. Helical or jig-saw type systems [28,29].
3. Multiturn ion optical geometries using electrostatic sectors [30].

In Osaka University, the multiturn type TOF mass spectrometers, which have a figure of eight flight path, are mainly designed and constructed. The first multiturn TOF mass spectrometer "MULTUM Linear plus" was constructed. In the next section, we will introduce the overview and the developing history.

3 MULTITURN TOF MASS SPECTROMETERS AT OSAKA UNIVERSITY

Figure 1 shows the development history of the multiturn TOF mass spectrometer at Osaka University. We designed and constructed the first multiturn TOF mass spectrometer "MULTUM Linear plus" as a laboratory model for cometary exploration [31]. The system consists of four discrete units, each comprising an electrostatic quadrupole lens and a cylindrical electrostatic sector. The total path length of one cycle is 1.284 m. The entire system was fixed on a base plate of 40 cm × 40 cm. Using electron ionization (EI) for gas analysis, a maximum mass resolution of 350,000 (m/z 28 of N_2^+) was achieved after 500 cycles (~645 m total flight length) using this instrument. This system had fairly complex operation based on the 28 electrostatic quadrupole lenses that were required. In a next generation multiturn mass spectrometer, we studied more simplified optical geometries. As a result, a new geometry of "MULTUM II" was designed and constructed [32]. In the MULTUM II geometry, no quadrupole lenses were used. MULTUM II consisted of only four toroidal electrostatic sectors. The number of components used was dramatically reduced. The total path length of one cycle was 1.308 m. The maximum mass resolution of 250,000 (m/z 28 of N_2^+) was achieved after 1200 cycles (~1500 m in flight length). MULTUM II was equipped with not only the EI ion source but also a matrix-assisted laser desorption/ionization (MALDI) ion source for biological applications. Using the MALDI ion source, a mass resolution of 61,000 was achieved for a deca peptide of angiotensin I.

Recently, we have been developing various types of TOF mass spectrometers based on the MULTUM II technology. The first instrument was a tandem TOF mass spectrometer "MULTUM-TOF/TOF" for the structural analysis of biomolecules [33]. The second instrument was used for imaging MS. Imaging MS is a novel visualization method using MS to create a mass-dependent picture of an analyzed surface [34]. The stigmatic imaging was performed with "MULTUM-IMG" [35,36]. Another instrument was equipped with an ionization source for secondary ion mass spectrometry so that the instrument was used for high spatial resolution (approximately submicrometer) imaging MS. The third revision instruments were miniaturized multiturn TOF mass spectrometers "MULTUM-S" and infiTOF (MULTUM-S II) [37]. MULTUM-S was the first prototype. This instrument was manufactured using a wide-use lathe and milling machine, resulting in a lack of manufacturing precision and assembly accuracy. The infiTOF was an optimized design of the ion optics, manufacturing precision and packaged

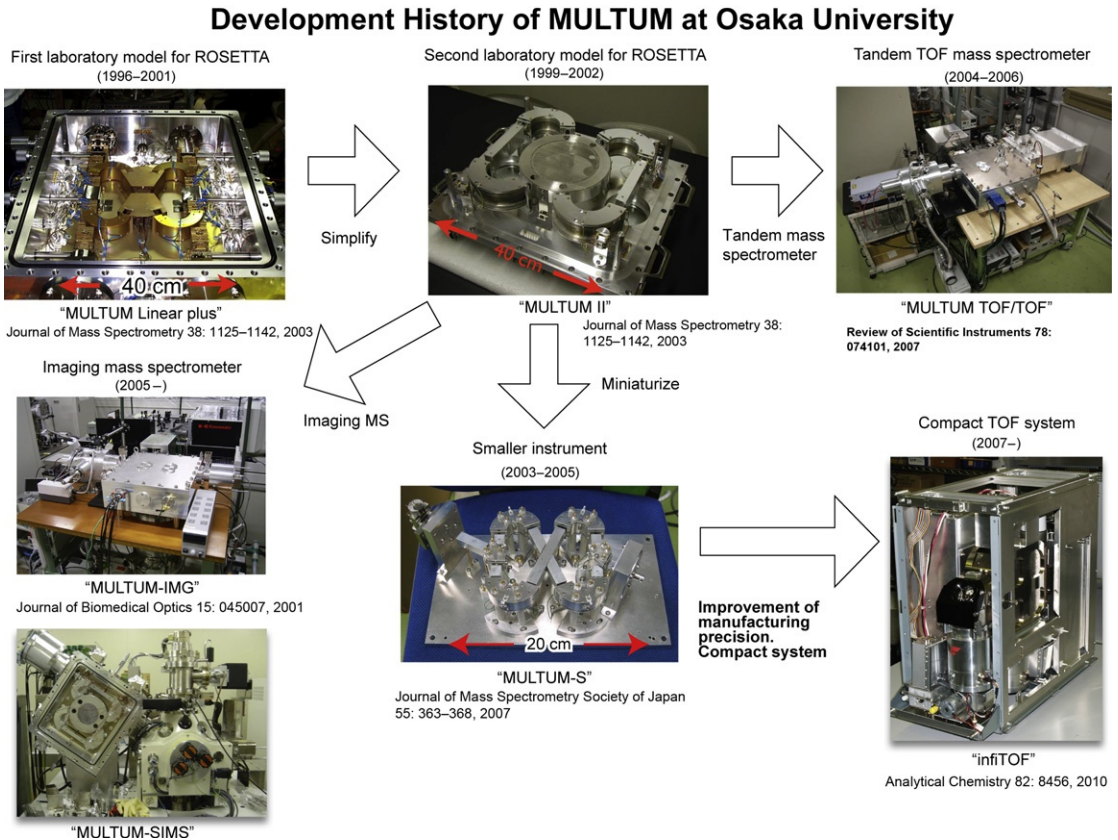

FIGURE 1 Development history of MULTUM series at Osaka University.

as a desktop type system. The detailed descriptions of infiTOF are found in the next section.

4 MINIATURIZED MULTITURN TOF MASS SPECTROMETER "infiTOF"

In this section, we explain the system and novel characteristics of the recently developed miniaturized TOF mass spectrometer "infiTOF."

4.1 Overview of infiTOF System

Photographs of infiTOF system are shown in Figure 2. The size of the analyzer is less than 20 cm × 20 cm (Figure 2A). The photographs of the whole system and the gas chromatography (GC)-infiTOF system are shown in Figure 2B and C. The developed system consists of the following: the ion source, multiturn mass analyzer, vacuum system, and high voltage circuit unit. The complete mass spectrometer weighs 36 kg, and the total size of the

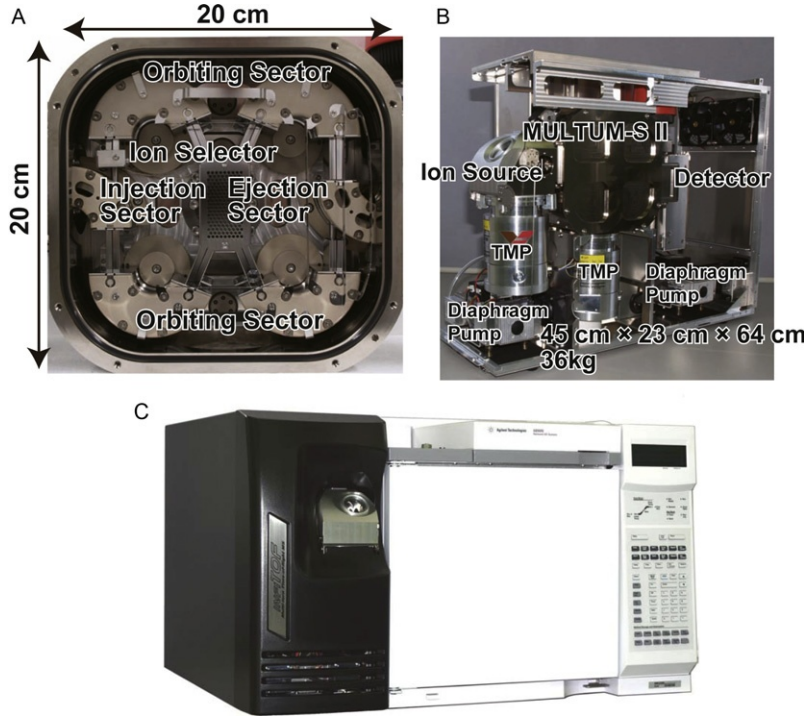

FIGURE 2 Photographs of infiTOF: (A) the inside of the analyzer, (B) the outside of the system, and (C) the GC-infiTOF system.

instrument is 45 cm × 25 cm × 64 cm. The equipped ionization source is a two-stage acceleration ion source of EI type introduced by Wiley and McLaren [38]. The accelerated ions are focused using the Einzel lens. After focusing, the ions are injected into the multiturn TOF mass spectrometer.

The main geometry of the analyzer was the same as MULTUM II. In the miniaturized instruments, it is important how ions are introduced with high efficiency into the mass analyzer from the ion source. Additionally, introduced ions need to travel with stability in the closed orbit to obtain high mass resolution. In the previous MULTUM system described in Figure 1, the ion beam passed through small holes in the outer electrodes of two of the electric sectors. When ions were injected or ejected, the voltages applied to the sector electrodes were switched. To prevent the reduction of resolution due to instability of the power supply for switching, we offer higher stability (<50 ppm) [33].

In order to overcome the problem of ion injection/ejection and stable traveling, infiTOF has two additional sectors shown in Figure 2A. Since these sectors were specialized for ion injection/ejection, the static voltage is simply applied to the orbiting sectors. For this reason, the electrical circuits for infiTOF system could be simplified and miniaturized.

4.2 Data Acquisition Methods

Block diagrams of the timing control and an illustration of the analyzer are shown in Figure 3. Ions are injected into the orbiting trajectory by applying voltage to Injection Electrode, and then the applied voltage to Injection Electrode is turned off after all ions are injected into the orbiting trajectory. When voltage is applied to the Ejection Electrode, ions are detected. Therefore, the switching timing of the Ejection Electrode controls the number of cycles. This variable flight path length in a compact space is the main advantage of the infiTOF system.

This feature becomes a powerful tool for specific ion measurement; however, an "overtaking problem" will occur in complex mixture analysis such as when analyzing PCBs. During orbiting, lighter ions take over the heavier ions because the ion velocity depends on the mass of the ion. To avoid the overtaking problem, the measuring mass range was divided into several segments. In this multisegment mode, the Ion Gate located in the orbiting trajectory controls the mass range. Therefore, the timing of Ion Gate and Ejection Electrode is individually configured as shown in Figure 3. Our software automatically calculates the timing of Ion Gate and Ejection Electrode based on the mass range and the number of cycles. Finally, the obtained segmented mass spectra are merged into one spectrum by software.

The mass resolution of this system is tunable by altering the number of cycles. Under multisegment mode, the mass range of each segment narrows as increasing the number of cycles. Additionally, data size increases as the number of segments increases. Both low mass resolution and high mass resolution

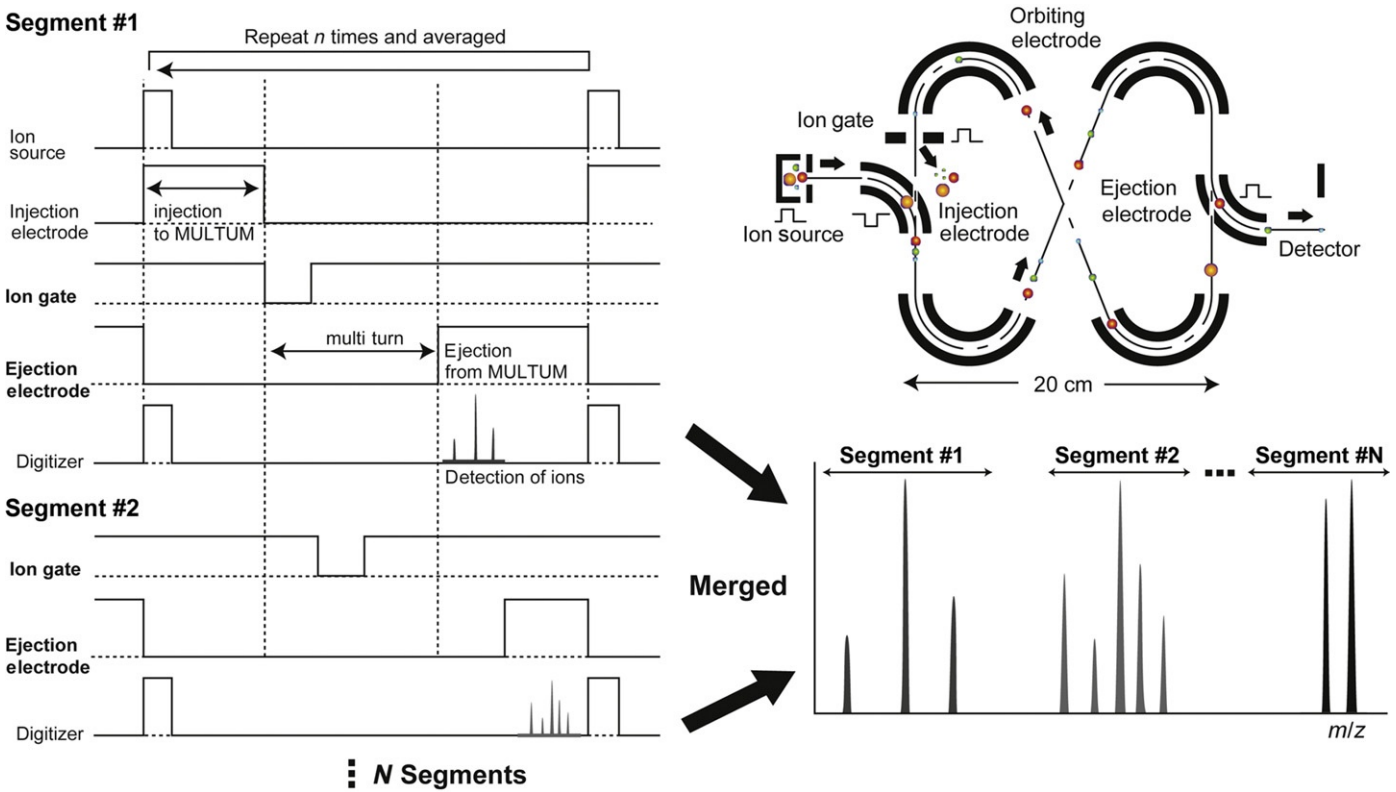

FIGURE 3 The block diagram of timing controls. To avoid the overtaking problem, the mass spectra were segmentally obtained over different mass ranges. The mass range and the number of cycles are controlled by changing timing of the ion gate potential and the ejection sector potential, respectively. The mass range is automatically calculated, and obtained spectra are merged as one spectrum.

should be used, "as the situation demands" due to these characteristics. A measurement as a screening tool in the wide mass range configuration should be performed using low mass resolution. On the other hand, when the operators measure specific molecules, high mass resolution would be preferable.

4.3 Ultra-High Mass Resolution Mass Spectra on infiTOF System

Here, we explain the tunable mass resolution capability with some measurement results. The first result is a separation of helium ion (He^+) and deuterium ion (D_2^+). Since both He and D_2 have a nominal mass of *m/z* 4, He and D_2 were detected as one peak in the low mass resolution mass spectrum (Figure 4A). To obtain this spectrum, the standard gases of He and D_2 were directly introduced into the ionization chamber via the needle valve. Before the introduction, these two gases were mixed in a gas-sampling bag. Figure 4B is the high mass resolution mass spectrum at 10 cycles. The values of the accurate mass were

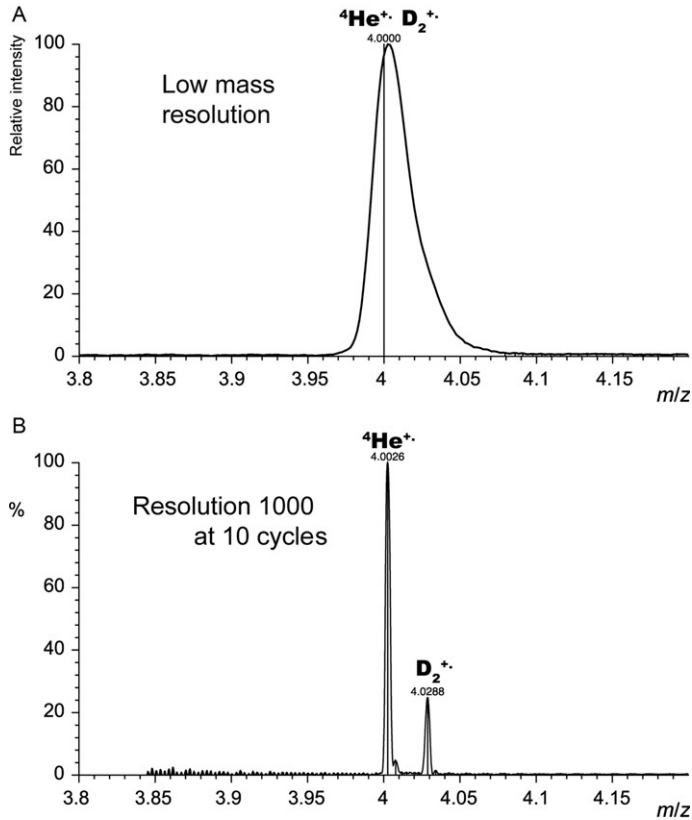

FIGURE 4 Separation of ^4He and D_2 doublet: (A) low mass resolution mass spectrum and (B) high mass resolution mass spectrum. The number of cycles was 10 cycles.

4.0026 for He and 4.0288 for D_2. Although the mass difference between He and D_2 was 0.025 u, infiTOF was able to easily separate He and D_2 even in the miniaturized instrument.

infiTOF also has a powerful potential for greenhouse gas analysis, especially carbon dioxide (CO_2), nitrous oxide (N_2O), and methane (CH_4). In the conventional methodology for field study, researchers collect samples in the field, bring them into laboratory, and then analyze using laboratory-equipped instruments. Using an alternative procedure, researchers prepare, and place several detectors to detect each species directly in the field. However, with a great variety of detectable species and portability afforded by infiTOF, it will have powerful merits for field study in the future. This high-quality gas analyzer will also help the reduction of systematic error attributed to different detectors and measurement conditions.

Figure 5 is a high mass resolution mass spectrum of CH_4 at 10 cycles. In this spectrum, the peak of CH_4 is separated from the oxygen peak (both nominal masses are *m/z* 16) derived from the fragment ion of the oxygen molecule or di-charged ion of oxygen molecule. The obtained mass resolution was 3200. We consider that this feature is merit for monitoring of CH_4 without oxygen contamination.

Furthermore, real-time monitoring of N_2O is required to elucidate the generating mechanism and investigate its trend of spread. N_2O is known as a greenhouse gas, and the warming effect is about 310 times larger than CO_2. Furthermore, N_2O is one of the ozone-depleting substances [39]. If we try to carry out real-time monitoring of N_2O and CO_2 simultaneously using MS, a mass spectrometer with high mass resolution is required because the nominal mass of N_2O is the same as that of CO_2. However, the difference in accurate mass between CO_2 and N_2O is 0.0112 u; therefore, these two peaks were expected to be separated in infiTOF. In this experiment, a mixture

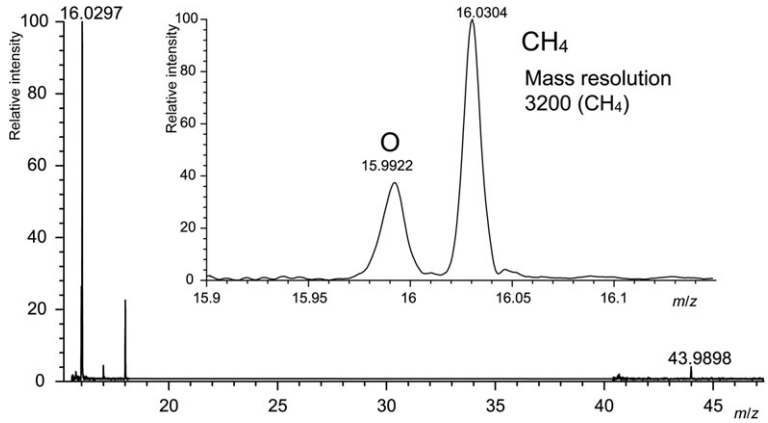

FIGURE 5 High-resolution mass spectrum of O and CH_4.

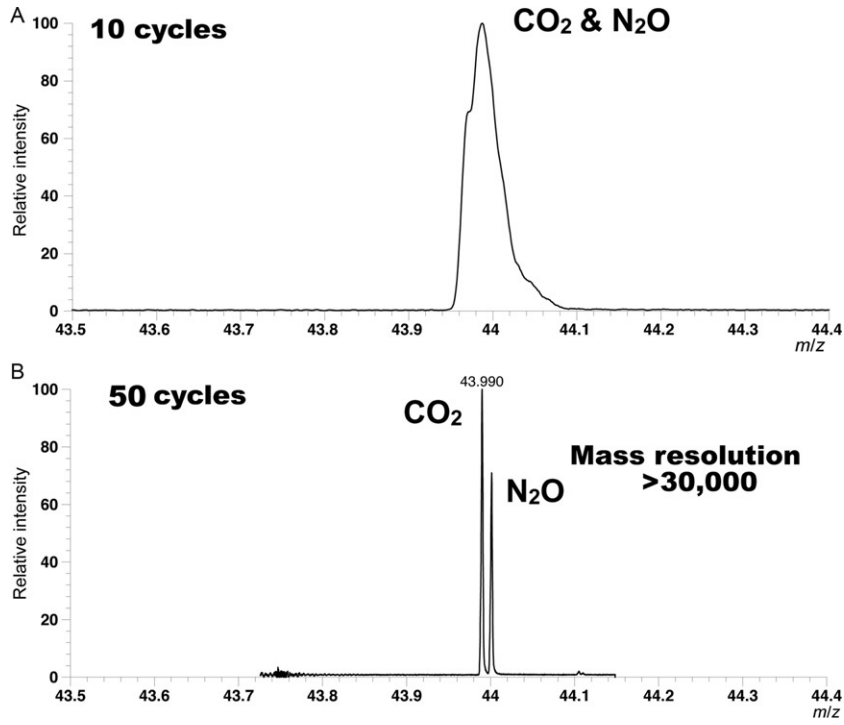

FIGURE 6 Separation of CO_2 and N_2O doublet: the spectrum at (A) 10 cycles and (B) 50 cycles.

of ultrapure CO_2 and N_2O (49.4%:50.6%) was introduced into the EI ion source via the needle valve. Figure 6 shows the difference in obtained mass spectra by changing the number of cycles. Figure 6A shows the mass spectrum of the CO_2 and N_2O doublet peak after 10 cycles. In this cycle, the doublet peak did not separate due to the lack of mass resolution. After 50 cycles (Figure 6B), these two peaks were completely separated, and consequently, the obtained mass resolution was 30,000. The sensitivity for N_2O in this high mass resolution mode was approximately 220 ppb (data not shown). This ultra-high mass resolution was the first achieved in a miniaturized mass spectrometer for these compounds. This mass resolution was comparable to those of laboratory-equipped instruments. We would like to note that the maximal time to acquire one spectrum was 1 ms. Therefore, high mass resolution accompanying fast data acquisition will be helpful for the on-site real-time monitoring.

Achieving high mass resolution is advantageous when trying to determine accurate masses. In the previous study, a mass accuracy of 2.3 ppm was achieved [37]. Capability of accurate mass measurement in the miniaturized mass spectrometer is another advantage to infiTOF.

5 PCBs ANALYSIS IN GC-infiTOF SYSTEM

PCBs are used in capacitors and transformers because of their superior dielectric and fire-retarding properties. In addition, they are utilized in heat transfer fluids, hydraulic fluids, lubricant oils, pesticides, paints, and plastics. Though they are employed in numerous commercial and industrial applications, it is known that PCBs are a catalyst to many adverse health effects. Many investigations have substantiated that PCBs have polluted the environment globally as a result of either intentional or unintentional release. Consequently, the environmental monitoring of PCBs contained in a number of materials is performed in various countries [40–42].

Currently, the methods of analysis for PCBs are complicated because time-consuming pretreatment and cleanup steps are required. In addition, the sample preparation techniques are commonly based on more than one chromatographic separation. By contrast, the pretreatment for gas chromatography–high-resolution mass spectrometry (GC–HRMS) is rather simple. The concept of GC–HRMS for accurate and congener-specific determination of dioxins and dioxin-like CB analysis is well established. The analysis was traditionally carried out by using magnetic sector mass spectrometers to provide sensitive and selective detection. Although the sensitivity and selectivity offered by GC–HRMS are unrivaled, the throughput is low, the cost is high, and the instruments have a large laboratory footprint. Furthermore, magnetic sector mass spectrometer setup and method development can be fairly complicated relative to other MS technologies.

In recent years, U.S. Environmental Protection Agency provided reports on the needs for on-site PCB monitoring using miniaturized or portable analyzers. Therefore, we consider that on-site PCB monitoring is required, and ultra-high mass resolution of infiTOF can be a good alternative to magnetic sector mass spectrometers.

5.1 Performance Evaluation Using Hepta-CB Standard

GC was performed using an Agilent 6890N. A FORTE HT8 column (12 m × 0.22 mm i.d.; film thickness 0.25 μm, SGE, Australia) was included in the GC-infiTOF system (Figure 2C). The GC column temperature was programmed to rise from 100 to 320 °C at a rate of 40 °C/min, which resulted in a total GC run time of 5.5 min. The inlet temperature was kept at 320 °C, and helium was used as a carrier gas at the constant flow rate of 70.0 cm/s. A sample of 1.0 μL was injected into an inlet set to splitless mode. The ion source temperature and the interface temperature between GC and the mass spectrometer were kept at 280 and 320 °C, respectively. The pressure in the analyzer chamber was kept at 1.2×10^{-4} Pa. For the ionization method, the filament current was 4000 mA and ionization voltages were variable. The optimized ionization voltage is discussed later. The obtained mass range

was from m/z 180 to 505 and set to 10 segments to obtain each molecular peak of PCB congeners.

As a starting point, we investigated the optimal ionization conditions to evaluate the performance of this system. Here, the acceleration energies of electrons were regarded as a variable parameter. The ion signal dependence (10, 15, 16, 18, 20, 38, and 70 eV) on the electron energy is shown in Figure 7A. The intensity of a peak corresponding to m/z 393.8, "base peak in the spectrum of hepta-CB," was used for quantification. Compared with 70 eV (typical electron energy for EI), the intensities around 20 eV were nine times larger. Therefore, all experiments described as follows were performed at an ionization energy of 18 eV.

Under this condition, the linear signal response for infiTOF was tested. The hepta-CB congener was diluted with isooctane. In the linearity study, mixing ratios of analytes from 1 ppb to 1 ppm were prepared. A sample of 1.0 μL was injected into the GC system and was measured at 20 cycles through the analyzer (high mass resolution mode). During this experiment, the parameters, including the gain of the detector (secondary electron multiplier 14,880, ETP, Ermington, Australia a subsidiary of the SGE group), were fixed. Peak intensities were plotted as a function of the solution concentration (1 ppb, 10 ppb, 100 ppb, and 1 ppm) (Figure 7B). The linear signal response was $R^2 = 0.999$ from 5 ppb to 1 ppm under the same experimental conditions.

Mass spectra acquired at 2 and 20 cycles are shown in Figure 7C and D, respectively. In the mass spectrum at two cycles, the FWHM of the peak was wide so that complete separations of adjacent isotopic peaks could not be achieved. After 20 cycles, the isotope peaks were clearly separated from each other. The FWHM of the peak was 0.038 Da, and the mass resolution achieved was greater than 10,000, which is comparable to the mass resolution used in magnetic sector instruments for HRMS analysis.

In a previous study, we found that approximately 98% of ions survived during each cycle. The loss of ions was attributed to collisions between residual gas in the analyzer and the ions. Therefore, the limit of detection (LOD) needed to be evaluated when performing HRMS at 20 cycles. To evaluate the LOD in this system, a voltage of 3200 V was applied to the detector. The total ion chromatogram (TIC) and mass chromatogram of 1 ppb are shown in Figure 7E. In these chromatograms, hepta-CB at 1 ppb was found to be detectable with a signal-to-noise ratio >2.

5.2 Fast GC/HRMS Using a Comprehensive PCBs Mixture

We applied the fast GC/HRMS of a comprehensive 66 PCBs mixture using the multisegment mode. Ten segments were configured to detect each molecular ion of mono- to deca-CB congeners. The TIC of the PCB mixture is shown in Figure 8. All components of the PCB mixture were detected within 5.5 min. In the obtained TIC, the intense peaks were found around

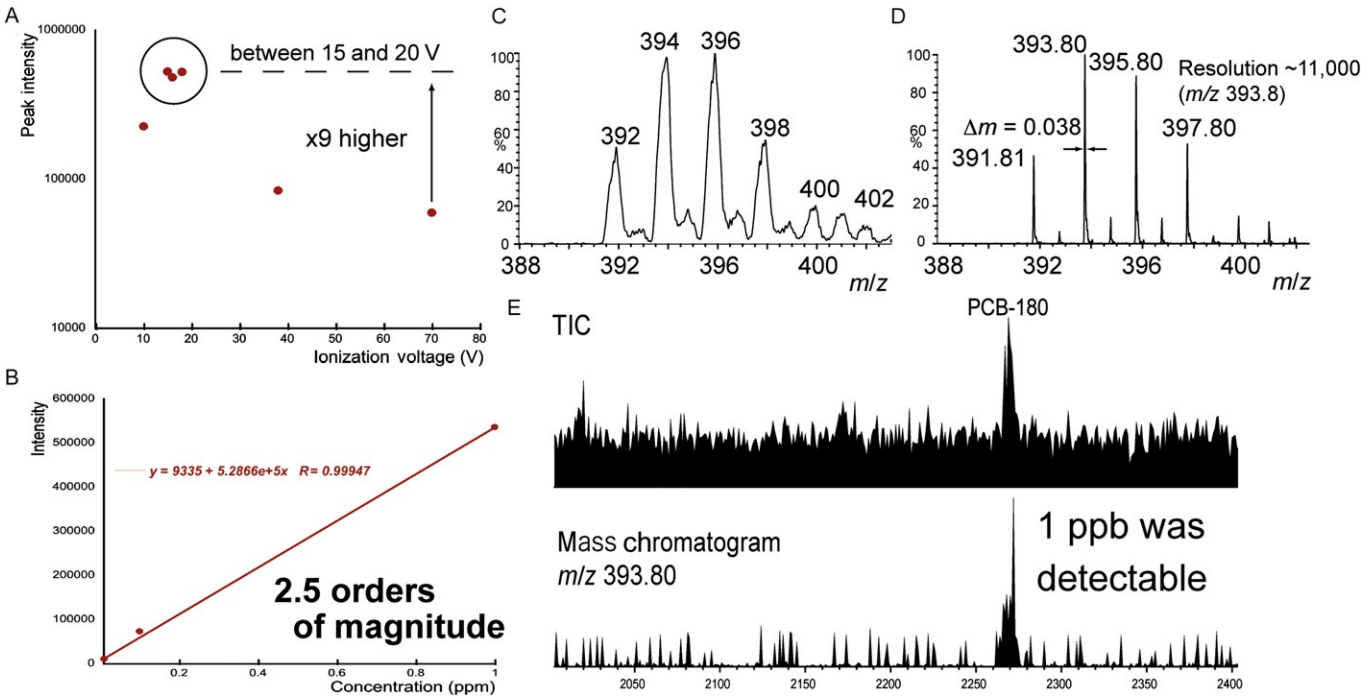

FIGURE 7 (A) Peak intensity variation over different ionization voltages. (B) Signal response curve from 1 ppb and 1 ppm. Comparison of hepta-chlorobiphenyl mass spectra: (C) 2 cycles (low mass resolution <400) and (D) 20 cycles (high mass resolution ~11,000). (E) Total ion chromatogram and the mass chromatogram (*m*/*z* 393.80 ±0.02) of 1 ppb.

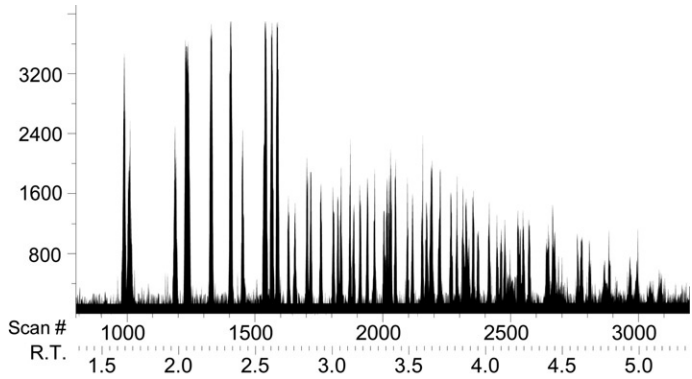

FIGURE 8 Total ion chromatograms of 66 PCBs mixture measured with the GC-infiTOF system. All components were detected within approximately 5 min.

1.5–2.5 min and were derived from mono- and di-CB congeners. Mono-CB and di-CB congeners were included at a concentration of 2 ppm. In the averaged mass spectra of each congener, we found all spectra had mass resolutions of approximately 10,000.

It is important to note that the signal-to-noise ratio in higher boiling point components was gradually falling as shown in Figure 8. In other words, the width of the peak detected in the mass chromatogram was broader. This property was significant in higher boiling point constituents of nona-CBs and deca-CB. This characteristic was attributed to GC-induced mass discrimination, because of an unrepresentative sampling from the injector to the column of low and high molecular weight components.

5.3 Separation of Contaminant Peaks in the Dielectric Coolant Fluid

As it was previously mentioned, the attributes of this instrument were the compact enclosure and tunable mass resolution. These attributes lead to the idea that PCB congeners could be detected without the effects of matrix interferences, in the event enough sample purification could not be performed during an on-site measurement. In this experiment, we investigated the influence of matrix contaminant peaks on PCBs signal peaks at different mass resolutions. Measurements were taken at 2 cycles during low-mass resolution and at 20 cycles for high mass resolution. The dielectric coolant fluid was diluted 1000-fold with *n*-hexane and was used as the matrix analyte. We obtained two chromatograms: one from the diluted fluid and another one from the spiked PCB congeners into the native diluted fluid. The final concentration of the PCB mixture was 0.02 ppm for mono- and di-CBs, and 0.01 ppm for the others. Results during low mass resolution are shown in Figure 9.

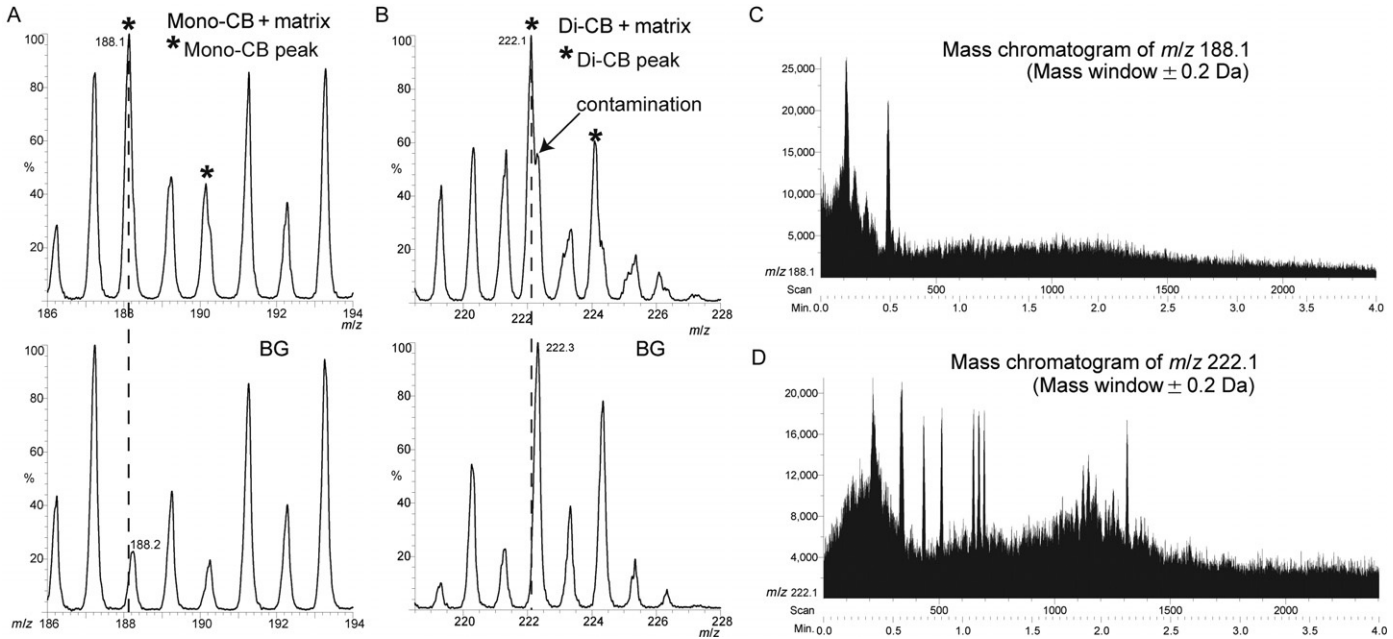

FIGURE 9 Effects of background (BG) interference in the low mass resolution mass spectra. The PCB mixture spiked in diluted dielectric fluids was used as the analyte. The comparison of mass spectra with and without PCBs: (A) mono-CBs and (B) di-CBs. Signal peaks of PCBs are represented by an asterisk. Extracted mass chromatograms of (C) *m/z* 188.1 and (D) 222.1. In all mass chromatograms, the mass window was set at ±0.2 Da.

Figure 9A and B show mass spectra that compare the spiked fluid and the blank fluid samples. Since there were high-intensity contaminant peaks at low temperature (data not shown), the focus here was on the influence of the contaminant peaks as they related to the mono- and di-CB peaks. These contaminant peaks were derived from organic substances included in the coolant fluid. Mass ranges of presented mass spectra were enlarged around *m/z* of base peak of mono- and di-CBs. Comparing background spectra, which is represented as "BG" in each spectrum, mono- and di-CB peaks were not separated from contaminant peaks. This incomplete separation was attributed to the broad peak width. In addition, the small mass difference of 0.1 Da was not enough to separate ions in low mass resolution. In Figure 9A, analyte peaks and contaminant peaks were completely overlapped. In Figure 9B, we could find a bump derived from the matrix peak at the shoulder of *m/z* 222.1 (indicated by an arrow). Obtained extracted mass chromatograms of mono- and di-CBs are displayed in Figure 9C and D, respectively. Here, the mass window of extracted mass chromatograms was set to ±0.2 Da. In the obtained mass chromatograms, the baseline noise, "due to the intense contaminant peak," existed from start to 2.5 min. During this retention time, the peaks derived from mono- and di-CB congeners were overlapped with the baseline noise.

Results of high mass resolution are shown in Figure 10. A peak set at *m/z* 188.04, 189.04, 190.04, and 191.04 corresponds to signal peaks of mono-CB (Figure 10A), and additional peak set at *m/z* 222.01, 223.01, 224.01, 225.01, and 226.01 corresponds to signal peaks of di-CB (Figure 10B). It can be seen that background spectra signal peaks and contaminant peaks were clearly separated. The difference of *m/z* between signal peaks and contaminant peaks were 0.15 Da in the mono-CB spectrum and 0.26 Da in the di-CB spectrum. By acquiring data with the complete separation of signal peaks and contaminant peaks, extracted mass chromatograms with high mass selectivity were available. In Figure 10C and D, extracted mass chromatograms of *m/z* 188.04 and 222.01, which have a mass window of ±0.02 Da, are displayed. For comparison, mass chromatograms of contaminant peaks (*m/z* 188.19 and 222.28) are included as insets. In the mass chromatograms of *m/z* 188.04 and 222.01, the baseline level in each chromatogram was dramatically reduced as compared to the low mass resolution results. These chromatograms were in good agreement with mass chromatograms of mono-CB and di-CB using the comprehensive 66 PCBs mixture (data not shown), even after simple pretreatment. These results demonstrate that GC-infiTOF produces correct HR mass chromatograms for PCBs even in the presence of significant interferences such as dielectric fluids. This feature along with the compact design of the system will be an advantage for rapid on-site detection of PCBs.

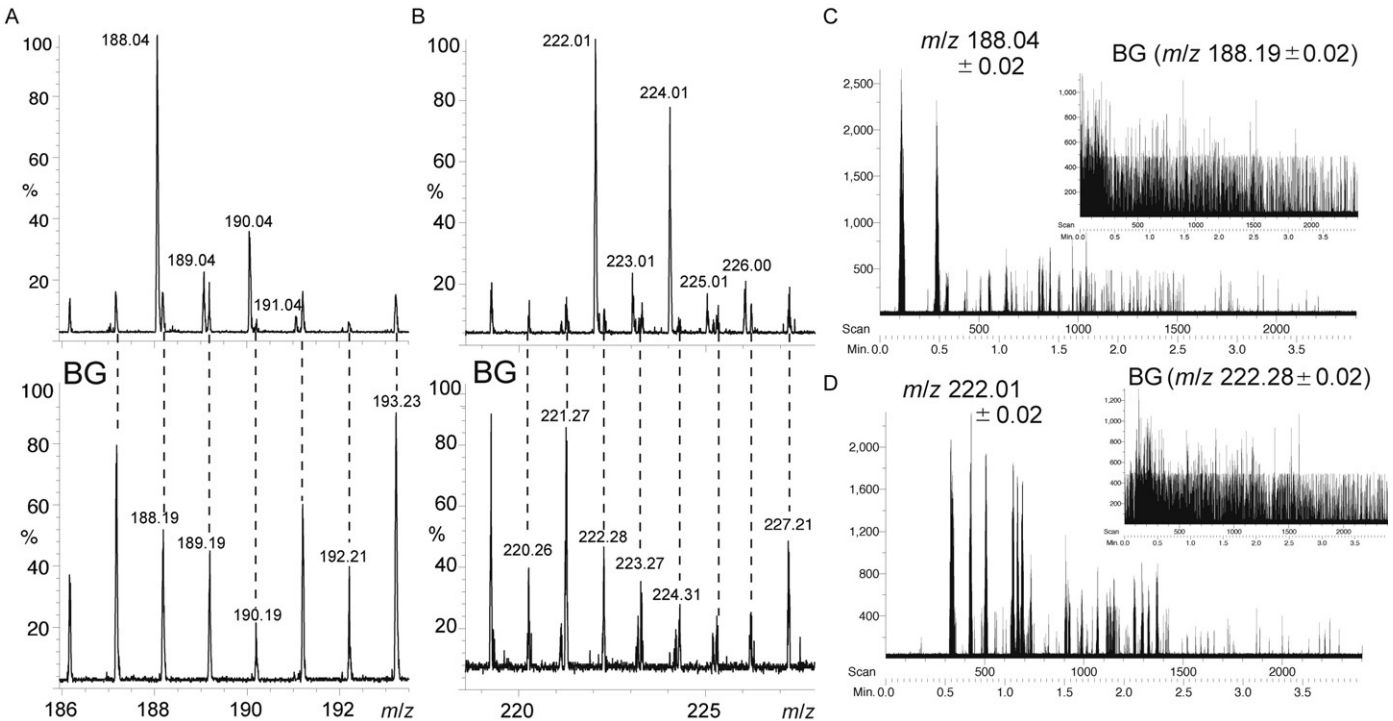

FIGURE 10 Effects of background (BG) interference in the high mass resolution mass spectra. (A) mono-CBs and (B) di-CBs. High-resolution extracted mass chromatograms of (C) *m/z* 188.06 and (D) *m/z* 222.04. Extracted mass chromatograms of *m/z* 188.19 and 222.27, which are background interference peaks, are displayed as insets. In all mass chromatogram, the mass window was set at ±0.02 Da.

6 CONCLUSIONS

To date, there are no commercially viable miniaturized mass spectrometers that can offer both portability and high mass resolution suitable for environmental analysis such as greenhouse gases and PCBs. In this chapter, we explained and demonstrated the performance of the infiTOF system as a novel analytical instrument with respect to environmental chemistry. From the point of view of routine high mass resolution and LOD for on-site measurements, the potential utility of PCBs analysis using infiTOF will be a better alternative to magnetic sector instruments. In the current setup, a commonly used GC system was equipped with infiTOF, but by miniaturizing the mass spectrometer as well as the GC systems, the high-performance field portable PCB analyzer will become a reality in the near future.

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Chapter 14

Environmental Applications of Soft Ionization with GC–TOFMS and GC–QTOFMS

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1 INTRODUCTION

Soft ionization techniques for gas chromatography include: (a) chemical ionization [1], which uses a reagent gas and lower energy electrons (10–20 eV) than conventional electron ionization (EI), (b) field ionization [2], and (c) photoionization [3]. Photoionization is achieved either by resonance-enhanced multiphoton ionization (REMPI) with intense UV-laser pulses in the wavelength range of 193–350 nm [4,5] or by the more universal single-photon ionization (SPI) method with vacuum ultraviolet (VUV) light from lamps or lasers in the wavelength range of 110–150 nm [5]. Rapid switching between EI and REMPI [6] or between EI and soft SPI [7] has been reported.

The soft ionization source used in this study incorporates a microplasma discharge as a source of VUV photons to accomplish SPI. The VUV emission is due to resonance fluorescence of excited atomic species that comprise the microplasma discharge. The wavelength of the resonance fluorescence (Figure 1) is dependent upon the electronic structure of the excited species;

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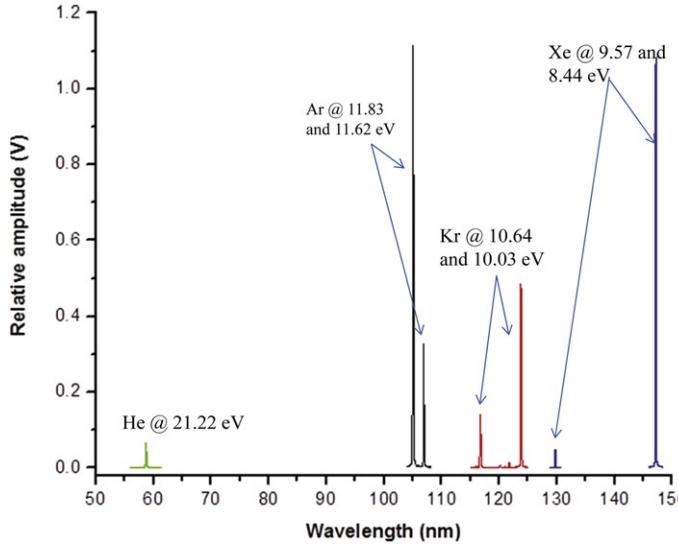

FIGURE 1 Resonance lines of various plasma gases—reflectivity versus wavelength. The relative amplitudes are not corrected for variation in grating.

therefore the energy of the VUV photon can be tuned by choice of plasma gas to values in the range of 104–150 nm, corresponding to photon energies between ~8 and 12 eV. Furthermore, our soft ionization source utilizes a windowless design that allows operation with the full range of rare gas mixtures, including those with emission lines below the ~105–115 nm cutoff of VUV transmissible materials such as LiF and MgF_2. A review of microplasma developments for analytical instrumentation was published by Karanassios [8] and details on a microfabricated microplasma source, in which plasma is generated in a 25-μm discharge gap formed in a ring-shaped microstrip transmission line, are given by Hopwood *et al.* [9].

An ionization source is generally considered "soft" when the number of intact molecular ion species produced in the source is significantly greater that the number of fragment ions, for a given analyte. Fragmentation is generally reduced at lower ionization energies because there is less energy transferred to the analyte in excess of what is required for ionization, and therefore there are less unimolecular decay pathways that are accessible to the activated complex. The ionization source used in this study can be operated in a manner that promotes "soft" ionization for a given analyte by choosing a plasma gas with the smallest resonance emission energy that is also greater than the vertical ionization energy of the analyte. The intensity of the molecular ion species that is observed is then also dependent on the thermodynamic stability of the ionic species.

In addition to the "soft" ionization mode, our ionization source can also be operated in a selective mode, where the photoionization energy is chosen to be less than the vertical ionization energy of the solvent used for analysis or the components of the sample matrix. Finally, it is also possible to choose a photon energy that is much higher than the vertical ionization energy to promote fragmentation, thus providing MS/MS like analysis.

There is a vast amount of information published on various polycyclic aromatic sulfur heterocycles (PASHs) in petroleum using different analytical tools including Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) using EI, electrospray ionization (ESI), field desorption/ionization, and atmospheric pressure photoionization [10]. To date, more than 60,000 different compounds containing C, H, N, O, and S have been identified [10]. ESI works well for analytes that are strong acids and strong bases, but it was found to be "blind" to aromatic hydrocarbons, thiophenes, and furans [10]. An ambient ionization/desorption technique known as the ambient sonic spray ionization mass spectrometry, which can be run in both positive and negative mode, provided similar compositional information as ESI when applied to crude oil samples [11]. Similarly, an atmospheric pressure low-temperature plasma probe, which desorbs and ionizes compounds from a petroleum sample without any sample preparation, was also reported recently [12]. PASHs reported in petroleum include thiophene, benzothiophene, dibenzothiophene (DBT), methyldibenzothiophenes (MDBTs), dimethyl-dibenzothiophenes (DMDBTs), trimethyl-dibenzothiophenes (TMDBTs), naphthothiophenes, benzonaphthothiophenes (BNTs), methyl-benzonaphthothiophenes (MBNTs), phenan throthiophene, chrysenothiophene, and dinaphthothiophene [13].

In this chapter, we focus our discussion on the analysis of aliphatic hydrocarbons, specifically on $C_{24}H_{50}$ hydrocarbon, and several PASHs in a NIST certified standard reference material (SRM), which is a Texas light-sour crude oil certified only for sulfur content, and a NIST certified mixture of polynuclear aromatic hydrocarbons (PAHs) isolated from coal tar that was certified for PAHs and 11 PASHs [14,15].

2 EXPERIMENTAL

2.1 Chemicals and Reagents

DBT (99.4%), 4,6-dimethyldibenzothiophene (97%), 2-methylthianaphthene (97%), 3-methylbenzothiophene (liquid, 96%), and a composite solution of 15 aliphatic hydrocarbons at 1 mg/mL in dichloromethane:carbon disulfide 85:15, which contained the $C_{24}H_{50}$ hydrocarbon and was identified as "Connecticut *n*-Hydrocarbon Mix" were obtained from Sigma–Aldrich (St. Louis, MO, USA). The NIST SRM 2721 is a light-sour crude oil that was passed through a 10 μm filter and blended before being ampouled by NIST. The only certified values for this oil are: S @$1.5832 \pm 0.0044\%$; Hg @41.7 ± 5.7 ng/kg, which

were obtained by isotope dilution thermal ionization mass spectrometry and cold vapor isotope dilution inductively coupled plasma mass spectrometry, respectively [14]. This oil was diluted with dichloromethane prior to analysis by GC–TOFMS. The NIST SRM 1597a is a complex mixture of PAHs isolated from coal tar and dissolved in toluene. In addition to PAHs, the NIST SRM 1597a has been certified for dibenzothiophene, three naphthothiophenes (i.e., naphtho[1,2-b]-, naphtho[2,1-b]-, and naphtho[2,3-b]thiophene), four MDBTs (i.e., 1-, 2-, 3-, and 4-MDBT), and three benzo[b]naphthothiophenes (i.e., benzo[b]naphtho[2,1-d]-, benzo[b]naphtho[1,2-d]-, and benzo[b]naphtho [2,3-d]-thiophene) [15].

Spectroscopic-grade dichloromethane was purchased from J.T. Baker (Phillipsburg, NJ, USA). Research Plus grade Xe and ultra-high purity 10% Kr in helium were purchased from Scott Specialty Gases (Plumsteadville, PA, USA) and ultra-high purity Ar was purchased from Airgas USA, LLC (Long Beach, CA, USA).

2.2 GC-MPPI High-Resolution TOF and QTOF Mass Spectrometer

The analysis of PASHs in the two NIST SRMs was performed on a research GC–TOF mass spectrometer equipped with a prototype microplasma photoionization (MPPI) source, operated in positive ion mode, and interfaced to an Agilent 7890 GC (Agilent Technologies, Santa Clara, CA, USA). The TOF mass spectrometer was a modified Agilent 6200 Accurate Mass TOF LC/MS system with a flight path of 2 m and a 4-GHz data acquisition system. The analysis of aliphatic hydrocarbons was performed on Agilent's 7200 QTOF mass spectrometer with a removable prototype MPPI source, and interfaced with an Agilent 7890 GC. Spectral data were averaged at a rate of 5 scans/s and the mass range for data acquisition was from *m/z* 42 to 600. The mass axis was calibrated daily using perfluorotributylamine, which was delivered to the MPPI source via the GC–MS transfer line. The mass resolution of the TOF mass spectrometer and QTOF mass spectrometer was approximately 10,000 (full width at half maximum, FWHM) at *m/z* 271.9867, which is the molecular ion of octafluoronaphthalene. The MPPI source schematic and other operational details are given elsewhere [16].

Samples were introduced via a 30 m × 0.25 mm id × 0.25 μm film thickness HP-5MS capillary column from Agilent Technologies. The oven temperature for the separation of a mixture of aliphatic hydrocarbons, including $C_{24}H_{50}$, was programmed from 35 to 150 °C (hold for 1 min) at 5 °C/min, 150–200 °C at 6 °C/min, and then to a final temperature of 280 °C at 16 °C/min, where it was held for 18 min. Helium was used as carrier gas at a flow rate of 1.2 mL/min. The injector temperature was 280 °C, the source temperature was 175 °C, and the GC–MS transfer line temperature was 280 °C. The injector, fitted with a double tapered liner, was set in splitless mode. For analysis of

the NIST 2721 crude oil and the NIST 1597a coal tar, the oven temperature was programmed from 50 to 300 °C at 10 °C/min, followed by another temperature ramp to 310 °C at 15 °C/min, and it was held at 310 °C for 5 min. The injector temperature and the GC–MS transfer line temperature were set at 300 °C. The flow rate of the carrier gas was 1.2 mL/min.

Data processing was performed using MassHunter Qualitative analysis software (Agilent Technologies, version B.05.00) and possible ion formulae were obtained using the Qual Formula Calculator algorithm incorporated in the software. The option "odd-electron ion" was selected for the molecular ion (if present) and "odd- and even-electron ion" was selected for the fragment ions.

3 RESULTS AND DISCUSSION

3.1 Aliphatic Hydrocarbons

Fragmentation can result from unimolecular decay of a molecular ion species that is activated by an energetic ionization event. Figure 2 shows the mass spectra obtained from the aliphatic hydrocarbon $C_{24}H_{50}$ by both Ar- and Kr-MPPI TOF mass spectrometry. The spectrum obtained by Kr-MPPI (Figure 2B) shows a base peak at *m/z* 338.3926, which corresponds to molecular ion $C_{24}H_{50}^{+\cdot}$. Note that the molecular ion species is only observed with a relative intensity of 2.5% by EI at 70 eV [17]. The mass spectrum obtained by Ar-MPPI (Figure 2A) yields 80% relative intensity of the molecular ion species. Observed in both mass spectra are a series of homologous fragments with a mass difference of 14.0156 u corresponding to the loss of CH_2 radical. The mass spectrum of $C_{24}H_{50}$ obtained by Kr-MPPI (10.0/10.6 eV) exhibits significantly less fragmentation than that obtained by Ar-MPPI (11.6/11.8 eV), or standard EI (70 eV) that results in further fragmentation.

Figure 3 shows mass spectra of $C_{24}H_{50}$ obtained by Ar-MPPI and QTOF mass spectrometry, which employs a quadrupole mass filter in radio frequency only mode and a collision cell using nitrogen as the collision gas. The three mass spectra show that the relative abundance of the molecular ion is significantly reduced in QTOF analysis with respect to that observed in Ar-MPPI TOF analysis (Figure 2A). The three mass spectra show the existence of an effect that relates the relative intensity of the molecular ion and the birth potential of the nascent ions. The three mass spectra were collected at birth potentials with respect to collision cell of 3, 4, and 5.9 V. The relative intensity of the molecular ion varied from 20% at 3 V to $<1\%$ at 5.9 V, whereas it is observed at 80% with a TOF mass spectrometer.

To further investigate this phenomenon, we carried out experiments with and without the collision gas and also incorporated a MgF_2 window into our windowless source design to determine the possible effect of Kr^+ ions. All mass spectra of $C_{24}H_{50}$ in Figure 4 were obtained by Kr-MPPI QTOF mass

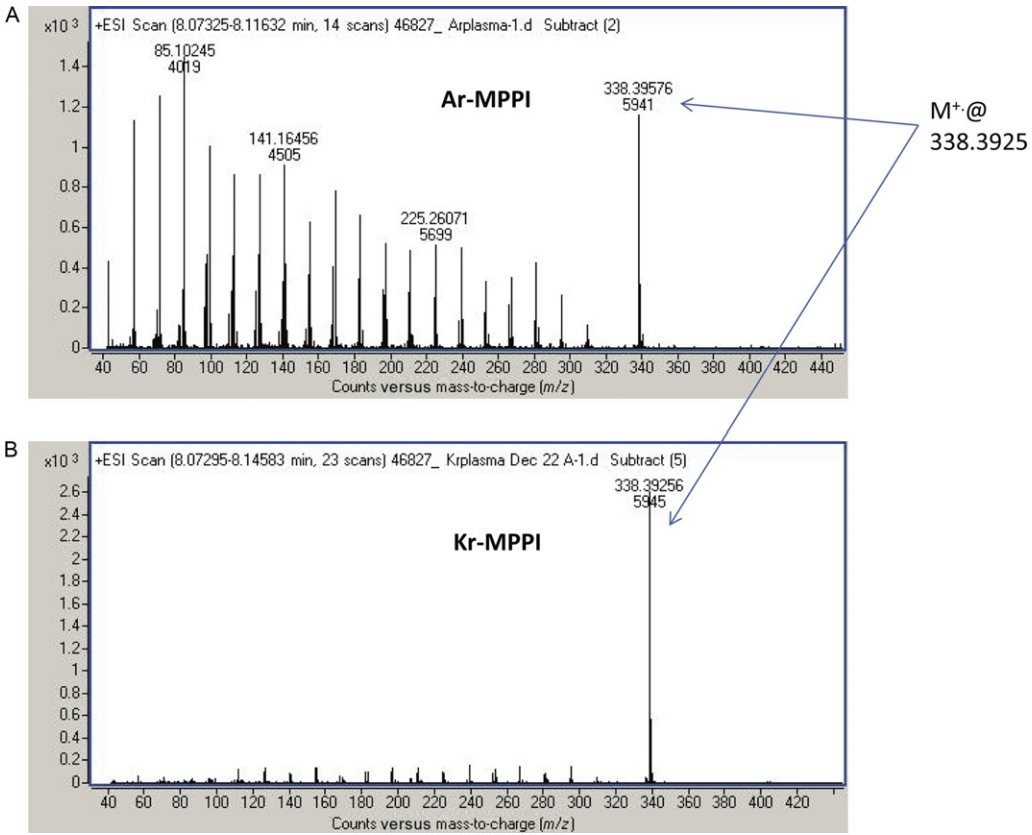

FIGURE 2 (A) Ar-MPPI and (B) Kr-MPPI spectra of $C_{24}H_{50}$ aliphatic hydrocarbon obtained with a TOF mass spectrometer.

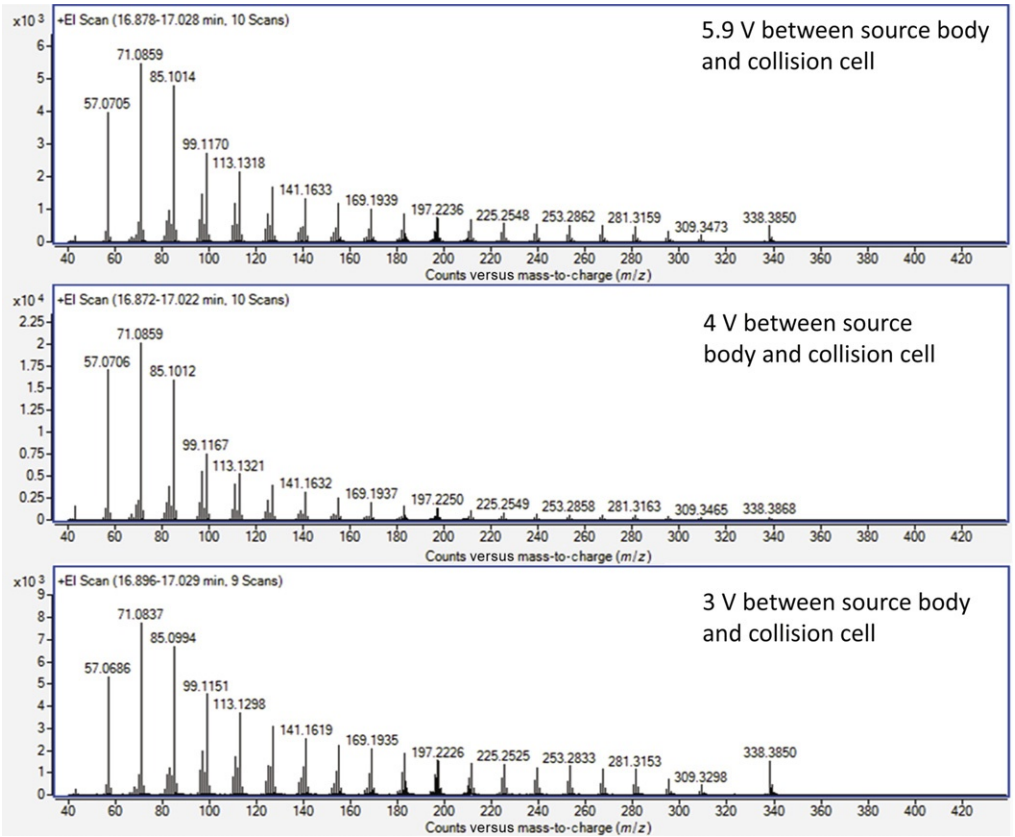

FIGURE 3 Ar-MPPI spectra obtained at various birth potentials obtained with a QTOF mass spectrometer.

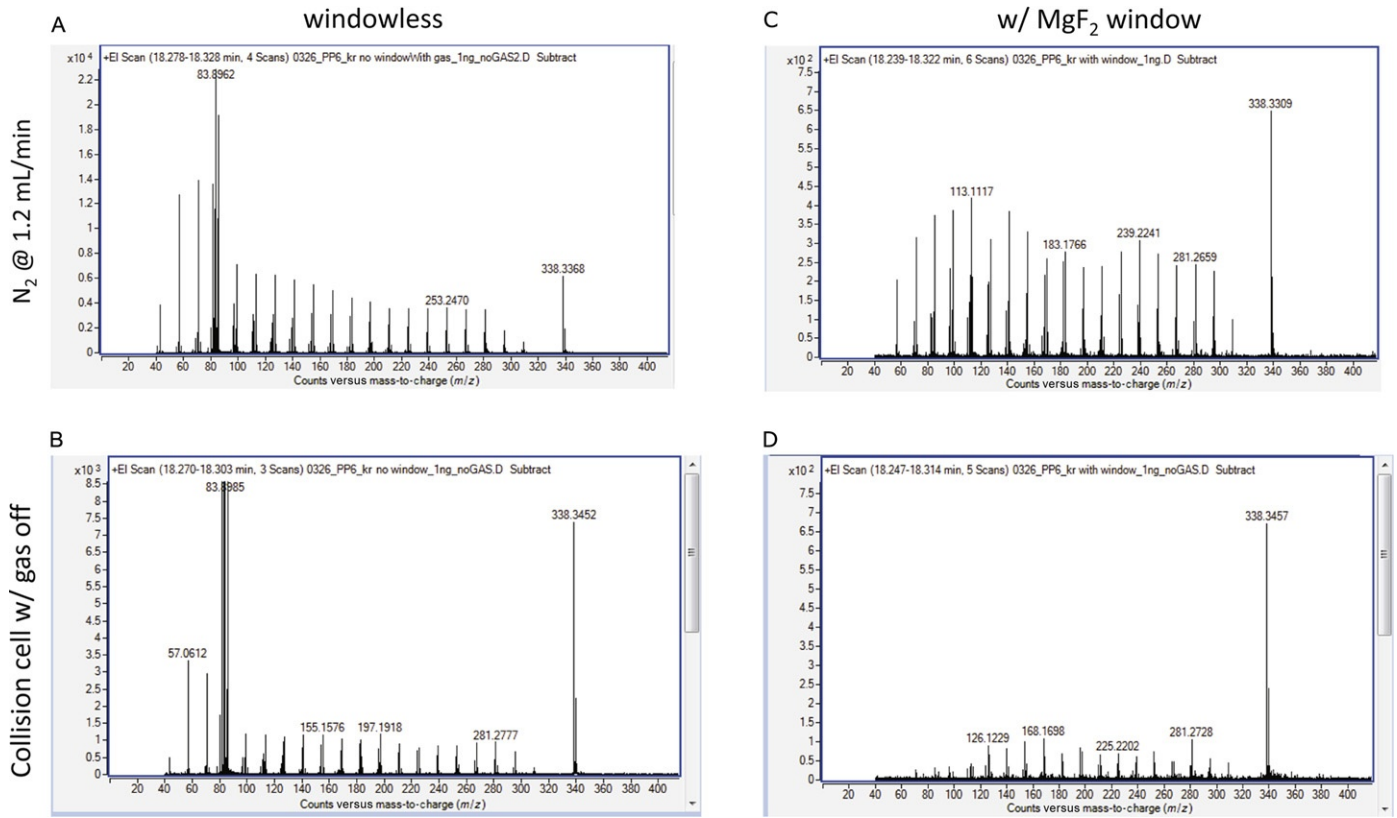

FIGURE 4 Kr-MPPI spectra of $C_{24}H_{50}$ aliphatic hydrocarbon obtained with the windowless source (with collision gas in graph A and without the collision gas in graph B) and with the MgF_2 window (with collision gas in graph C and without the collision gas in graph D). The source body birth potential is 44 V and the collision cell dc voltage is 40 V.

spectrometry with a 4 V birth potential. In Figure 4, graphs (A) and (B) were obtained under windowless conditions, and graphs (C) and (D) show mass spectra taken with a MgF_2 window acting as mechanical barrier between the plasma gas and the analyte stream. The relative intensity of the molecular ion observed with the QTOF mass spectrometer in Figure 4A (windowless and with collision gas present) is significantly less than that observed with the TOF mass spectrometer, which does not have a collision cell (Figure 2B). Figure 4B shows a mass spectrum taken without gas in the collision cell and without the MgF_2 window. Note that the relative intensity of the molecular ion is significantly enhanced, the Kr^+ ions are present, and the relative intensities of the $CH_3(CH_2)_n^+$ fragment ions are less than in Figure 4A. With a mechanical barrier in place, graphs (C) and (D), the possibility of Penning ionization from collisions with metastable Kr atoms [18] and ion molecule reactions, from collision with Kr ions is eliminated. Penning ionization occurs when a gas-phase Kr atom excited to a metastable state interacts with the target molecule M, which has an ionization energy lower than the internal energy of the metastable Kr atom, and leads to the formation of an odd-electron ion M^+, an electron, and a neutral Kr atom. The mass spectrum in Figure 4D yields less fragmentation without the collision gas, resembling that shown in Figure 2B. Spatially resolved measurements of the density of metastable excited atoms in the plume of an Ar microplasma operated at a pressure of 1 Torr were done by Xue *et al.* [19], who reported that the density of metastable excited atoms follows axial and angular distributions consistent with vacuum gas expansion as predicted by the classic rarified flow theory [19].

3.2 PASHs

Analysis of PASHs in the NIST certified materials was performed only by GC–TOFMS using both Ar-MPPI and Xe-MPPI. The latter ionization technique turned out to be very selective as shown in Figure 5, because the aliphatic hydrocarbons in the crude oil were not ionized by the Xe plasma. Half of the total ion chromatogram (TIC) for Ar plasma is dominated by fragment ions corresponding to $C_4H_9^+$ (m/z 57.0699), whereas the corresponding TIC for Xe plasma shows no response, because the aliphatic hydrocarbons have ionization energies of >9.8 eV and are not ionized with Xe plasma. DBTs, methylated DBTs, BNTs, and methylated BNTs as well as a few bicyclic sulfides were identified in the NIST crude oil by using extracted ion chromatograms (EICs) corresponding to the molecular ions of these compounds. Table 1 lists the PASHs that were found in the NIST 2721 crude oil using Xe-MPPI and Table 2 lists the PASHs and PAHs that were found in the NIST 1597a coal tar. Due to lack of reference PASH standards, we used the compounds certified by NIST in the coal tar as our reference standards. Figure 6 shows the individual EICs for PASHs found in the NIST 2721 oil and the corresponding Xe-MPPI spectra are shown in Figure 7.

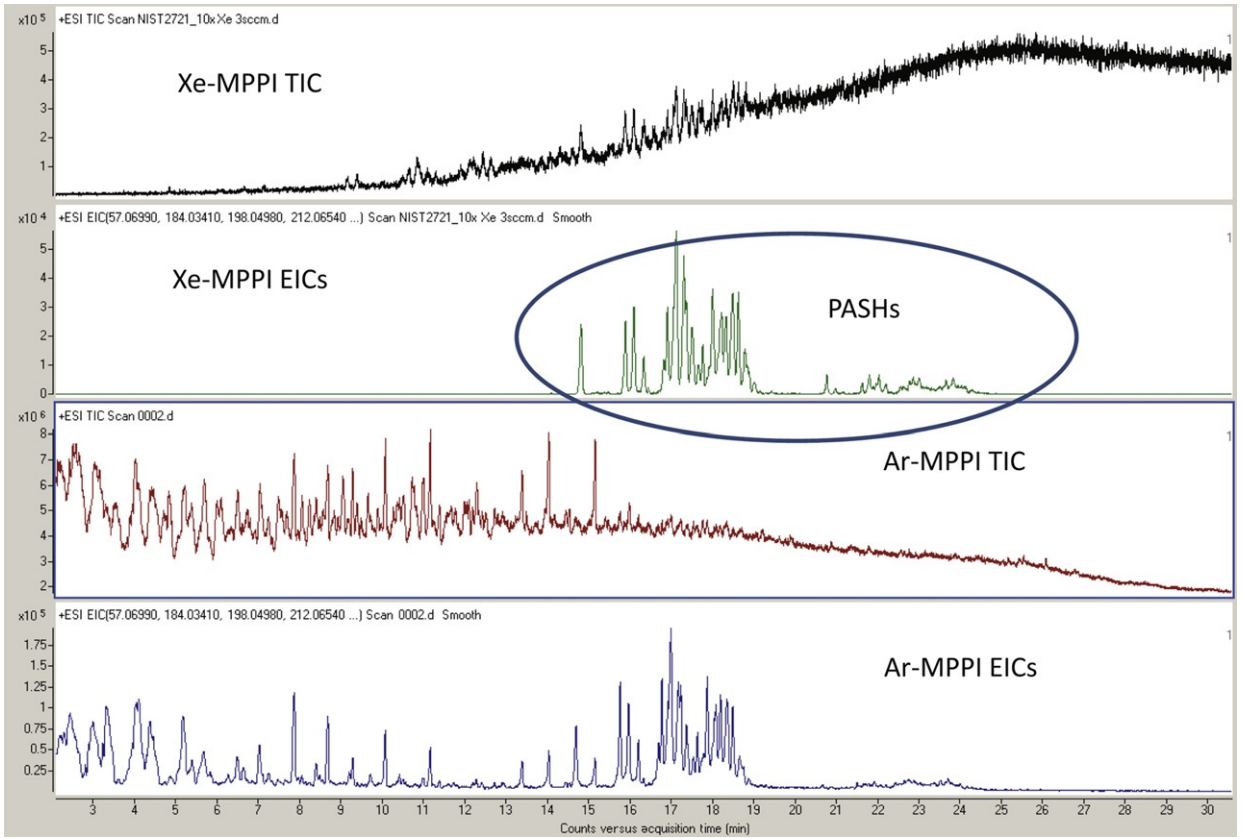

FIGURE 5 Xe-MPPI versus Ar-MPPI TICs and EICs (*m/z* 57.0699, 184.0341, 198.0498, 212.0654, 226.0817, 234.0498, 248.0654, 262.0811, and 276.0967) of the NIST 2721 crude oil.

TABLE 1 Molecular Formulae, *m/z* for the Monoisotopic Masses (Calc. and Exp.) of Various Methylated DBTs and BNTs Found in the NIST 2721 Crude Oil

Compound Identification	Molecular Formula	Calc. *m/z*	Exp. *m/z* (Xe-MPPI)	Retention Time (min)
DBT	$C_{12}H_8S$	184.0341	184.0341	14.81
MDBTs	$C_{13}H_{10}S$	198.0498	198.0498	15.90
			198.0534	16.09
			198.0502	16.33
DMDBTs[a]	$C_{14}H_{12}S$	212.0654	212.0654	16.71, 16.91
			212.0723	17.12, 17.21 17.35,
			212.0712	17.38
			212.0703	17.51, 17.66
			212.0701	17.92
TMDBTs	$C_{15}H_{14}S$	226.0817	226.0811	17.76, 18.00
			226.0836	18.22, 18.32
			226.0856	18.49, 18.63
			226.0867	18.79, 18.84
			226.0850	18.89, 19.03
BNTs	$C_{16}H_{10}S$	234.0498	234.0537	20.77
			234.0547	20.98
			234.0554	21.16
MBNTs	$C_{17}H_{12}S$	248.0654	248.0667	21.81, 21.89
			248.0703	21.62, 22.03
			248.0747	22.18
DMBNTs	$C_{18}H_{14}S$	262.0811	262.0802 262.0804	22.57, 22.65, 22.78, 22.90
			262.0826 262.0868 262.0837 262.0791	22.98 + other unresolved isomers at 23.34
TMBNTs	$C_{19}H_{16}S$	276.0967	276.0970	23.31, 23.39
			276.1021	23.66, 23.83
			276.1081	23.95, 24.04
			276.0965	24.10, 24.14
			276.1060	
			276.1044	
			276.0948	

Continued

TABLE 1 Molecular Formulae, *m/z* for the Monoisotopic Masses (Calc. and Exp.) of Various Methylated DBTs and BNTs Found in the NIST 2721 Crude Oil—Cont'd

Compound Identification	Molecular Formula	Calc. *m/z*	Exp. *m/z* (Xe-MPPI)	Retention Time (min)
276 PAHs	$C_{22}H_{12}$	276.0934	276.0966–276.0991	26.26–27.19[b]
TetramethylBNTs	$C_{20}H_{18}S$	290.1124	290.1084–290.1174	23.93–25.41
290PAHs	$C_{23}H_{14}$	290.1090	290.1116–290.1177	27.19–28.10

[a]*The GC retention time of 4,6-dimethyldibenzothiophene reference standard is 16.77 min.*
[b]*This is the GC retention time of window for benzo[ghi]perylene and indenopyrene.*

By using GC–TOFMS and Xe-MPPI we detected in the NIST 2721 crude oil: DBT, 3 of the 4 possible MDBTs, 9 of the possible 16 DMDBTs, 10 of the possible 28 TMDBTs, 3 BNTs, 5 out of 30 possible MBNTs, and several of the many possible DMBNTs and TMBNTs using Xe-MPPI and high-resolution TOFMS (see Table 1 for the GC retention times of the various isomers). Benzothiophene (*m/z* 134.0185, ionization energy of 8.17 ± 0.03 eV [17]) was not detected in the NIST crude oil or the NIST coal tar with Xe-MPPI, nor was it certified by NIST in the coal tar. Several isomers of tetramethylBNTs (exp. *m/z* 290.1084–290.1174), eluting between 23.93 and 25.41 min, were found in the NIST crude oil, but they were chromatographically resolved from the $C_{23}H_{14}$ PAHs (exp. *m/z* 290.1116–290.1177) which elute between 27.40 and 28.10 min. If these tetramethylBNTs with calc. *m/z* 290.1124 would not be separated chromatographically from the $C_{23}H_{14}$ isomers with calc. *m/z* 290.1090, a mass resolution greater than 85,000 would be needed to distinguish them.

The identification of PASHs was greatly facilitated by the fact that the aliphatic hydrocarbons are not ionized with Xe-MPPI, as their ionization energies are >9.8 eV. Except for DBT (ionization energy 8.14 ± 0.3 eV [17]) and dimethyl-9H-thioxanthene (ionization energy 7.7 eV [17]), the ionization energies of the PASHs in Table 1 are not known. The ionization energies of such PASHs are definitely lower than 8.44 eV, as these compounds are ionized with Xe plasma, and most likely even below 8 eV, because the ionization energies of PAHs with molecular weights of 178–252 u are from 7.2 to

TABLE 2 Molecular Formulae, *m/z* for the Monoisotopic Masses (Calc. and Exp.) of Various Methylated DBTs and BNTs, and PAHs Found in the NIST 1597a Coal Tar

Compound Identification	Molecular Formula	Calc. *m/z*	Exp. *m/z* (Xe-MPPI)	Retention Time (min)	Certified by NIST[a]
DBT Naphthothiophene (3 isomers[b])	$C_{12}H_8S$	184.0341	184.0386	14.73	x
			184.0371	15.14	x
			184.0406	15.30	x
Phenanthrene Anthracene	$C_{14}H_{10}$	178.0777	178.0833	15.05	x
			178.0841	15.19	x
MDBTs (4 isomers[b])	$C_{13}H_{10}S$	198.0498	198.0534	15.79	x
			198.0566	15.99	x
			198.0494	16.10	x
			198.0546	16.16	x
Methyl phenanthrene or methylanthracene (6 isomers[b])	$C_{15}H_{12}$	192.0934	192.1020	15.88	x
			192.0978	16.25	x
			192.0981	16.53	x
DMDBTs	$C_{14}H_{12}S$	212.0654	212.0722	17.00	
Fluoranthene Pyrene	$C_{16}H_{10}$	202.0777	202.0833	17.95	x
			202.0841	18.45	x
TMDBTs	$C_{15}H_{14}S$	226.0817	ND	17.76–19.03	

Continued

TABLE 2 Molecular Formulae, *m/z* for the Monoisotopic Masses (Calc. and Exp.) of Various Methylated DBTs and BNTs, and PAHs Found in the NIST 1597a Coal Tar—Cont'd

Compound Identification	Molecular Formula	Calc. *m/z*	Exp. *m/z* (Xe-MPPI)	Retention Time (min)	Certified by NIST
4H-cyclopenta[c,d] pyrene	$C_{18}H_{10}$	226.0777	226.0824	21.24	x
BNTs (3 isomers[b])	$C_{16}H_{10}S$	234.0498	234.0538	20.68	x
			234.0556	20.89	x
			234.0534	21.08	x
			234.0559	21.47	
MBNTs	$C_{17}H_{12}S$	248.0654	248.0685	21.71	
			248.0800	21.81	
			248.0718	22.06	
DMBNTs	$C_{18}H_{14}S$	262.0811	ND	22.57–23.34	
TMBNTs	$C_{19}H_{16}S$	276.0967	ND	23.31–24.14	
Benzo[ghi]perylene[c] Indeno[1,2,3-cd] pyrene[c]	$C_{22}H_{12}$	276.0934	276.0966	26.26	x
			276.0991	26.45	x
			276.0986	26.95	
			276.0987	27.19	

ND, not detected with Xe-MPPI.
[a]*Compounds certified by NIST are marked with "x."*
[b]*Number of isomers reported by NIST.*
[c]*The retention times assigned to benzo[ghi]perylene and indeno[1,2,3-cd]pyrene have not been verified with reference standards.*

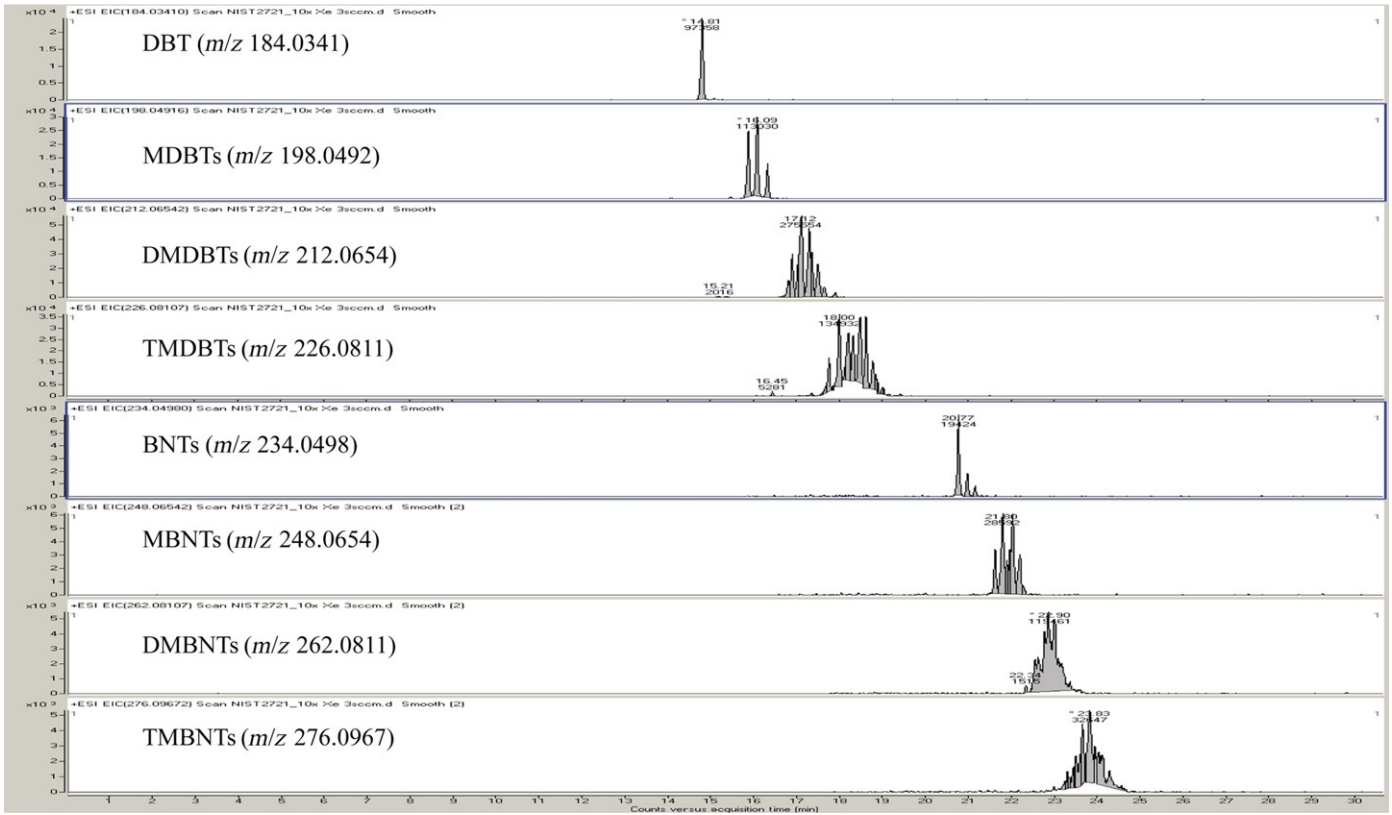

FIGURE 6 EICs of DBT (*m/z* 184.0341), MDBTs (*m/z* 198.0492), DMDBTs (*m/z* 212.0654), TMDBTs (*m/z* 226.0817), BNTs (*m/z* 234.0498), MBNTs (*m/z* 248.0654), DMBNTs (*m/z* 262.0811), and TMBNTs (*m/z* 276.0967) found in the NIST 2721 crude oil using Xe-MPPI.

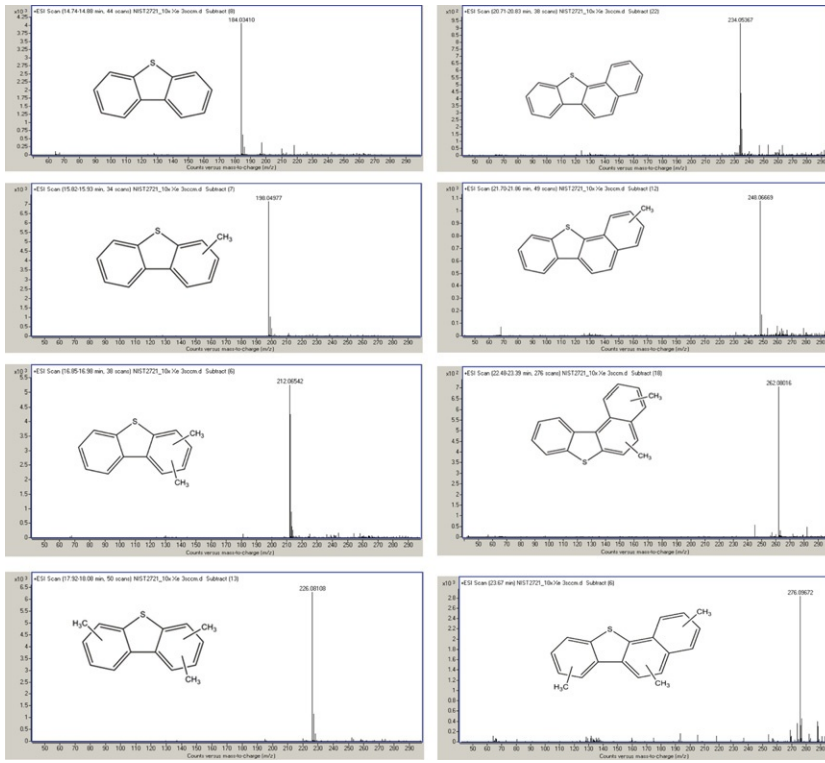

FIGURE 7 Xe-MPPI spectra of PASHs and methyl-substituted PASHs found in the NIST 2721 crude oil.

8.03 eV [17]. Elemental sulfur S_8, which could interfere with the determination of PASHs, is also not ionized with Xe-MPPI because its ionization energy is 9.04–9.6 eV [17]. The GC conditions used in this study were not optimized to separate the various isomers of the methylated DBTs and BNTs, many of which are not even available commercially, but the high-resolution mass spectral data obtained with Xe-MPPI TOFMS indicate that indeed these compounds are present in the NIST 2721 oil.

The Xe-MPPI spectra of PASHs and methyl-substituted PASHs found in the NIST 2721 crude oil that are shown in Figure 7 yield abundant molecular ions and no observable fragmentation. Unlike EI, where methylated DBTs such as TMDBTs yield mass spectra in which the ($M-CH_3$) cations are quite abundant [20], there are no such fragment ions in the Xe-MPPI spectra of the methylated DBTs. Due to lack of reference standards, only DBT and 2,6-dimethyldibenzothiophene were positively confirmed in the NIST crude oil

from their high-resolution mass spectra and by retention time match against a reference standard. Should naphtho[1,2-b]thiophene, naphtho[2,1-b]-thiophene, and naphtho[2,3-b]thiophene be present in either the NIST crude oil or the NIST coal tar, only two of the naphthothiophenes would be resolved chromatographically from DBT, according to published GC retention indices (RI) [21]. Because the experimental RI of DBT is 296.01 and the naphthothiophenes listed above have RIs of 295.80, 300.00, and 304.47, respectively, we expect that, under our GC conditions, DBT coelutes with naphtho[1,2-b] thiophene, and the other two naphthothiophenes reported by NIST in the coal tar are the peaks at retention times of 15.14 and 15.30 min, respectively (see Table 2).

Figure 8 shows EICs of tentatively identified compounds, like $C_{10}H_{14}$ (a C_4-alkyl benzene), $C_{12}H_{12}$ (a C_2-alkyl naphthalene), $C_{13}H_{14}$ (a C_3-alkyl naphthalene), or possibly a $C_{10}H_{18}S$, $C_{11}H_{12}S$ (possibly trimethylbenzothiophene) and possibly another sulfide $C_{11}H_{20}S$, obtained with both Xe and Ar plasma. Other two sets of methylated PASHs include isomers of $C_{16}H_{16}S$ and $C_{20}H_{18}S$, which are listed as possibly tetramethylated DBTs and BNTs, and will need to be identified with reference standards.

The enhanced selectivity provided by the Xe plasma, as shown in the TIC in Figure 5, leads to defined peaks in this very complex chromatogram of the crude oil, and the soft ionization-TOFMS leads to enhanced molecular ions with an accurate *m/z* from which a formula could be generated. Furthermore, by using the ionization energies of specific isomers or related compounds, certain isomers that have ionization energies above that of the plasma gas (see Figure 1) can be ruled out because such compounds would not be ionized. For example, only *p*-cumene and tetramethylbenzene are listed for $C_{10}H_{14}$ in Table 3 because isomers such as *n*-butyl benzene and *t*-butyl benzene with ionization energies of 8.68 eV would not be ionized with a Xe plasma.

In petroleum, once the molecular formula is established for a compound at lowest mass in a series, then a homologous series of compounds having the same heteroatom and number of rings and double bonds can be easily identified by Kendrick Analysis [22,23]. For example, once the two methylnaphthalenes were identified (*m/z* 142.0777, $C_{11}H_{10}$) in the NIST oil, the formula assignment for molecular ions in Table 3 with exp. *m/z* 156.0971–156.1021 as $C_{12}H_{12}$, and for ions with exp. *m/z* 170.1136–170.1175 as $C_{13}H_{14}$ seemed appropriate, because the mass difference between the experimental values for $C_{13}H_{14}$ and $C_{12}H_{12}$ corresponds to the CH_2 radical. Nonetheless, there is also the possibility that we are dealing with a series of bicyclic sulfides, which are known to be present in petroleum [24], and as such we also assigned formulas $C_9H_{16}S$ and $C_{10}H_{18}S$, respectively. Likewise, there is one more set of isomers in Table 3 with exp. *m/z* 184.1262–184.1299 that seem to fit the same pattern to which we assigned both $C_{14}H_{16}$ and $C_{11}H_{20}S$. The various positional isomers of these methylated naphthalenes or bicyclic sulfides would

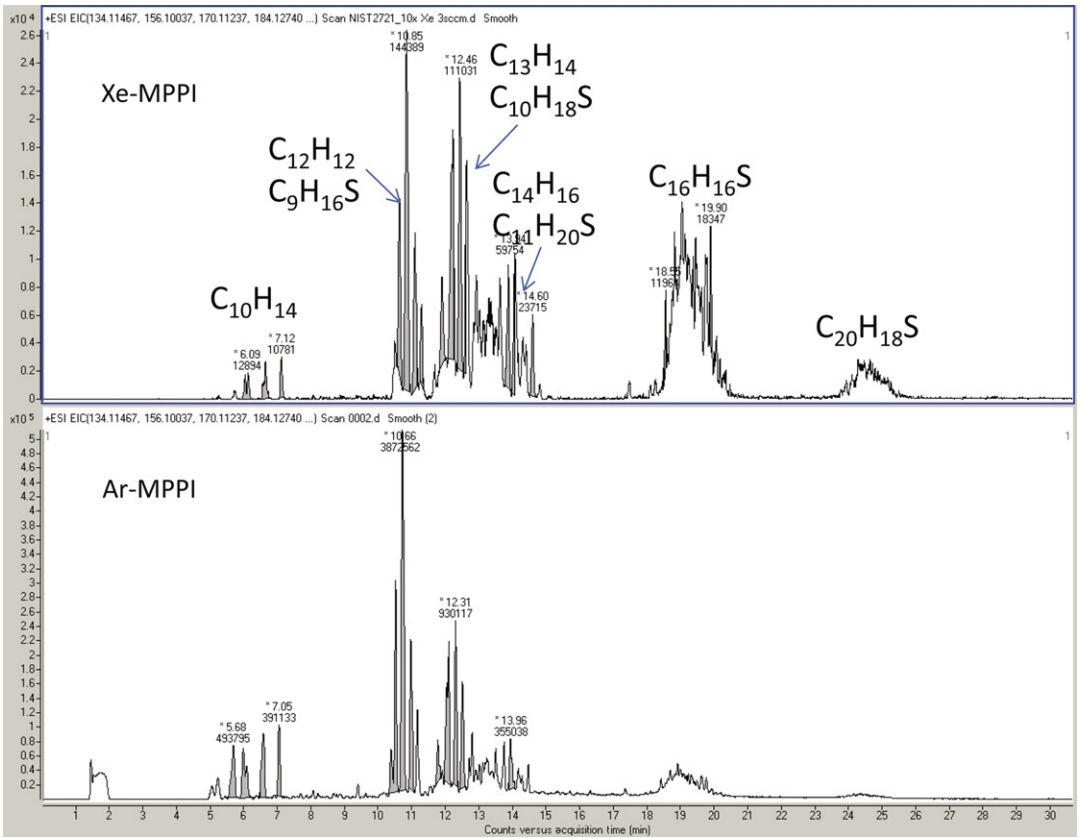

FIGURE 8 Xe-MPPI versus Ar-MPPI—EICs (*m/z* 134.1147, 156.1004, 170.1124, 184.1274, 240.0967, and 290.1124) for compounds tentatively identified in the NIST 2721 crude oil.

TABLE 3 Molecular Formulae, *m/z* for the Monoisotopic Masses (Calc. and Exp.) of Other Compounds Tentatively Identified in the NIST 2721 Crude Oil[a]

Compound Identification	Molecular Formula	Calc. *m/z*	Exp. *m/z* (Xe-MPPI)	Exp. *m/z* (Ar-MPPI)	Ionization Energies (eV) (from Ref. [17])
p-Cumene Tetramethylbenzene	$C_{10}H_{14}$	134.1090	134.1156	134.1117	8.29
			134.1153	134.1116	8.06 ± 0.03
			134.1147	134.1120	
			134.1148	134.1125	
1-Methylnaphthalene 2-Methylnaphthalene	$C_{11}H_{10}$	142.0777	142.0837	142.0813	7.96 ± 0.03
			142.0833	142.0809	7.91 ± 0.03
Dimethylnaphthalenes[a]	$C_{12}H_{12}$	156.0934	156.1004	156.0971	7.89–8.2 (2,3-dimethylnaphthalene)
			156.1011	156.0977	
C_9-bicyclic sulfide	$C_9H_{16}S$	156.0967	156.1021	156.0970	
			156.0971	156.0950	
Trimethylnaphthalenes	$C_{13}H_{14}$	170.1090	170.1136	170.1134	7.1 (trimethylazulene)
			170.1151	170.1129	
C_{10}-bicyclic sulfide	$C_{10}H_{18}S$	170.1124	170.1172	170.1132	
			170.1175	170.1135	
Trimethylbenzothiophenes	$C_{11}H_{12}S$	176.0654	176.0699	176.0707	8.1–8.23[b]
			176.0759	176.0687	

Continued

TABLE 3 Molecular Formulae, *m/z* for the Monoisotopic Masses (Calc. and Exp.) of Other Compounds Tentatively Identified in the NIST 2721 Crude Oil—Cont'd

Compound Identification	Molecular Formula	Calc. *m/z*	Exp. *m/z* (Xe-MPPI)	Exp. *m/z* (Ar-MPPI)	Ionization Energies (eV) (from Ref. [17])
Tetramethylnaphthalenes	$C_{14}H_{16}$	184.1246	184.1286	184.1274	
			184.1299	184.1278	
C_{11}-bicyclic sulfide	$C_{11}H_{20}S$	184.1280	184.1295	184.1280	
			184.1262	184.1285	
TetramethylDBTs	$C_{16}H_{16}S$	240.0967	240.0992	240.0995	7.93[c]
			240.0998	240.1021	
			240.0991	240.1060	
			240.1029	240.1011	
			240.1048	240.1031	
TetramethylBNTs	$C_{20}H_{18}S$	290.1124	290.1174		<8.44
			290.1084	290.1169	
			290.1133	290.1180	
			290.1116	290.1071	
			290.1109	290.1123	
			290.1107	290.1164	

[a]*One of the isomers is 2,6-dimethylnaphthalene that was certified by NIST.*
[b]*Value given is for 2,5-dimethylthiophene. Trimethylbenzothiophene is expected to have an ionization energy less than that of 2,5-dimethylthiophene because of the aromatic ring.*
[c]*Value given is for 2,3,4,5-tetramethylthiophene. TetramethylDBTs are expected to have an ionization energy less than that of 2,3,4,5-tetramethylthiophene because of the aromatic ring.*

have to be identified by retention time match with reference standards. The mass accuracy of the experiments with Xe-MPPI was slightly off, as the TOF mass spectrometer was calibrated with PFTBA using an Ar plasma, because PFTBA has an ionization energy of 11.3–11.7 eV [17] and it is not ionized by radiation from Kr or Xe plasmas. Work is in progress to identify compounds that can be used for mass calibration with Kr and Xe plasmas.

4 CONCLUSIONS

Soft ionization used with gas chromatography TOF mass spectrometry allows for the selective ionization of aliphatic hydrocarbons and PASHs in certified standard reference oil NIST SRM2721 and coal tar NIST SRM1597a. Soft ionization is accomplished by photoionization from VUV resonance lines produced by Xe, Kr, and Ar microplasmas. The enhanced selectivity provided by the Xe plasma leads to defined peaks in a very complex chromatogram obtained by GC–TOFMS of an oil sample and a coal tar, and soft ionization leads to enhanced molecular ions with an accurate m/z from which a molecular formula could be generated. When dealing with petroleum, once the molecular formula is established for a compound at lowest mass in a series, then a homologous series of compounds having the same heteroatom and number of rings and double bonds can be easily identified.

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Chapter 15

Compound-Specific Stable Isotope Analysis of Natural and Produced Hydrocarbon Gases Surrounding Oil and Gas Operations

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1 INTRODUCTION

Recent advances in the technologies of horizontal drilling and hydraulic fracturing have revolutionized the oil and gas industry, bringing new life to depleted or previously unprospective oil and gas fields. Total United States production of natural gas increased from 18.9 trillion cubic feet (Tcf) in 2005 to 24.0 Tcf in 2011, an increase of 27%, and the largest sustained increase in gas production since the 1960s (www.eia.gov). Much of this

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new gas production can be traced to successful development of unconventional shale plays such as the Marcellus Shale in Appalachia and the Barnett Shale in Texas. The increase in production has driven natural gas prices to recent historic lows (http://www.eia.gov/naturalgas/). As a result, industry has shifted attention to economically more valuable liquids-rich plays, using new technologies to extract oil and condensates from "tight" (low permeability) rocks. Examples of these tight oil plays include the Bakken formation in the Williston basin of North Dakota, Montana and Saskatchewan, the Eagle Ford Shale of Texas, and the Niobrara/Codell formations in the Wattenberg field in Colorado (Figure 1).

Increasing media spotlight and public concern regarding environmental and human health impacts has accompanied the development of unconventional oil and gas resources. Much of this concern centers on contamination of shallow groundwater and soils by migration of stray hydrocarbons and other fluids associated with industry operations, including hydraulic fracturing [1]. Hydraulic fracturing involves pumping fluids and proppants (usually sand) at high-pressure deep underground to induce and prop open artificial fractures in rock formations, thereby improving flow and ultimate recovery

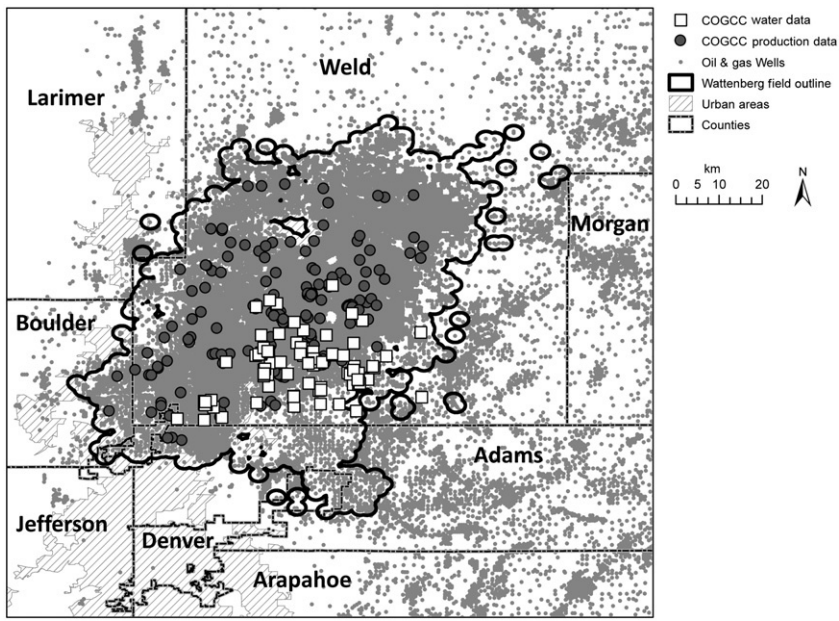

FIGURE 1 The Wattenberg field near Denver, Colorado, has seen a resurgence of drilling activity. There are at present 22,000 well records in the field, an average density of 14 wells per section (1 section = 2.6 km^2). Also shown are COGCC water and production wells with isotope data, as discussed in Section 5.2. *Oil and gas well records are from the Colorado Oil and Gas Conservation Commission (http://cogcc.state.co.us/).*

of oil and gas [2]. The environmental impact of hydraulic fracturing has generated much controversy [3–6]. However, there have been few clearly documented cases in which hydraulic fracturing has directly caused upward migration of gas or fluids from deep underground hydrocarbon reservoirs into the shallow subsurface [1]. A more common and well-documented problem involves migration of hydrocarbon gas through the annular space around the casing of faulty or poorly cemented well bores [1,7–9]. This can be especially problematic in oil and gas fields with numerous old and abandoned well bores [10–12].

With increasing development of unconventional oil and gas resources, there is growing need for objective hydrocarbon gas identification and fingerprinting methods. Besides the documented health and explosion hazards posed by the presence of natural gas in groundwaters [1,7,9,13], stray gas can also indicate the existence of natural or potentially man-made hydraulic connectivity with deep underground hydrocarbon reservoirs [14–16]. As gas migrates more readily than fluids, specifically when gas concentration exceeds solubility and ebbulates from formation fluid (methane solubility in water: 26 mg/l at 1 atm., 20 °C), the presence of stray gas in the shallow subsurface may forewarn of a potential conduit for upward migration of formation fluids (e.g., brines, oil) or production fluids (hydraulic fracturing and flowback fluids) [15]. Determination of gas origin is therefore critical to well site environmental monitoring and remediation efforts.

Baseline water quality monitoring regulations have been implemented in some state and provincial jurisdictions across North America, notably Alberta, Pennsylvania, and Colorado. Recently, the Colorado Oil and Gas Conservation Commission (COGCC; http://cogcc.state.co.us) approved regulations requiring water wells surrounding new drill sites be tested, before and after drilling, for a suite of water quality parameters including stable carbon and hydrogen isotope composition of methane in the water. Almost no soils or sediments are completely devoid of hydrocarbons, and many groundwaters contain *in situ* methane generated by natural microbial activity [17–19]. The stable isotopic composition of microbial methane is distinct from that of the methane in thermogenic natural gas which is usually found in deeper geological formations [20,21]. Therefore, the COGCC and similar regulatory protocols are designed to monitor for the effects of drilling and production operations on the presence and origin of hydrocarbon gas in groundwater.

The isotopic composition of methane may differentiate between microbial and thermogenic gas, however, this identification method is sometimes ambiguous [12,13]. For example, the isotopic composition of methane can overlap with that of low maturity thermogenic gas [12] and is also susceptible to secondary alteration effects in the shallow subsurface, thereby confounding interpretations of gas origin [22,23]. A different problem arises from the presence of naturally occurring thermogenic gas in relatively shallow geological formations and in contact with shallow aquifers, such as in coal seams or as

residual thermogenic gas generated millennia ago in the deep subsurface but more recently brought to the surface by tectonic events and erosion of overburden [6,8]. In this case, a more detailed geochemical fingerprint may be the only means of differentiating between different sources of thermogenic gas. Compound-specific stable isotope analysis of a wider range of natural gas alkanes (methane, C_1; ethane, C_2; propane, C_3; butane, C_4; pentane, C_5) provides a more complete and robust geochemical fingerprint of natural gas compared with the stable isotope composition of methane alone. With detailed isotopic fingerprinting, the source of stray gas may be determined more reliably [10–12,24].

This chapter deals with compound-specific stable carbon and hydrogen isotope analysis of hydrocarbon gases for the purpose of fingerprinting and discriminating between different sources of natural gas. Given the ongoing development of unconventional oil and gas resources, increasing awareness about environmental impacts, and implementation of baseline water monitoring regulations in Colorado and other jurisdictions, the topic is timely and relevant. The general concepts of hydrocarbon gas sampling, stable isotope theory and definitions, compound-specific stable isotope analysis using gas chromatography–isotope ratio mass spectrometry (GC-IRMS), and data interpretation methods are briefly reviewed. The chapter ends with a short case study of production gas and water samples from the Wattenberg field of Colorado, in order to demonstrate the application of gas stable isotope fingerprinting techniques in an active oil and gas production field.

2 HYDROCARBON GAS SAMPLING METHODS

Establishing stable isotopic signatures of hydrocarbon-bearing reservoirs is a critical component of any baseline monitoring program. Samples may be collected during well drilling and completion operations, or from completed production wells and pipelines [9]. It is important to note that hydrocarbon gases are associated with all stages of hydrocarbon generation. Therefore, gas samples can be collected from oil or gas wells alike.

Samples collected while drilling are obtained from gases exsolved from circulating drilling fluids, using gastight sampling bags, or with specialized flow-through manifolds that can accommodate double-ended sampling cylinders. This allows for isotopic characterization of background and show gas at defined intervals along the length of a well bore [24,25]. The resulting mudgas isotope log is an effective way to characterize formation gas within producing and nonproducing geological formations from surface to total depth (Figure 2).

Mudgas isotope logs primarily represent the “free gas” within the pore spaces of reservoir formations. Depending on the permeability and organic composition of the rock, adsorbed gases may constitute a compositionally and isotopically distinct gas phase [26]. Adsorbed gases can be sampled by immersing drill cuttings, sidewall cores, or core chips in water in a sealed

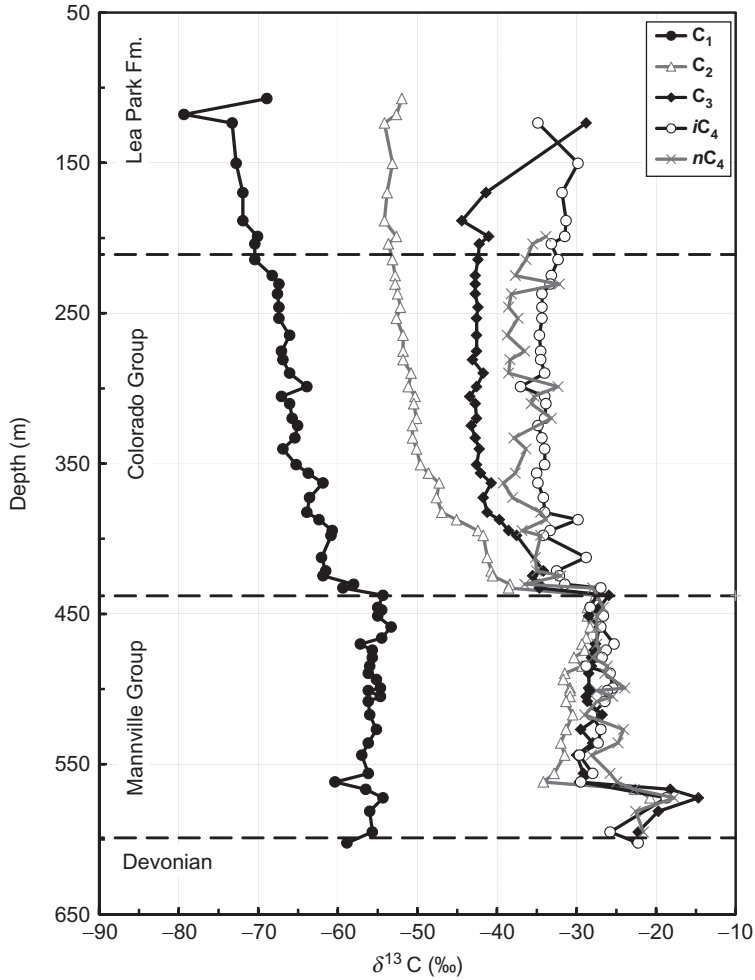

FIGURE 2 Example mudgas isotope log from heavy oil region of eastern Alberta, Canada, showing $\delta^{13}C$ of C_1 through C_4 alkanes. Mudgas samples were collected while drilling and subsequently analyzed for $\delta^{13}C$ in the laboratory. Variations with depth reflect compartmentalization of hydrocarbons in reservoirs with different maturation, migration, and alteration histories. Colorado Group hydrocarbons were generated and accumulated mainly *in situ*. Mannville Group hydrocarbons were migrated from remote source rocks and subsequently severely biodegraded [10,11,24]. The abrupt change across the top of the Mannville illustrates how $\delta^{13}C$ can be used to fingerprint different sources of natural gas. *Redrawn from Ref. [24].*

container and allowing sufficient time for the adsorbed gas to desorb and equilibrate in the headspace [27–29]. Care must be taken to sterilize the container contents in order to prevent secondary gas production by microbes [30]. Samples representative of adsorbed gas can also be obtained from gas emitted from sleeved and capped core samples.

One important consideration that can impact analytical results is the type of mud system used during drilling. Drilling muds serve many functions, including cooling and cleaning the drill bit, carrying the cuttings to the surface, and providing hydrostatic pressure to prevent the escape of formation fluids into the well bore. The two main categories of these muds are water-based mud and oil-based mud (OBM). The amount of hydrocarbon gas soluble in water is $<1\%$ of the amount that is soluble in oil at the same temperature, and this disparity can lead to dissimilar analytical results, so caution must be exercised when comparing results between wells drilled with different mud systems [31]. The use of OBM and other hydrocarbon-containing mud additives can also contribute to the "wet" (C_2 and higher molecular weight) hydrocarbon composition of the gas analyzed [32].

During the production phase of an oil or gas well, gases representative of the producing hydrocarbon reservoir may be sampled directly from the well head. The surface casing vent or "bradenhead," which is the annular space between the production casing and the surface casing, is another sample collection source [33]. Measurable gas or fluid pressure at the bradenhead may indicate gas migrating up through a poor-quality section of the cement between the steel casing and the well bore, or it could be coming from a shallow gas source that is above the traditional reservoir interval where cemented well bore integrity is required by regulatory agencies. Sources of near-surface gas may generate soil bubbles, which can also be sampled to determine the source of the gas [11,24].

The free or dissolved hydrocarbon gases in water may also be sampled [34]. Measurements are made on gas equilibrated into the headspace above a water sample following established protocols [35]. Water samples with sufficient free gas form a gas headspace naturally. For waters with lower concentrations of dissolved gas, a headspace of known volume is created by simultaneously injecting high-purity helium and withdrawing an equal volume of water and allowing sufficient time for the headspace gases to equilibrate [35]. Sterilization is necessary to prevent subsequent microbial gas production in water sampling protocols [12].

3 STABLE ISOTOPE BACKGROUND

3.1 Stable Carbon and Hydrogen Isotopes

Isotopes are nuclei with the same number of protons but a different number of neutrons [36]. "Lighter" isotopes have the same number of protons and neutrons. "Heavier" isotopes have one or more extra neutrons. Unlike radioactive isotopes, stable isotopes do not undergo radioactive decay, and therefore their abundance is not a function of time. The stable isotopes of carbon and hydrogen in C_1–C_5 alkanes are the subject of this chapter (Table 1).

TABLE 1 Stable Isotopes of Hydrogen and Carbon [37]

Isotope	Protons	Neutrons	Atomic Mass	Natural Abundance (atom%)
^{1}H	1	0	1.00782503	99.985
^{2}H	1	1	2.01410178	0.015
^{12}C	6	6	12.00000000	98.90
^{13}C	6	7	13.00335483	1.10

3.2 Definitions

Because it is easier to measure ratios of heavier-to-lighter isotopes rather than their absolute abundances, and for convenience of reporting, stable isotope composition is reported in delta (δ) notation, which is an expression of the ratio of heavier-to-lighter isotopes in a sample normalized to that of an international reference material (Table 2).

Carbon isotopes are reported as $\delta^{13}C$, as calculated by Equation (1):

$$\delta^{13}C = \left[\left(\left({}^{13}C/{}^{12}C\right)_{\text{sample}}/\left({}^{13}C/{}^{12}C\right)_{\text{standard}}\right) - 1\right] \times 1000‰ \quad (1)$$

The $\delta^{13}C$ values are reported on the internationally recognized Vienna Pee Dee Belemnite (VPDB) reference scale. As the original PDB has been exhausted, alternative carbonate reference materials of known $\delta^{13}C$, relative to the original PDB, define the VPDB scale (Table 2) [38]. By definition, $\delta^{13}C$ of VPDB = 0‰.

Hydrogen isotopes are reported as δD, as calculated by Equation (2):

$$\delta D = \left[\left(\left({}^{2}H/{}^{1}H\right)_{\text{sample}}/\left({}^{2}H/{}^{1}H\right)_{\text{standard}}\right) - 1\right] \times 1000‰ \quad (2)$$

δD values are reported on the Vienna Standard Mean Ocean Water (VSMOW) reference scale, defined by VSMOW = 0‰ and Standard Light Antarctic Precipitation Water (SLAP) = −428‰ (Table 2) [39].

Stable isotope composition is expressed relative to the abundance of the heavy isotope, such that materials relatively higher in $\delta^{13}C$ or δD are termed "heavier" or "enriched," while materials relatively lower in $\delta^{13}C$ or δD are "lighter" or "depleted" [41].

3.3 Stable Isotope Fractionation

Stable isotopes of a given element differ slightly in atomic mass and quantum mechanical energies. Chemical bonds involving the light isotope of an

TABLE 2 Selected $\delta^{13}C$ and δD International Reference Materials and Laboratory Intercomparison Standards

Name	Compound	$\delta^{13}C$ VPDB	δD VSMOW
International reference material			
NBS-19[a]	Calcium carbonate	1.95[a]	
L-SVEC[a]	Lithium carbonate	−46.6[a]	
NBS-22[a]	Oil	−30.03[a]	
RM 8562[a]	Carbon dioxide	−3.72[a]	
RM 8564[a]	Carbon dioxide	−10.45[a]	
RM 8563[a]	Carbon dioxide	−41.59[a]	
VSMOW	Water		0[b]
SLAP	Water		−428[b]
Laboratory intercomparison standards			
NG1 (coal gas)[c]	Methane	−34.18 ± 0.10	−185.1 ± 1.2
	Ethane	−24.66 ± 0.11	−156.3 ± 1.8
	Propane	−22.21 ± 0.11	−143.6 ± 3.3
	n-Butane	−21.62 ± 0.12	
	iso-Butane	−21.74 ± 0.13	
	Carbon dioxide	−5.00 ± 0.12	
NG2 (microbial gas)[c]	Methane	−68.89 ± 0.12	−237.0 ± 1.2
NG3 (oil-related gas)[c]	Methane	−43.61 ± 0.09	−167.6 ± 1.0
	Ethane	−40.24 ± 0.10	−164.1 ± 2.4
	Propane	−33.79 ± 0.09	−138.4 ± 3.0

[a]*Reference [38].*
[b]*Reference [39].*
[c]*Reference [40].*

element are easier to break than those of the heavier isotope. Therefore, chemical reactions tend to favor the light isotope. This leads to isotopic fractionation of materials in nature [37]. Equilibrium fractionation applies to reversible reactions, such as the condensation and evaporation of water in a closed system. Kinetic fractionation applies to irreversible or incomplete reactions, which tend to dominate in geological processes [36]. Methane exhibits one of the largest natural ranges in $\delta^{13}C$ (∼−100‰ to −20‰) and δD (−400‰ to −100‰) due to large and variable kinetic isotope fractionation

associated with generation of natural gas by microbes and thermocatalytic cracking of organic molecules [21]. These processes are discussed in Section 5.1.

4 ANALYTICAL METHODS

4.1 Instrumentation

Stable isotopes of light elements are usually measured by isotope ratio mass spectrometry (IRMS) of simple gases such as H_2 or CO_2. IRMS is a well-established technology dating to the original dual-inlet gas source mass spectrometer design of Alfred Nier [41,42]. The basic principle of IRMS is to accelerate ionized molecules of gas through a magnetic field in order to separate the molecules by their mass-to-charge (m/z) ratio (Figure 3). Beams of isotopically lighter ions ($^1H^1H^{+\bullet}$, $m/z=2$; $^{12}C^{16}O^{16}O^{+\bullet}$, $m/z=44$) bend more than beams of isotopically heavier ions ($^1HD^{+\bullet}$, $m/z=3$; $^{13}C^{16}O^{16}O^{+\bullet}$, $m/z=45$). Beams are focused into Faraday collectors that continuously record the electronic current generated by each beam. The ion beam current ratio generated by a sample is integrated, background-subtracted, and normalized against the ion beam current ratio generated by a reference gas in order to calculate δ-values according to Equation (1) or (2). A complication specific to the analysis of δD arises from the presence of H_3^+ ions formed by collisions in the ion source of an IRMS, as shown in Equation (3):

$$H_2^{+\bullet} + H_2 \rightarrow H_3^+ + H^\bullet \tag{3}$$

H_3^+ ions interfere with the measurement of ^1HD. Instrument-derived proportionality between H_3^+ and the partial pressure of H_2 is used to correct for this interference [43].

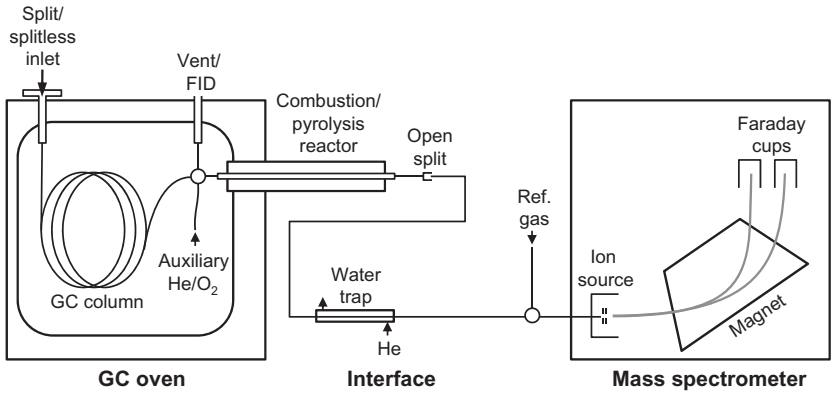

FIGURE 3 Schematic of a GC-IRMS system. *Redrawn from Ref. [41].*

Compound-specific isotope analysis of hydrocarbon gas involves the separation and purification of component alkanes, conversion of each component to CO_2 or H_2 gas, and analysis by IRMS [44,45]. This is usually accomplished with a specialized inlet system, consisting of gas chromatograph (GC), reactor furnace and purification trap, and IRMS, connected in series (GC-IRMS; Figure 3) [41,46]. GC-IRMS is a relatively new technique, with commercial systems only available for about the past 15 years.

Gas chromatographic separation of hydrocarbons and other gases is performed using a capillary column (typically, porous layer open tubular, or PLOT, columns) with helium (He) carrier gas in a temperature-controlled GC [45,47]. Samples may be introduced to the GC using custom-configured plumbing and valves [48], or by gastight syringe operated manually or with an autosampler. Manual syringe injections of methane without sample loss or change in $\delta^{13}C$ have been demonstrated [47]. GC parameters are optimized for baseline separation of alkane components (Figure 4). The GC cycle time, typically 10–20 min, sets the upper limit on the rate of sample throughput.

As alkanes elute from the GC, they are passed through a combustion reactor for conversion to CO_2, or a pyrolysis furnace for conversion to H_2. Various reactor configurations exist. Typical combustion reactors are made with vitreous alumina tubing packed with twisted strands of nickel oxide (NiO) and platinum (Pt) wire. NiO acts as the O_2 donor for the combustion reaction

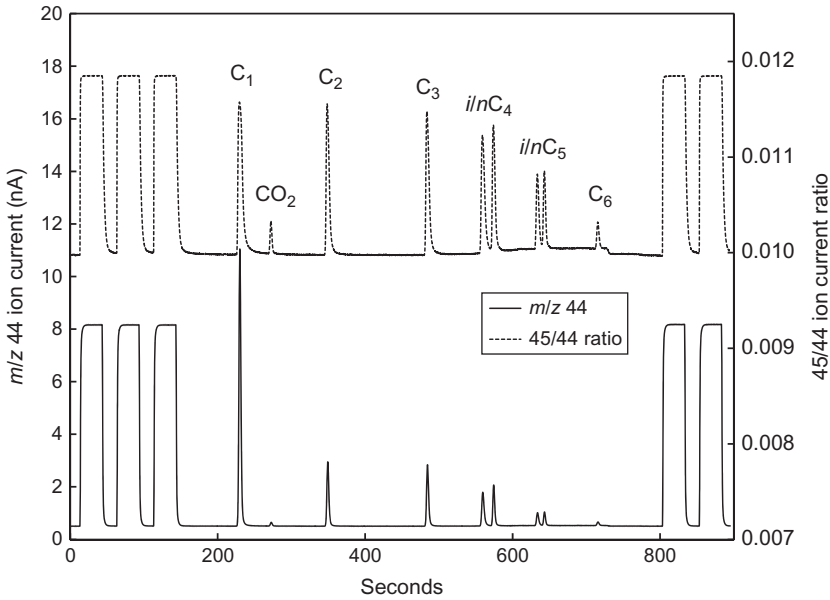

FIGURE 4 Example GC-IRMS chromatogram for a sample of thermogenic natural gas. Pulses of pure reference CO_2 (square-shaped peaks) bracket the sample peaks, which represent C_1 through C_6 (hexane) alkanes combusted to CO_2.

and Pt serves as a catalyst. When heated to ~1150 °C, alkanes are combusted to CO_2 and H_2O quantitatively [49]. The reactor effluent is then stripped of H_2O by passing through a tubular hydrophilic Nafion™ membrane [49,50]. This step is necessary to prevent H_2O from entering IRMS, where it can interfere with the source electronics as well as protonate CO_2 molecules and corrupt the isotopic measurements [51]. Typical pyrolysis reactors are made with empty alumina tubing. When heated to ~1420 °C, alkanes (and H_2O) are pyrolyzed to H_2 and graphite, which deposits as a microfilm on the inside of the tube [52]. Combustion and pyrolysis reactor configurations, temperatures, gas flow rates, and number of conditioning runs are optimized to achieve quantitative conversion [49,52]. Nonquantitative conversion leads to isotopic fractionation and residual fragment ions that could interfere with CO_2 or H_2 isotopic mass spectra [46,49,52].

GC-IRMS offers significant advantages in terms of automated sample preparation and measurement, high sample throughput, minimal sample size requirements (0.1–5 nmol for CO_2, 10–50 nmol for H_2), and good precision (0.1–0.3‰ for $\delta^{13}C$; 2–5‰ for δD) [41]. For higher precision work, offline preparation of CO_2 and H_2 gas, followed by dual-inlet IRMS, may be necessary. Dual-inlet IRMS instruments involve repeated differential measurement from sample and standard gas streams. Precision is typically 0.01‰ for $\delta^{13}C$ and 0.5‰ for δD [42], but at least 1 µl of purified CO_2 or H_2 gas is required [53]. The necessary offline preparation drastically reduces sample throughput and introduces potential operator errors.

Operating conditions for Dual Inlet or GC-IRMS systems (physical space, controlled temperature and humidity, and electrical and vacuum requirements) necessitate setup in the laboratory. Analyses, therefore, must be made on samples collected in the field and stored and transported to the lab in appropriate gastight sample containers.

Recent developments in laser spectroscopy provide a completely novel way of measuring stable isotopes. Laser instruments use the unique infrared absorption properties of different isotopically substituted molecules [54]. Commercial vendors now offer portable, easy-to-use instruments capable of measuring the $\delta^{13}C$ of C_1 (or C_{2+} alkanes combusted to CO_2), with a precision approaching that of GC-IRMS, and analytical run times of <1 min [55], thus allowing for the possibility of real-time measurements in the field [56]. With improvements in analytical dynamic range and analyte capabilities beyond C_1 and CO_2, this technology could see widespread application in the oil and gas industry.

4.2 Stable Isotope Analysis of Hydrocarbons—Special Considerations

Analysis of the $\delta^{13}C$ and δD of natural gas alkanes poses special analytical challenges introduced by the wide range of concentrations typically

encountered. GC-IRMS systems described in the literature often are designed to run samples, such as gaseous C_1 or CO_2, which have low and/or relatively invariant natural concentrations [47,48,57]. Hydrocarbon gases, on the other hand, can range from trace levels (<100 ppm), typical of "no-show" zones of a mudgas profile, to essentially 100%, in gas shows or production streams [44,45]. Further, the concentrations of C_{2+} components can range from equal to that of C_1 in a thermogenic "wet" gas (Figure 4), to trace or nonexistent levels in a "dry" microbial or postmature thermogenic gas [21].

Low-concentration samples require special precautions to avoid mass spectral interferences caused by the presence of air [47,48,57]. The N_2 in air is partially converted to N_2O ($m/z = 44$) in the combustion reactor of a GC-IRMS system, which can interfere with the measurements of CO_2 derived from the combustion of C_1. Air and C_1 are difficult to separate on PLOT columns at GC starting temperatures >40 °C (Figure 5). Baseline separation can be improved by running the GC at subambient temperatures with the use of a cryo-focusing device to cool the head of the analytical column with liquid nitrogen (Figure 5) [47,48,57]. The wider separation introduced by cryo-focusing allows the air to be vented to the laboratory atmosphere by means of a heart split valve located upstream of the combustion reactor. Once the air has vented, analysis proceeds by closing the heart split valve and heating the column to normal operating temperature. Valve timing must be optimized to allow sufficient time for IRMS ion beam stabilization before the pulse of CO_2 from the combustion of C_1 enters the IRMS. Cryo-focusing also decreases the width and increases the height of the C_1 peak, thereby improving signal-to-noise ratio and analytical precision. For concentrations <1000 ppm, further cryogenic enrichment methods may be employed [58].

Evolution in IRMS design has led to improvements in instrument dynamic range. Nevertheless, the amount of sample introduced into the IRMS must be large enough to obtain a measurable ion current response, but without overloading the detector. Ideally, hydrocarbon concentrations are premeasured on a standalone GC, following established methods [59]. Once the concentrations of gas components are known, sample volume and split ratio on the GC inlet are adjusted specific to each sample to achieve the desired ion current response. Samples of thermogenic dry gas may need to be analyzed twice, first using low volume/high split ratio to measure C_1, then with higher volume/low split ratio combined with a heart split cut of the C_1 peak for measurement of C_{2+} components.

4.3 Standardization

Routine stable isotopic analyses can be made with very high precision relative to natural variability in natural gas $\delta^{13}C$ and δD. However, consistent accuracy, essential for the production of reliable data that can be compared across multiple laboratories, is more difficult to achieve [53]. Routine stable isotope

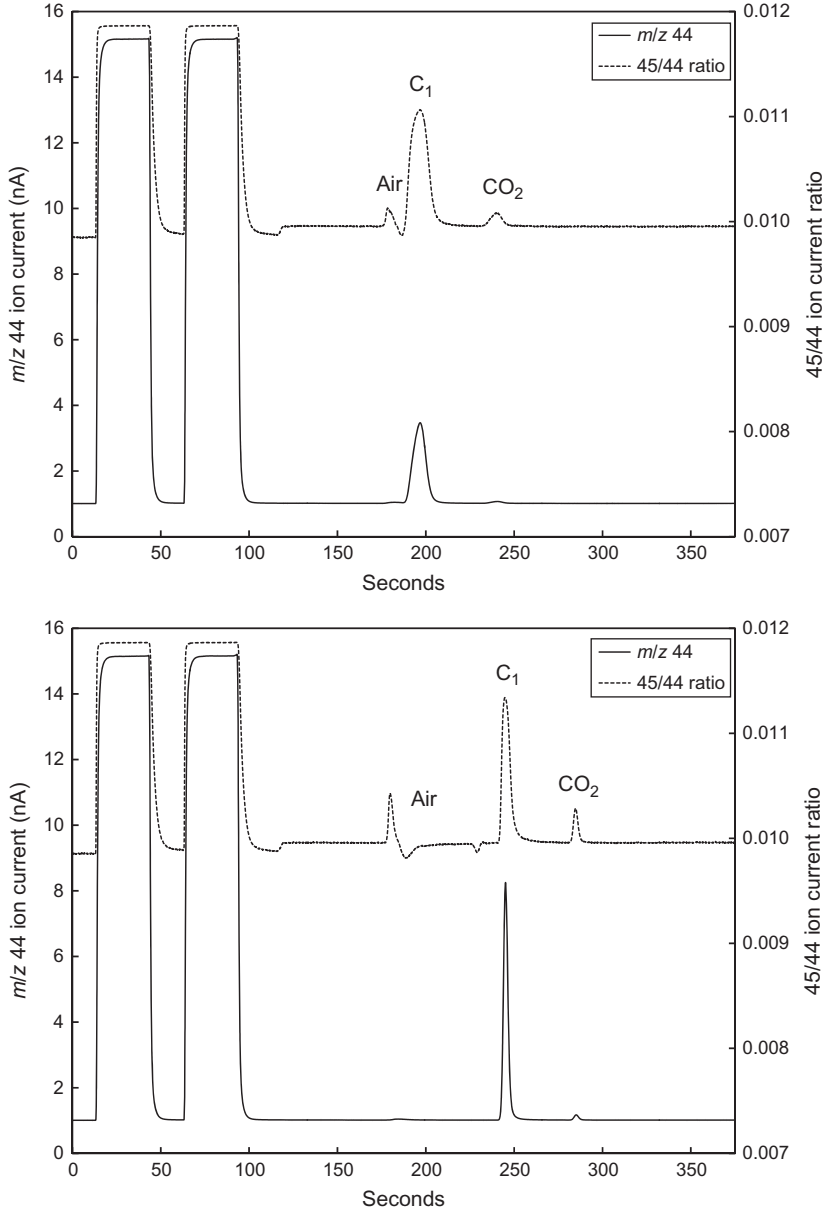

FIGURE 5 GC-IRMS chromatograms showing the elution of air, methane, and carbon dioxide with (lower panel) and without (upper panel) cryo-focusing. Cryo-focusing improves baseline separation and peak shape. Analytical parameters: 0.5 ml of 2500 ppm methane in air, injected with a split ratio of 10:1 onto an Agilent HP Plot Q column held at 50 °C for 3.5 min, then ramped at 30 min to 240 °C, with a column flow of 1.5 ml/min. For cryo-focusing, the head of the GC column was held at −150 °C for 1 min after injection and then heated instantaneously to 50 °C.

measurements are calibrated against laboratory reference gases of known $\delta^{13}C$ and δD introduced to the IRMS during an analysis (Figure 4). Ideally, these reference gases are periodically calibrated against international reference materials on the VPDB and VSMOW scales (Table 2).

Post-analysis corrections for instrument drift and linearity are made from repeated analysis of identical working samples or standards interspersed through an analytical sequence. GC-IRMS data are further calibrated using laboratory working or intercomparison standards following the identical treatment principle [53]. Isotopic fractionation introduced by sample injection, GC, or combustion/pyrolysis cancel out when a sample is calibrated to a standard of the same chemical nature and measured the same way. Because natural gas spans a wide range of $\delta^{13}C$ and δD, multipoint calibration standards, bracketing the range of sample values, are necessary. Beginning in 1984, the International Atomic Energy Agency (IAEA) informally distributed a suite of three natural gas $\delta^{13}C$ standards, comprising an isotopically heavier coal gas (NGS-1), an isotopically intermediate oil-associated gas (NGS-2), and an isotopically light microbial gas (NGS-3) (Table 2) [60]. These gases were then transferred to the National Institute of Standards and Technology (NIST) in the USA for formal distribution as laboratory intercomparison standards [61]. However, the original gases have been exhausted and are no longer available from NIST. The current lack of IAEA- and NIST-distributed natural gas reference materials is problematic and hopefully will be resolved in the future. Meantime, natural gas reference standards can be purchased from commercial sources. Recently, round robin interlaboratory measurements of the $\delta^{13}C$ and δD of coal gas, oil-associated gas, and microbial gas sourced from China were performed (Table 2); these gases are available for distribution in limited quantities [40].

5 INTERPRETATION OF NATURAL GAS STABLE ISOTOPES

The following sections draw on a compilation of natural gas isotope data from the literature and the United States Geological Survey (USGS) Energy Geochemistry Database (http://energy.cr.usgs.gov/prov/og/). This compilation serves to demonstrate natural gas molecular and isotopic distribution across the spectrum of natural gas types, from a wide range of geographic locations and conventional/unconventional reservoir lithologies. Because data on the $\delta D_{C_{2+}}$ of gas hydrocarbons are uncommon in the literature, the discussion focuses on the interpretation of $\delta^{13}C_{1-5}$ and δD_{C_1} data. The literature/USGS data are shown in a series of classic interpretive plots (Figure 6), along with production and water sample data from a Wattenberg Field case study (Section 5.2).

5.1 Natural Gas Types

Natural gas may be grouped into three primary types: microbial, thermogenic, and abiogenic [62]. Microbial and thermogenic gas is found in economic

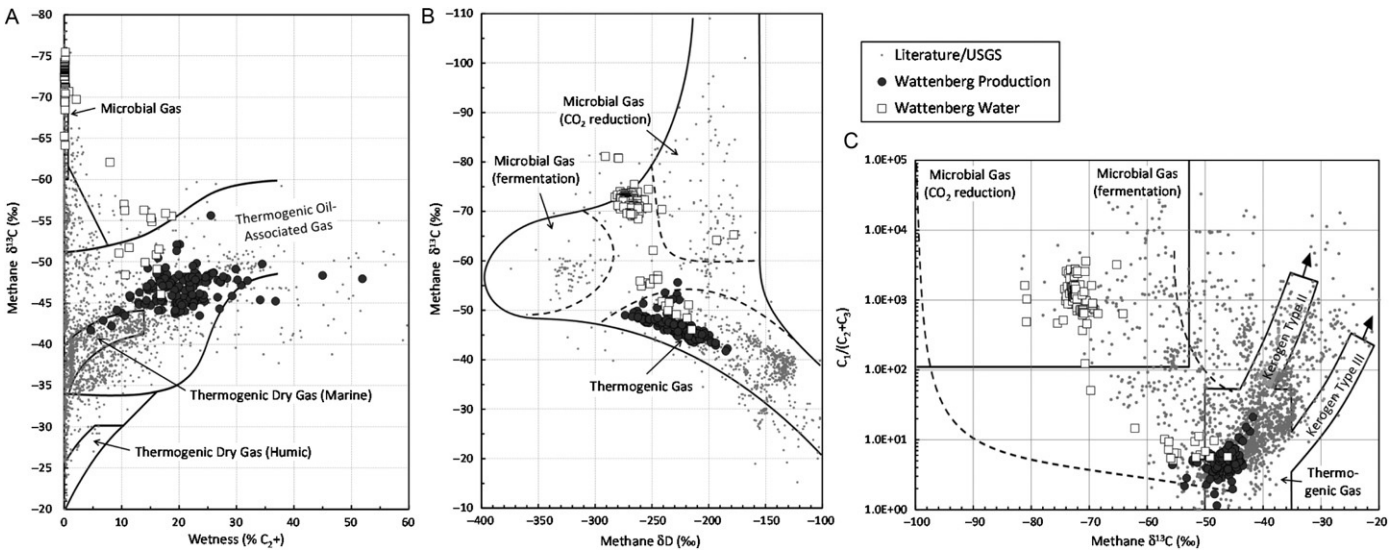

FIGURE 6 (A) Schoell [21] plot showing distinctions between microbial and thermogenic gas and emphasizing different maturity levels of thermogenic gas. The "wetness" parameter is a measure of the % C_2 and higher alkanes in the gas. (B) Whiticar [62] plot showing distinctions between thermogenic and different types of microbial gas based on the $\delta^{13}C$ and δD of methane. (C) Modified Bernard *et al*. [63] plot emphasizing variability within microbial gas, and effects of mixing (dashed lines) and maturation (arrows) [62]. *Data from USGS, literature, and COGCC (Wattenberg Field) sources, as discussed in text. Literature data from Refs. [11,17,45,64–76].*

reservoirs worldwide. Abiogenic gas is found in crystalline rocks and crustal fluids, but not in economic quantities [77], and therefore is not considered further. Primary gases have a single source as opposed to secondary gases that have been altered by mixing of gas sources, migration, degradation, or other fractionation processes [62].

5.1.1 Microbial Gas

Microbial gas is composed primarily of methane generated by fermentation of methyl-type substrates (e.g., acetate, a breakdown product of complex organic molecules) or by reduction of CO_2 in anoxic environments [17,78,79]. Generation of methane from methyl-type fermentation occurs naturally in near-surface, usually freshwater environments (e.g., swamps, sewers, compost, landfills) by the following generalized reaction shown in Equation (4):

$$CH_3COOH \rightarrow CH_4 + CO_2 \tag{4}$$

Reduction of CO_2 under anoxic conditions occurs as shown in Equation (5):

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \tag{5}$$

Reduction of CO_2 is more common in marine environments and deeply buried glacial drift deposits. Microbial generation of ethane and propane has also been documented, although these constitute $<1\%$ of the total microbial gas [12,78,80]. Microbial gases are sometimes referred to as "biogenic" or "bacterial" gases. These terms are more ambiguous, because thermogenic gases originate from "biogenic" substrates [62], and methanogens belong to the class Archea [81].

Microbial methanogenesis strongly fractionates against ^{13}C [82]. As a result, microbial methane is strongly depleted in $\delta^{13}C$, typically $<-60‰$ [17,18,78]. Combined with a molecular composition of $<1\%$ C_{2+} alkanes, microbial gas is easy to identify on classic "Schoell" or modified "Bernard" cross plots (Figure 6A and C). Microbial ethane, if present in measurable quantities, is generally $<-40‰$ in $\delta^{13}C$ and may provide even more robust discrimination [12]. The δD of methane can further distinguish between methyl-type fermentation ($>-250‰$) and CO_2 reduction ($<-250‰$; Figure 6B) [17,78]. The $\delta^{13}CO_2$ and δD of water coexisting with methane may also help with gas genetic interpretation [17,64,79].

5.1.2 Thermogenic Gas

Thermogenic gases generate from thermocatalytic breakdown (cracking) of complex organic molecules as they are cleaved and subsequently saturated to form the C_1–C_5 alkanes of natural gas [62]. Gas may be generated from the direct cracking of source rock organic kerogen (primary cracking) or from oil (secondary cracking). Generation of thermogenic gases occurs over a gradient of maturation. During early and mature stages, methane and higher natural gas range alkanes are generated along with the molecules that make up

oil and condensate liquids. As the thermocatalytic reactions continue into later stages, all of the hydrocarbon molecules eventually are broken down, leaving methane as the final "dry gas" end product. It is important to distinguish organic matter type when describing and predicting gas wetness associated with maturation, as sapropelic (Type I/II) kerogen generates more C_{2+} hydrocarbons compared with humic (Type III) kerogen [83].

Along with molecular composition, the $\delta^{13}C$ of thermogenic gas is a powerful characterization and fingerprinting tool. The $\delta^{13}C$ of thermogenic methane ranges from $\sim -55‰$ to $-20‰$, which in most cases distinguishes thermogenic from microbial gas (Figure 6). The distribution of $\delta^{13}C$ among C_1–C_5 alkanes is determined mainly by (1) kinetic isotope fractionation associated with thermal cracking of kerogen and subsequent breakdown product molecules, (2) chemical structure and type of organic matter, and (3) isotopic composition of the precursor kerogen [84]. Cracking favors $^{12}C—^{12}C$ bonds over $^{12}C—^{13}C$ bonds, leading to predictable isotope distribution patterns among generated hydrocarbons [65,85–89]. For any given level of maturity, isotopic values are normally distributed as $\delta^{13}C_1 < \delta^{13}C_2 < \delta^{13}C_3 < \cdots < \delta^{13}C_{kerogen}$. With further maturation, the $\delta^{13}C$ of all C_1–C_5 components increases, ultimately approaching that of the source kerogen, until C_1 is the only remaining component. Kerogen $\delta^{13}C$ ranges from $\sim -35‰$ to $-20‰$ depending on the type and geological age [90]. "Chung" plots [65], in which $\delta^{13}C$ is plotted against reciprocal carbon number, provide a simple and powerful way to visualize the effects of maturation and different source kerogen signatures on isotopic distributions in natural gas (Figure 7A–C). Similar effects apply to the δD of thermogenic natural gas; however, isotopic distributions are complicated by hydrogen isotopic exchange between water and precursor organic molecules [91].

5.1.3 Secondary Gas

A variety of postgenetic or secondary processes impact the molecular and isotopic composition of natural gas, potentially confounding the genetic characterizations outlined above. These include (1) mixing of different gases, (2) physical fractionation due to migration, (3) chemical fractionation due to biodegradation, and (4) isotope "reversal" phenomena. Chung plots help to visualize and identify these effects (Figure 7) [66].

Physical mixtures of two or more gases display molecular and isotopic compositions determined by isotopic mass balance of the constituent gases. For example, the $\delta^{13}C_1$ of a thermogenic gas will be lowered by mixing with microbial C_1 (Figure 7D). Mixing of thermogenic gases of different sources or maturation histories may result in deviations from idealized linearity on a Chung plot (Figure 7E) [24,66,92].

Migration can alter the molecular and isotopic composition of hydrocarbon gases through complex interactions of diffusion, adsorption, and solubilization in pore fluids [84,93–95]. Lower molecular weight and isotopically

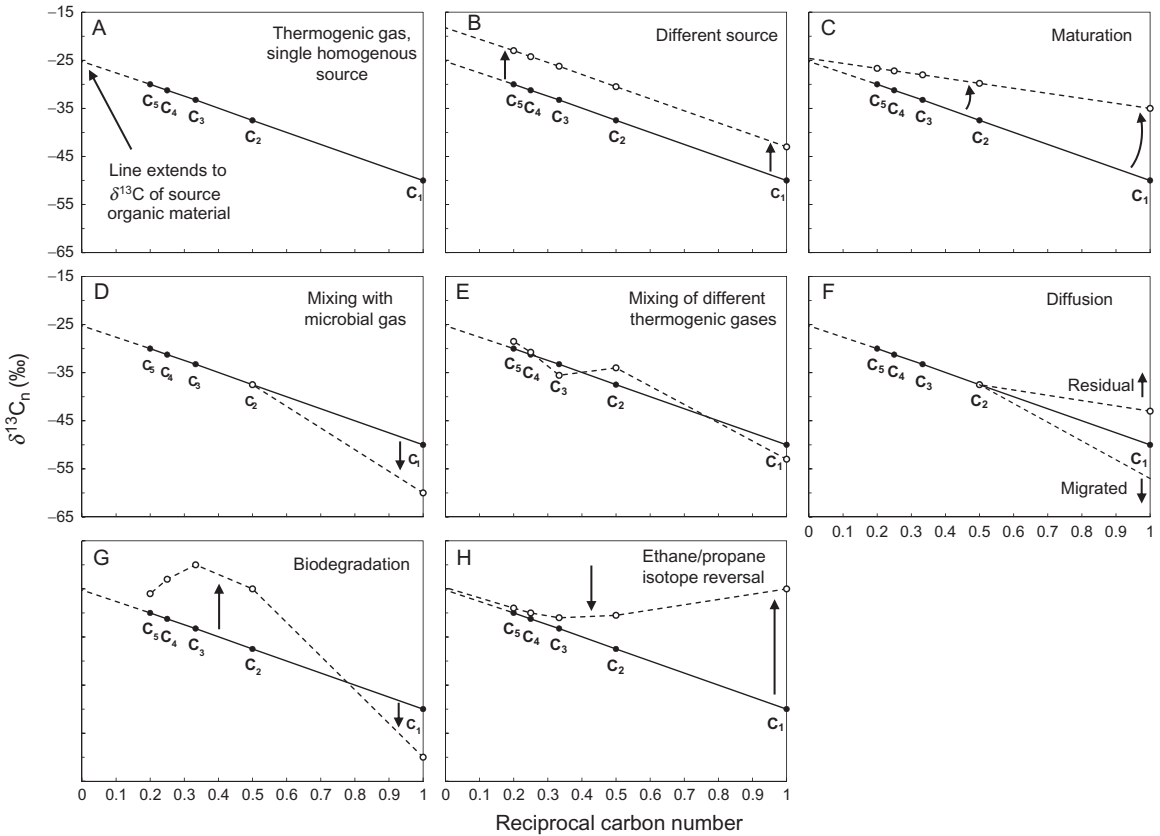

FIGURE 7 Chung [65] plots showing $\delta^{13}C$ of C_{1-5} alkanes in (A) an idealized thermogenic gas, and generalized effects of (B) different source organic materials, (C) maturation, (D) mixing with microbial methane, (E) mixing of different thermogenic gases, (F) methane diffusion, (G) biodegradation, and (H) isotope reversal. *Modified from Ref. [66].*

lighter species diffuse more readily [84,95–97]. In the field, diffusively migrated gases, therefore, tend to be drier and isotopically lighter than gases that generate and accumulate *in situ*, potentially confounding genetic interpretations [94,97]. However, the opposite effect has been observed upon filtration of gas through water-saturated rock, due to preferential dissolution and retention of ^{12}C in the pore water [84,98]. Diffusion of gases from a leaking sampling container can leave the residual gases with distinct isotopic enrichments (Figure 7F) [96]. In the shallow subsurface, migration effects are likely to be comingled with biodegradation [99].

Biodegradation describes a series of reactions by which microbes metabolize hydrocarbons to CH_4, CO_2, H_2, and other end products, either aerobically or anaerobically, at depths $\approx<1500$ m and temperatures $\approx<80\,^{\circ}C$ [92,100–102]. Natural gas range alkanes are usually metabolized in the following order of preference: C_3, $n C_4$, C_5, $i C_4$, C_2, C_1 [92,100–102]. Biodegradation also favors ^{12}C over ^{13}C, resulting in $\delta^{13}C$ enrichment of the residual alkanes [23,103,104]. C_1 is both consumed and produced during microbial degradation, with the rate of production sometimes exceeding that of consumption [105]. This can result in the net gain of secondary microbial C_1 [100,106,107]. Differential molecular selectivity combined with kinetic isotope fractionation and net production of secondary microbial C_1 produces characteristic biodegradation signatures on Chung plots (Figure 7G). Additional indicators of biodegradation may include $\delta^{13}CO_2 > 0‰$ and elevated C_2/C_3 and iC_4/nC_4 molecular ratios [18,92,104,107].

Finally, isotope reversal describes a phenomenon specific to closed system, overmature gas in which the normal thermogenic gas pattern of $\delta^{13}C_1 < \delta^{13}C_2 < \delta^{13}C_3$ is wholly or partially reversed (Figure 7H) [67–69]. The causes of isotope reversal have only just begun to be explored. Isotopically lighter C_2 and C_3 generated from secondary cracking of oil/condensate liquids during the later stages of thermal maturation has been proposed as a possible mechanism [108].

5.2 Case Study: Wattenberg Field of Colorado

A publicly available and continuously updated database hosted by the COGCC (http://cogcc.state.co.us/Home/gismain.cfm) serves as a generalized case study for the Wattenberg field of Colorado. At present, these data include natural gases from 149 oil and gas production wells completed in the Sussex, Niobrara, Codell, and J Sand formations, and the free or dissolved gas from 95 water samples collected from water wells (Figure 1). The 95 water samples contained sufficient C_1 for isotopic analysis; a much larger number of samples in the database had insufficient C_1 or were not measured for stable isotopes. Data were provided by the COGCC for informational purposes only, and it was not possible to verify the collection or analytical protocols for each sample. For water samples in particular, no information was provided on the

specifics of each sampling site, such as well integrity, age, or history of contamination. Therefore, this case study is not intended to identify particular instances of groundwater contamination that can be attributed to oil and gas drilling and production activities. Rather, it provides a general overview of the molecular and isotopic distribution of production gases and waters in an active oil and gas field and interpretive methods which may be used to fingerprint different sources of gas.

Data from the Wattenberg field illustrate some of the interpretive concepts outlined in Section 5.1. With values of $\delta^{13}C_1 > -55‰$ and $\delta^{13}C_2 > -40‰$, production gases in all cases may be characterized as thermogenic in origin, with possible mixing of microbial C_1 in a few samples (Figure 6). The relatively linear distribution of $\delta^{13}C_n$ values indicates little secondary isotopic alteration (Figure 8). The range in isotopic values is mainly a result of different source rocks, combined with vertical and lateral maturity gradients. Among production formations, $\delta^{13}C$ increases from the Upper Cretaceous Sussex formation down through the Lower Cretaceous J Sand, representing a vertical separation of ~1000 m (Figure 8). For any given location within the field, $\delta^{13}C$ signatures of noncommingled Sussex, Codell, and J Sand production gases are distinct, therefore providing unique fingerprinting for the source rocks that provided the hydrocarbons for these reservoirs. An unknown gas sample can be compared to these production gas fingerprints to determine if it originates in one of the production gas formations. Lateral variability in $\delta^{13}C$ results from the fact that the Wattenberg field is centered over a geothermal hot spot, with maturity increasing toward the center of the field [109]. For all production formations, $\delta^{13}C$ exhibits an increase toward the center of the field [110]. This highlights the importance of site selection when trying to match an unknown gas to a production formation, since a Sussex gas from the center of the field could have a similar isotopic signature as a J Sand gas from the margin of the field, due to lateral differences in thermal maturity.

Of the 95 water samples with measured $\delta^{13}C$ of methane and higher alkanes, 84% clearly contain microbial methane, where $\delta^{13}C_1 < -60‰$ as shown on the gas characterization plots in Figure 6. In $\delta^{13}C_1$ versus δD_{C_1} space, these gases mainly occupy a region midway between methyl fermentation and CO_2 reduction (Figure 6B), consistent with a shallow, *in situ* source of microbial methane. The remaining 16% of samples have either thermogenic or mixed microbial-thermogenic $\delta^{13}C_1$ values. For these mixed samples, $\delta^{13}C$ of C_2 and C_3 clearly overlap with thermogenic production gas isotopic signatures (Figure 8). Therefore, it may be concluded that these gases represent mixtures of microbial and thermogenic gases, rather than microbial methane that has been secondarily enriched in $\delta^{13}C_1$ through biodegradation [23,62]. Among the thermogenic and mixed microbial-thermogenic water samples, overlap of $\delta^{13}C_{2+}$ values with Wattenberg field production gases suggests that the water sample gases have not been significantly altered by secondary fractionation processes (e.g., migration, biodegradation)

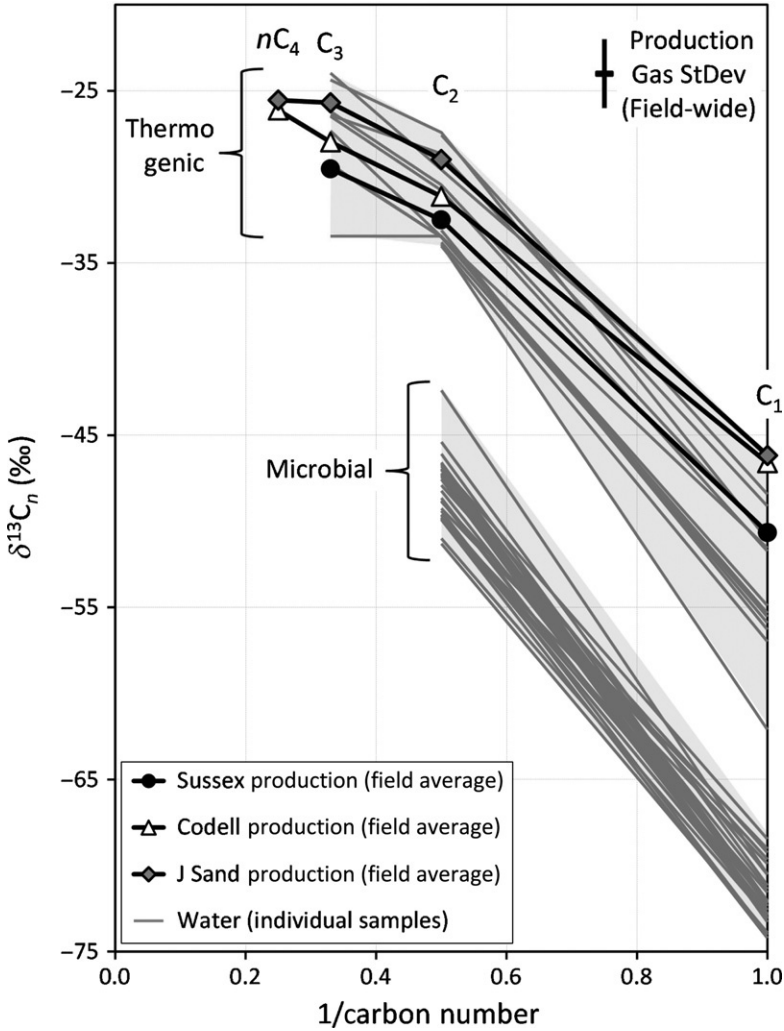

FIGURE 8 Chung *et al*. [65] plot showing Wattenberg field production gas and water sample $\delta^{13}C_n$ data available from the COGCC database. Production data show values for noncommingled Sussex, Codell, and J Sand, averaged by formation across the field. Indicated standard deviation reflects variability within a production formation due to lateral maturity gradient across the field. Water data are from 35 individual water samples in which $\delta^{13}C$ of C_2 or higher was reported. The $\delta^{13}C_{2+}$ data delineate water samples into populations of microbial and thermogenic gas (gray-shaded regions).

(Figure 8). The overlap between water sample and production gas $\delta^{13}C_{2+}$ values further suggests that some of these water samples may contain thermogenic gas which has migrated from a source at least as deep as the Codell, and perhaps as deep as the J Sand. Water samples collected in close proximity to a mudgas isotope profile through these formations may help to positively

identify the source of stray thermogenic gas in the water, whether from deep underground production formations or from relatively shallow, naturally occurring thermogenic gas accumulations [10,11,24].

This Wattenberg Field case study demonstrates the utility of these interpretive methods in determining unique sources of microbial and thermogenic gas. The relatively "wet" molecular composition of thermogenic production gases in the Wattenberg Field, with measurements of $\delta^{13}C$ up to C_4, allows for robust fingerprinting of different production gas formations. The $\delta^{13}C$ of C_2 and higher alkanes in water samples clearly differentiates between microbial and thermogenic sources of gas in the water. The thermogenic gas fingerprints in water samples are consistent with migration of stray gas originating from traditional Wattenberg production reservoirs; however, mudgas profiles from surface to the depth of at least the Sussex would be required to rule out the possibility that these thermogenic gases originate from shallower thermogenic sources. Additional supporting evidence, including geological and historical information, and evidence from nearby well completions and stimulation activities would be necessary to confirm the origins of the stray gas [6].

6 CONCLUSIONS

Responsible development of oil and gas resources requires effective environmental monitoring and remediation technologies. Compound-specific stable isotope analysis of the range of natural gas alkanes provides a robust fingerprint of unique gas origins. Samples of unknown gas may be compared to known nearby point sources, including underground hydrocarbon reservoirs sampled from the mudgas stream while drilling, casing or bradenhead gases, and production streams, to determine gas origin [9]. The wide range of analyte concentrations and stable isotope values typically encountered in natural gas samples necessitates specific GC-IRMS analytical considerations, including variable sample injection volumes, dilution factors, and multipoint isotope calibration. Natural gas stable isotope interpretive methods traditionally used for hydrocarbon exploration and development in the deep subsurface are directly applicable to understanding of the origin and transformation of stray natural gases in the shallow subsurface. Carbon isotopic analysis of ethane (C_2) and higher molecular weight alkanes is especially important in this context. By identifying unique sources of stray gas, natural gas isotope analysis, when used in conjunction with other lines of geological and historical evidence, is a powerful monitoring and remediation tool.

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Chapter 16

Analysis of Halogenated Flame Retardants by Gas Chromatography Coupled to LRMS, HRMS, MS–MS, and TOF-MS

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1 INTRODUCTION

Since fires are an important source of material and economical damages, and can also cause loss of human lives, flame retardants (FRs) have been used since many years in order to reduce the flammability of certain materials. For instance, the Egyptians used alum to reduce the flammability of wood [1]. Chemical compounds are used nowadays but the purpose is still the same: to prevent fires. These chemicals are applied to different kinds of materials

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such as textiles, plastics, wood, electronic furniture, and so on [2]. In addition, the recent developments in the polymeric industry have led to the introduction of a wide number of polymers which have rather different properties and applications. Moreover, most of them are derived from petroleum and, thus, are flammable. Therefore, the presence of the FRs to prevent fires is needed in almost all the materials that we are surrounded by. The National Bureau of Standards carried out several room combustion tests comparing different plastic materials with and without FRs. Results showed that materials containing FRs presented less heat and smoke release, together with lower generation of toxic gases generated during the combustion process [3].

There are more than 175 different types of FRs, divided into general groups: inorganic, organophosphorous, nitrogen-containing, and halogenated organic FRs [4]. Inorganic FRs are used in polymers and are considered immobile; organophosphorous are used in polymers and textiles; nitrogen-containing are used in polymers which contain nitrogen (e.g., polyurethane); finally, halogenated organic FRs are used in a wide range of materials such as polymers, textiles, furniture, and so on [1,5].

The combustion process has four main steps: preheating, volatilization/decomposition, combustion, and propagation. The FRs have different modes of action depending on the step of the combustion process they act on [6]. For example, some compounds can cause parallel endothermic reactions which can dilute the temperature of the flames. On the other hand, some compounds can act at the propagation step by capturing the free radicals produced in the third step inhibiting the propagation of the reaction. This is the way of action of the halogenated FRs (HFRs). However, not all of the halogenated compounds are highly effective. The most common HFRs are the brominated and chlorinated compounds, which meet the optimal conditions in terms of thermal stability. Among them, brominated compounds are most used since the bromine ion is heavier than the chlorine ion [7] and less flammable.

1.1 Brominated Flame Retardants

Global market demand of brominated FRs (BFRs) increases every year and it is estimated that more than 200,000 tons are produced every year. They are used in a wide range of materials such as electronic materials, textiles, furniture, or vehicles, where they represent a 5–30% of the total product weight [5]. The main BFRs are tetrabromobisphenol A (TBBPA), polybromodiphenyl ethers (PBDEs), and hexabromocyclododecane (HBCD) [8]. Since they are brominated, BFRs are usually toxic, persistent in the environment and with a great capacity of bioaccumulation [9]. They have also proved their capacity for global transport, for example, they have been found in the Arctic, far away from any area with an industrial activity [10]. In fact, many BFRs are listed as persistent organic pollutants (POPs). BFRs are often classified as reactive or additive, depending on the way they are used in the chemical

industry. Reactive FRs, like TBBPA, are added to the polymer matrix by a covalent bond, whereas additive FRs like PBDEs of HBCD are dissolved in the matrix and as a result they are weakly associated with the polymer [11]. Due to that, they can be easily released into the environment by leaching out of the product.

1.1.1 Polybrominated Diphenyl Ethers

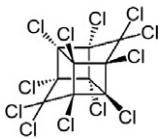
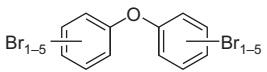
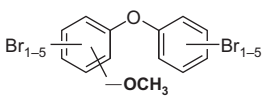
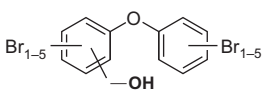
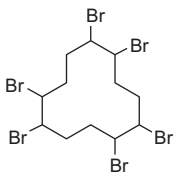
There are 209 different congeners of PBDEs, depending on the number of bromine atoms and their position in the aromatic rings [12] (Table 1). Since the hydrogen positions of PBDEs are similar to polychlorinated biphenyls (PCBs), the nomenclature proposed by Ballschmiter and Zell [12] is also used for PBDEs. Commercially, PBDEs can be found in three different commercial mixtures: penta-BDE mixture, which contains a 24–37% of tetra-BDEs, a 50–60% of penta-BDEs, and a 4–8% of hexa-BDEs; Octa-BDE mixture, which contains a 10–12% of hexa-BDEs, a 44% of hepta-BDEs, a 31–35% of octa-BDEs, and a 10–11% of nona-BDEs; and Deca-BDE mixture, which contains a 97% of BDE-209 and a 3% of nona-BDEs [16].

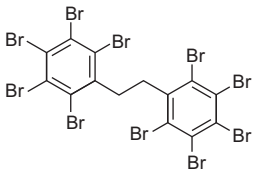
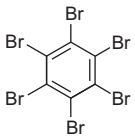
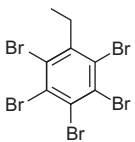
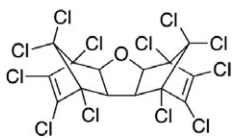
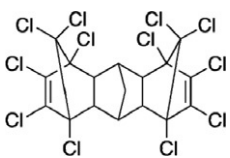
PBDEs were first detected in the environment in 1970 [17] and their production highly increased in middle 1990s, when polybrominated biphenyls (PBBs) were banned [18]. The scientific community became really aware of PBDEs when [19] they were reported in human breast milk, with levels increasing over the years, while the levels of other POPs were decreasing. However, nowadays the situation has changed. The penta-BDE and octa-BDE mixtures were banned by the EU in 2001 and since 2006 their presence in polymeric formulations and other compounds is being reduced both in Europe and in North America [20]. Deca-BDE is already banned in Europe, whereas there are exceptions for certain applications, and its production in North America is slowly decreasing and the plan is to stop its production by the end of 2013 [21]. In consequence, due to the recent restrictions over the PBDEs, other brominated compounds are proposed as substitutes of PBDEs. Some examples of these compounds, which are considered emerging BFRs, are HBCD, hexabromobenzene (HBB), pentabromoethylbenzene (PBEB), or decabromodiphenylethane (DBDPE) [22].

1.1.2 Decabromodiphenylethane

This compound is being used actually as a substitute of BDE-209, although its use is not as extended yet as BDE-209 due to its high cost [23]. However, it will probably become one of the most abundant BFRs in the thermoplastic industry [24]. In contrast with BDE-209, it does not produce furans or dioxins when it is exposed to natural sunlight [25] and its bioavailability seems to be lower than that of BDE-209 due to its high log K_{ow} (Table 1), which is a ratio of concentrations of the un-ionized compound both in octanol and water, and it is used to evaluate lipophilicity and it has been estimated as a value of 11

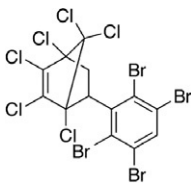
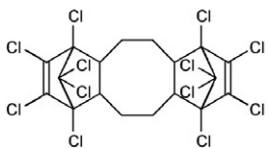
TABLE 1 Structural Information and Physicochemical Properties of HFRs

Family Name	Structure	Molecular Formula	Nominal Mass (g/mol)	Log K_{ow}	Water Solubility 25 °C (mg/L)	LC_{50} (μg/L)	References
Mirex		$C_{10}Cl_{12}$	546	7.01	4.76×10^{-4}	>100	[13]
PBDEs		$C_{12}HOBr_y$	248–950	5.88–12.1	4.4×10^{-3}–1.4×10^{-2}	2.37–1×10^5	[1]
MeO-PBDEs		$C_{13}H_xO_2Br_y$	278–902	10.1–13.5	n/a	n/a	[14]
OH-PBDEs		$C_{12}H_xO_2Br_y$	264–887	8.3–12.7	n/a	n/a	[14]
HBCD		$C_{12}H_{18}Br_6$	642	5.6	2.1×10^{-3}–4.9×10^{-2}	n/a	[15]

DBDPE		$C_{14}H_4Br_{10}$	972	11.1	n/a	n/a	[15]
HBB		C_6Br_6	552	6.11	1.6×10^{-2}	2×10^4	[15]
PBEB		$C_8H_5Br_5$	501	6.76	0.21	>500	[15]
Dec 602		$C_{14}H_4Cl_{18}O$	614	8.05	1.75×10^{-5}	n/a	[13]
Dec 603		$C_{17}H_8Cl_{12}$	638	11.2	2.45×10^{-8}	n/a	[13]

Continued

TABLE 1 Structural Information and Physicochemical Properties of HFRs—Cont'd

Family Name	Structure	Molecular Formula	Nominal Mass (g/mol)	Log K_{ow}	Water Solubility 25 °C (mg/L)	LC_{50} (μg/L)	References
Dec 604		$C_{13}H_4Br_4Cl_{16}$	693	11.5	3.75×10^{-8}	n/a	[13]
DP		$C_{18}H_{12}Cl_{12}$	654	11.3	1.68×10^{-8}	$>1 \times 10^{5}$	[13]

for DBDPE [26]. DBDPE was first found in the environment in 2000 [26], and since then it has been found in different environmental matrices, such as sediments [27] or sludge [28], and in different biological samples such as bivalves [29] or bird eggs [30]. The sources and behavior of DBDPE are still not clear [31], so more research is needed to completely know the environmental extent of this contaminant.

1.1.3 PBEB and HBB

PBEB is an additive that FRs used mostly in thermoset polyester resins (textiles, adhesives, electric coatings), and it was produced between the 1970s and 1980s in the United States [32]. It is classified as a low production volume chemical in the EU, and it is classified as persistent, toxic, and with bioaccumulation capacity [15].

On the other hand, HBB is mainly produced in Japan and China [33], whereas its production is not reported by the EU industry [15]. Both PBEB and HBB have been found in the environment in different matrices such as air, marine mammals, bird eggs, and blood.

1.1.4 PBDE Analogues: Methoxylated and Hydroxylated PBDEs

There are more than 4000 naturally produced halogenated compounds in the marine environment, such as methoxylated (MeO)-PBDEs or hydroxylated (OH)-PBDEs [34] (Table 1). MeO-PBDEs are produced by blue mussels, sponges, or red algae [35], and they can bioaccumulate and biomagnify through the food chains in the same way as PBDEs [36]. Due to their source, they are considered a problem only in the marine environment. Furthermore, their concentration has proved to be higher in deep seas than in coastal areas [37]. They have been found in marine animals, especially in top predators around the world [38]. On the other hand, it is thought that OH-PBDEs are metabolites from PBDEs, as they have been found in rats or fish [39]. However, it has been suggested that OH-PBDEs may also come from natural sources, that is, sponges [40]. Similar to MeO-PBDEs, they have been found in the aquatic environment [41] but also in terrestrial food chains [14]. To achieve a complete understanding of the danger that PBDEs represent to the environment, these two groups of compounds must also be taken into account in environmental analysis.

1.2 Halogenated Norbornenes

The halogenated norbornenes included different compounds such as Dechlorane plus ($C_{18}H_{12}Cl_{12}$, DP), Dechlorane 602 ($C_{14}H_4Cl_{12}O$, Dec 602), Dechlorane 603 ($C_{17}H_8Cl_{12}$, Dec 603), and Dechlorane 604 ($C_{13}H_4Br_4H_{16}$, Dec 604). They appeared as substitutes of Mirex ($C_{10}Cl_{12}$) when Mirex was banned as FR in 1976 due to its bioaccumulation capacity and its toxic effects (Table 1). There

are only two chemical facilities which report the production of these compounds: OxyChem (New York, USA) and Anpon Electrochemical Co. (China) [42].

DP exists as two stereoisomers, *syn*- and *anti*-DP, with an F_{anti} (ratio between the concentration of *anti*-DP and the total concentration of DP) value of 0.7 in commercial mixtures [43]. It is listed in the Canadian Domestic Substance List and it cannot be present in plastic materials in amounts higher than 35% of the total weight [44]. It is considered a low production volume chemical in the EU, in contrast with the United States, where it is considered a high production volume chemical [13]. It is used in wire coatings, plastics, computers, and televisions, and it has been proposed as an alternative to BDE-209 [13]. Thus, the studies about its occurrence and behavior, together with its toxicity, have increased in the past years. Since its first detection, in 2006 [45], it has been detected in a wide range of environmental and biological matrices, such as air, water, sludge, sediment, fish, mussels, or cetaceans, and also in human matrices such as hair, blood, or milk [13,45–48].

DP-related compounds are generated by the same Diels–Alder reaction than DP, but with a different precursor [13]. Dec 602, Dec 603, and Dec 604 were patented by OxyChem in the middle 1960s with the idea of improving the structures of the polymers and made them less flammable. Actually, Dec 602 and Dec 604 are listed in the Canadian Non-Domestic Substance List and in the European Chemical Substances Information System. They are less restricted than DP and sometimes they are used when the use of DP does not meet the legal requirements [49]. Meanwhile, there is a lack of information about the uses of Dec 603 as FR, but its presence as an impurity of Aldrin and Dieldrin has been reported [50]. These compounds were identified for the first time in sediment from the Great Lakes [51], and since then they have been found in different environmental and biological matrices such as sediment, air, sludge, bird eggs, and fish [13,51–55].

1.3 Toxicity

The toxicological effects of PBDEs have been widely reported [56–58]. They can act as endocrine disruptors which can cause several harmful effects, as well as damages in the neurological system. The mechanisms are not well defined, but they could include estrogenic and androgenic activity, variations in the bonds to some receptors such as pregnane X receptor and constitutive androstane receptor, and, probably, disruptions in the thyroid hormones [56]. They can also cause damages in the liver and in the neurological system, especially during its development. PBDEs can disrupt the natural homeostatic processes due to the similarity between their structure with the structures of the hormones triiodothyronine (T_3) and thyroxine (T_4) [59]. In animals, Octa-BDE mixture has a limited toxicity while there is evidence that Penta-BDE mixture has effects in the development of the nervous system [5]. In addition, low brominated BDEs tend to accumulate in the liver, causing

an increase of the enzymatic activity or histopathological changes [60]. BDE-209, the most brominated BDE, is the congener with more toxicological studies about it [57]. It has been proved that it can affect the normal development of the nervous system in rats, transferring from the mother to the fetus by the placenta [61]. Moreover, some BDE congeners present the capacity to form adducts with DNA molecules and, in consequence, modify the gene expression system and cause mutations [62].

As mentioned before, DBDPE has lower solubility than BDE-209. Thus, its bioavailability is also lower. However, the toxicokinetic properties of these two compounds seem to be different. Moreover, DBDPE can alter thyroid hormone homeostasis and interfere with the mRNA decoding [63].

Mirex toxicity has been widely evaluated due to its high persistence and its extended use, which has exposed human and other species during a long period. Different harmful effects, such as changes in liver weight or the inhibition of the reproductive capacity in rats, have been reported. Furthermore, it is carcinogenic in animals, so it is a probable carcinogen also in humans [64,65]. Despite the fact that DP was supposed to not have the same toxic properties as Mirex, the fact that it has been found in biological matrices suggests that it could also present toxicological properties. The information about the toxicity of DP or its analogues is still limited, but the number of studies is rising in recent years. For instance, a decrease in liver weight and ovaries was observed in female rats when they were dermally exposed to DP [66]. It seems to affect the mRNA expression [67] and increase the enzyme activity of CYP 2B2 [68].

2 SAMPLE TREATMENT

Since HFRs have, in general, similar physicochemical properties, as they are highly hydrophobic and fairly stable, the same methodologies used for the extraction of PCBs, PBBs, and other organic compounds are also used for the extraction of PBDEs, emerging BFRs, and halogenated norbornenes. Other compounds such as OH-PBDEs, which are more polar, need a more complex extraction to be determined by gas chromatography (GC). Some examples of the most used extraction and purification methodologies are shown in Table 2.

2.1 PBDEs, Emerging BFRs, and Halogenated Norbornenes

The number of studies reporting these compounds in water is small, due to their high hydrophobicity [79]. Nevertheless, there are several extraction methodologies used. The most used technique is liquid–liquid extraction using hexane, isooctane, and *tert*-butyl ether as extraction solvents [80]. The main problem that occurs with this technique is that it requires a high volume of sample and consumes a lot of solvent. Another technique which is widely

TABLE 2 Sample Preparation and Instrumental Analysis of Several HFRs in Different Matrices

Compounds	Matrix	Extraction	Cleanup	Chromatographic Column	Instrumental Technique	References
PBDEs	Sediment	Soxhlet hexane: DCM (3:1)	Acidified silica	HT-8 50 m × 0.22 mm × 0.25 μm	GC–NCI-MS	[69]
PBDEs, emerging HFRs, halogenated norbornenes	Sediment	PLE hexane: DCM (1:1)	Neutral alumina	DB-5MS 15 m × 0.1 mm × 0.1 μm	GC–NCI-MS GC–NCI-MS–MS	[70,71]
PBDEs, emerging HFRs, halogenated norbornenes	Air	Soxhlet hexane: acetone (1:1)	Alumina	DB-5MS 30 m × 0.25 mm × 0.25 μm	GC–NCI-MS	[72]
PBDEs, emerging HFRs, halogenated norbornenes	Sludge	PLE hexane: DCM (1:1)	Acid silica, basic alumina, activated carbon	DB-5MS 60 m × 0.25 mm × 0.25 μm	GC–HRMS	[73]
PBDEs, emerging HFRs, halogenated norbornenes	Biota	PLE Hexane: DCM (1:1)	1. Acid treatment 2. Alumina	DB-5MS 15m × 0.10 mm × 0.1 μm	GC–NCI-MS GC–NCI-MS–MS	[71]
Halogenated norbornenes	Biota	LE (hexane)	Silica and alumina	DB-5HT 5 m × 0.25 mm × 0.10 μm	HRMS	[74]
PBDEs, emerging HFRs	Bird eggs	Soxhlet (DCM)	1. GPC 2. Silica	DB-5HT 15 m × 0.25 mm × 0.10 μm	HRMS	[30]
Halogenated norbornenes	Bird eggs	MSPD	Acid and basic silica	DB-5HT 15 m × 0.25 mm × 0.10 μm	HRMS	[75]
PBDEs, natural BDEs	Biota	PLE Hexane: DCM (1:2)	GPC	DB-5 30 m × 0.25 mm × 0.25 μm	HRMS	[76]
PBDEs	Human hair	L-L-Hexane	Fluorisil	ZB-5MS 15 m × 0.25 mm × 0.1 μm	GC–EI-MS	[77]
PBDEs, halogenated norbornenes	Breast milk	L-L-Hexane: DCM (1:1)	GPC	DB-MS 15 m × 0.25 mm × 0.25 μm	GC–NCI-MS	[78]

used is solid-phase extraction (SPE), especially using C_{18} as the sorbent [81]. In the recent years, some new extraction techniques such as stir bar sorptive extraction (SBSE), cloud point extraction (CPE), and dispersive liquid–liquid microextraction (DLLME) have appeared as an alternative to the classical techniques for the extraction of HFRs in water samples [82–84].

Soxhlet and pressurized liquid extraction (PLE) are the most commonly used extraction techniques for extracting a wide number of organic compounds from solid matrices, normally using hexane:dichloromethane 1:1 or hexane: acetone 1:1 as extraction solvents [38,46,85,86]. Some new techniques such as solid-phase dispersion [87] or ultrasound-assisted leaching-dispersive solid-phase extraction followed by liquid–liquid microextraction (USAL-DSPE-DLLME) [88] are recently presented as an alternative for solid samples.

For biota samples, an acid treatment [30,45,71,89] or gel-permeation chromatography (GPC) have been used to remove lipids from the sample extract. Moreover, in some cases, multilayer columns of silica, alumina, or carbon silica were used to separate halogenated norbornenes from other halogenated compounds such as PBDEs [55,87,90,91].

In contrast with the rather extensive information of HFRs in the environment and aquatic and terrestrial organisms, there is less information regarding human biological samples. Again, Soxhlet and PLE are the main techniques used for the analysis of different solid human matrices such as hair [77]. When determining HFRs with different physicochemical properties in liquid matrices, such as blood or breast milk, liquid–liquid extraction is normally used to separate nonpolar compounds from the polar ones [92]. Other authors use SPE with two different cartridges in order to perform the extraction and cleanup simultaneously [93].

2.2 MeO-PBDEs and OH-PBDEs

The simultaneous determination of methoxylated and hydroxylated compounds is not easy due to their different physicochemical properties. While MeO-PBDEs have similar properties than PBDEs and hence can be extracted and determined by GC using the same methodologies applied for PBDEs, in the case of OH-PBDEs, a previous derivatization step is needed. This step is often a methylation using a derivatization agent such as diazomethane [94]. However, it is not as simple as that, because the new MeO-PBDEs generated may interfere with the natural MeO-PBDEs that could be also present in the sample. Derivatization can be done at different times. Some authors divide the resulting sample extract in two parts and perform the derivatization only in one part. In this way, MeO-PBDEs and other organic compounds are determined together and OH-PBDEs are determined by the analysis of the MeO-BDEs contained in the derivatized fraction. On the other hand, other authors prefer to carry out the derivatization after the analysis of the extract. Then, they reinject the derivatized sample and the difference between the two MeO-BDE values is the amount of OH-PBDEs in the sample.

There are some authors who avoid the derivatization step by analyzing directly the OH-PBDEs by liquid chromatography mass spectrometry (LC–MS) [95,96]. This means that there is no need to use derivatization agents, which are normally carcinogenic and difficult to handle. Furthermore, the derivatization has other disadvantages such as the low reproducibility that is normally obtained. However, the detection limits obtained by LC–MS are higher than those obtained by GC–MS.

3 INSTRUMENTAL ANALYSIS

3.1 Chromatographic Separation

GC is the most widely used separation technique for the analysis of HFRs due to the vapor pressure and polarity of these compounds [97]. Capillary columns are the most commonly used to obtain an appropriate resolution and separation [98]. The choice of the column is critical because the type of retention gap, connector, column length, or stationary phase can have a great influence in the analysis of HFRs [99]. Nonpolar stationary phases such as DB-5 are used for the separation of these compounds (Table 2). The best separation is obtained with columns of 30–50 m, but BDE-209 is lost by thermal degradation during the injection and also due to the long retention time in these columns [26]. The optimal length for the analysis of BDE-209 is 15 m [86]. Other factors, such as the film thickness, which is recommended to be between 0.1 and 0.2 mm, are important for the analysis of higher brominated compounds [92]. Moreover, some stationary phases, such as DB-XLB, are not useful for the analysis of compounds with high molecular mass [26].

Occasionally, authors make a compromise and chose one length or another depending on the compounds they are analyzing. For example, when analyzing MeO-PBDEs by a 15 m column, there is a coelution between 6-MeO-BDE-47 and BDE-77, which is usually used as an internal standard. Thus, when analyzing biota samples, where BDE-209 tends to be low, some authors choose a 30 m column in order to have a proper separation of the MeO-BDE congeners [38]. In this case, BDE-209 cannot be determined because of thermal degradation.

Another important step in the analysis of HFRs with GC is the injection system. Splitless, on-column, and programmable temperature vaporization are the three most common systems found in the literature [100]. Temperature of the injection port is also important. Usually, it is settled between 250 and 300 °C. If it is too high, the compounds with high molecular masses are degraded [92].

Recently, new alternative techniques such as two-dimensional gas chromatography (GC × GC) have been considered for the analysis of PBDEs, motivated by the increase in resolving power that the two different columns provide and by the fact that this technique has been successfully applied for the analysis of polyhalogenated contaminants [99]. All the coelution problems

that may occur in the analysis of organohalogenated compounds are solved by using GC × GC coupled to time-of-flight (TOF)-MS [101]. However, the use of this technique is not common due to its high cost compared to traditional GC–MS.

Finally, the use of LC–MS should also be taken into account, in spite of it being not as usual as GC–MS. In theory, the thermal degradation of the less stable compounds can be avoided using LC–MS, as suggested by Bacaloni *et al*. [102]. Normally, nonpolar stationary phases are used, as in GC–MS. C_{18} columns seem to meet the ideal conditions for the analysis of HFRs by LC [96]. However, the number of authors who prefer LC–MS against GC–MS is still small for the compounds that are able to be analyzed by GC–MS. Meanwhile, other compounds like HBCD or TBBPA are usually analyzed by LC–MS. HBCD has different diastereoisomers and the analysis of this compound by GC–MS is almost impossible due to the interconversion of the diastereoisomers when the temperature is higher than 160 °C or the decomposition at temperatures above 240 °C [103]. Consequently, HBCD can be quantified only as a total value of all the diastereoisomers by GC–MS, which is not an option since they have a different behavior in the environment. Thus, HBCD diastereoisomers are normally analyzed by LC–MS with electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) [104].

3.2 Mass Spectrometric Determination

The three most extended techniques for the analysis of HFRs are MS, tandem mass spectrometry (MS–MS), and high-resolution MS (HRMS). HRMS is the one which provides better results in terms of selectivity and sensitivity, and it is the main choice for authors who have access to this kind of instrument. On the other hand, its cost is considerably higher than MS and MS–MS instruments and, consequently, not all the laboratories can afford this technique (Table 3).

3.2.1 Single Quadrupole Mass Spectrometry

The most advantageous technique is GC–MS working in negative chemical ionization (NCI) or electron ionization (EI) mode. EI is considered the main ionization source in GC–MS, but sometimes it is not the best option. Because not all the analytes are capable of producing a stable negative ion, NCI provides a higher sensitivity for the analytes that can ionize, thus being higher than that afforded by EI. Therefore, halogenated compounds are normally analyzed using this ionization mode due to their high electronegativity [105]. Furthermore, the selection of the reagent gas is also important. Methane and ammonia are the two most common reactive gases used. The use of ammonia and methane does not influence the signal of BFRs [106]. However, the use of methane provides better limits of detection (LOD) than those

TABLE 3 Comparison of the Main Used Techniques for the Analysis of HFRs

	NCI-MS	EI-MS	IT-MS–MS	QqQ-MS–MS	TOF-MS	HRMS
Sensitivity	a	b	a	a	a	c
Selectivity	b	a	a	a	a	c
Accuracy	a	c	c	c	a	c
Cost	b	b	a	a	a	c

[a]*Medium.*
[b]*Low.*
[c]*High.*
Modified from Ref. [103].

obtained working with ammonia for halogenated norbornenes. This behavior could be explained by the fact that these two gases have different proton affinities, and ammonia could form a complex with Cl^- that could change the equilibrium in the source [71]. Moreover, another difference in the conditions for BFRs and halogenated norbornenes is the source temperature. While for BFRs high temperatures, for example, 250 °C, are normally used, especially for the highly brominated BDEs [107], it has been demonstrated that the intensity of the molecular ion of DP decreases critically at this temperature due to the high fragmentation of the molecular ion. Thus, the optimal source temperature for DP must be low, for example, 150 or 175 °C [71,108]. On the other hand, NCI-MS has a poor selectivity for BFRs because the ions commonly monitored are *m/z* 79 and 81, while more specific fragments are monitored in EI (Table 4). As a consequence, mass labeled standards cannot be used in NCI-MS. Moreover, since only one bromide ion is monitored, there are several coelutions that may become a serious problem.

Another option is to work in GC–MS using EI as ionization mode. This presents two main advantages: the fragmentation of the molecules can be used for identification purposes, and the use of ^{13}C-labeled compounds is possible for BFRs, making the quantification process more accurate. However, there is a significant loss of sensitivity compared with NCI-MS. Instrumental limits of detections (iLODs) using NCI for tri-, tetra-, penta-, hexa-, and hepta-BDEs were 89, 76, 80, 61, and 128 fg injected, while for EI were, in the same order, 690, 1960, 4300, 2550, and 13,580 fg injected [106]. As can be seen, there is a significant difference. This is especially critical for deca-BDE-209 determination. Since BDE-209 is usually the most abundant PBDE congener in environmental samples, its detection is crucial for complete information of the behavior of PBDEs in the environment. Moreover, the ions monitored for BDE-209 identification when using NCI-MS are *m/z* 487 and 489 (Table 4), and thus, the labeled standard ^{13}C-BDE-209 can be used for quantification

TABLE 4 Ion Fragments or Transitions Monitored for the Determination of HFRs by Different MS Techniques

Compound	Technique	Fragments
Tri-BDEs	GC–MS (NCI)	79/81
	GC–MS (EI)	406/408
	GC–MS–MS	409–249/407–247
	GC–HRMS	405.8026/407.8006
Tetra-BDEs	GC–MS (NCI)	79/81
	GC–MS (EI)	484/486
	GC–MS–MS	486–326/488–328
	GC–HRMS	483.7131/485.7121
Penta-BDEs	GC–MS (NCI)	79/81
	GC–MS (EI)	564/566
	GC–MS–MS	566–406/568–408
	GC–HRMS	563.6215/565.6195
Hexa-BDEs	GC–MS (NCI)	79/81
	GC–MS (EI)	642/644
	GC–MS–MS	646–486/648–488
	GC–HRMS	641.5320/643.5300
Hepta-BDEs	GC–MS (NCI)	79/81
	GC–MS (EI)	722/724
	GC–MS–MS	561–402/563–404
	GC–HRMS	721.4405
Deca-BDE	GC–MS (NCI)	487/489
	GC–MS (EI)	797/801
	GC–MS–MS	799–640/801–642
	GC–HRMS	957.1699/959.1679
Tri-MeO-BDEs	GC–MS (NCI)	79/81
	GC–MS–MS	n/a
	GC–HRMS	435.8133/437.8113

Continued

TABLE 4 Ion Fragments or Transitions Monitored for the Determination of HFRs by Different MS Techniques—Cont'd

Compound	Technique	Fragments
Tetra-MeO-BDEs	GC–MS (NCI)	79/81
	GC–MS–MS	516–356/516–358
	GC–HRMS	513.7237/515.7217
Penta-MeO-BDEs	GC–MS (NCI)	79/81
	GC–MS–MS	596–434/596–433
	GC–HRMS	n/a
Tri-OH-BDEs	GC–MS (NCI)	79/81
	GC–MS–MS	n/a
	GC–HRMS	421.7976/423.7956
Tetra-OH-BDEs	GC–MS	79/81
	GC–MS–MS	n/a
	GC–HRMS	501.7061/503.704
DBDPE	GC–MS (NCI)	79/81
	GC–MS (EI)	485/487
	GC–MS–MS	485–325/485–404
	GC–HRMS	969.2063/971.2043
HBB	GC–MS (NCI)	79/81
	GC–MS (EI)	550/552
	GC–MS–MS	550–390/552–313
	GC–HRMS	549.5058/551.5038
PBEB	GC–MS (NCI)	79/81
	GC–MS (EI)	485/487
	GC–MS–MS	485–325/485–406
	GC–HRMS	499.6266/501.6246
Dec 602	GC–MS (NCI)	612/35
	GC–MS–MS (NCI)	612–35/612–37
	GC–HRMS	271.8102/273.8072
Dec 603	GC–MS (NCI)	638/35
	GC–MS–MS (NCI)	638–35/638–37
	GC–HRMS	262.8570/264.8540

TABLE 4 Ion Fragments or Transitions Monitored for the Determination of HFRs by Different MS Techniques—Cont'd

Compound	Technique	Fragments
Dec 604	GC–MS (NCI)	79/81
	GC–MS–MS (NCI)	460–79/504–79
	GC–HRMS	417.7026/419.7006
DP	GC–MS (NCI)	654/35
	GC–MS–MS (NCI)	654–35/654–37
	GC–HRMS	271.8102/273.8072

n/a: not available.

(*m/z* 497 and 499), which makes the results obtained for BDE-209 more accurate [70]. This is why some authors still prefer to use NCI-MS for the analysis of BFRs in environmental samples. In fact, some authors propose to determine HFRs using NCI-MS and then confirm the values obtained by EI-MS [109]. Figure 1 shows a comparison between EI-MS and NCI-MS.

Finally, another option is to use HRMS with EI as an ionization source. Then, the high sensitivity obtained by monitoring the exact mass of the compound is complemented with the selective fragmentation gained by the use of EI. However, HRMS is an expensive technique and not all the laboratories can afford it, which supposes a problem when comparing data among laboratories. In Table 4, the main ions and fragments used for GC–MS and GC–HRMS for the different groups of compounds are shown.

3.2.2 *Tandem Mass Spectrometry*

Due to the high cost of HRMS, the importance of the development of methodologies by low-resolution MS that provide similar LODs has been increased in recent years. One possibility is the use of MS–MS. This technique provides a great selectivity due to the fact that a transition between a parent ion and a fragment ion is monitored instead of a single ion (Table 4). Actually, most of the authors prefer working with EI-MS–MS compared with NCI-MS, even if this means to have slightly higher LODs [110–112]. The main advantage of EI-MS–MS is that selecting two transitions per compound instead of the two ions by single quadrupole MS makes this technique considerably more selective and quantification can be done by an isotope dilution technique. Moreover, when analyzing environmental and biotic samples, the noise is highly reduced and thus the sensitivity is increased. This is really important when analyzing samples with low levels of contamination. Pirard *et al.* [111] developed a

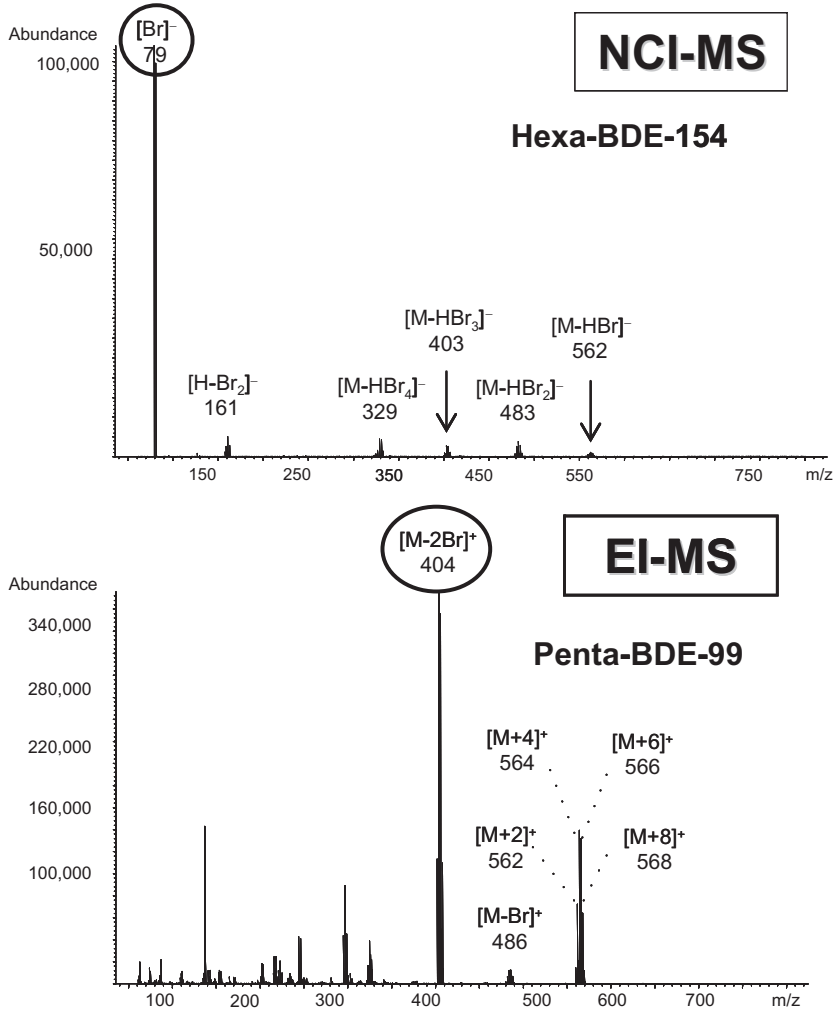


FIGURE 1 Comparison of BDE fragmentations using NCI and EI.

methodology using MS–MS for the analysis of PBDEs in fish samples. While HRMS was better in terms of precision, selectivity and accuracy, the method LODs (mLODs) were similar for both methodologies. An example of the effect of the selectivity of MS–MS compared to MS is showed in Figure 2.

Other analytical approaches, such as ion trap (IT)-MS, have been also developed. The applicability of IT-MS–MS has proved to be highly specific when working in EI mode [14,113,114]. The use of IT-MS shows different fragmentation and isolation patterns for PBDEs with the same degree of bromination [113]. Although this difference was not observed for the less brominated PBDEs, for tetra- to deca-BDEs there was a different fragmentation

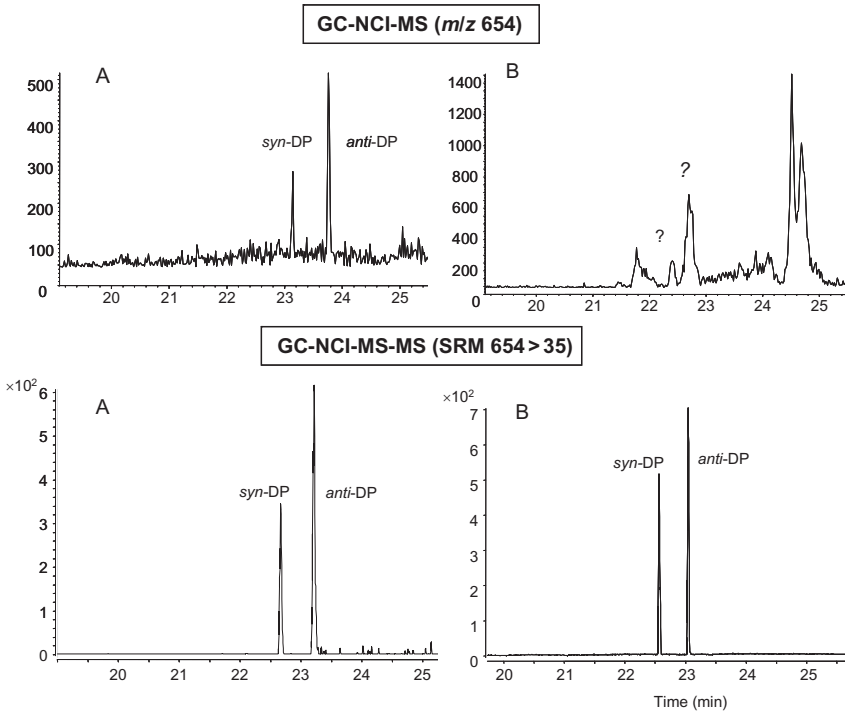


FIGURE 2 Comparison between GC–MS and GC–MS–MS for the analysis of DP in (A) sediment and (B) fish samples. *Modified from Ref. [71].*

pattern for *ortho*- and non-*ortho*-substituted PBDEs. The loss of two bromine atoms was seen for the *ortho*-substituted BDEs, in contrast with the pattern of non-*ortho*-BDEs, where the molecular ion cluster was the most intense peak in the spectrum. The analysis by IT-MS–MS also provides similar LODs than HRMS in the case of MeO-PBDE analyses [115]. Losada *et al.* [112] also found that fragmentation patterns of MeO-PBDEs analyzed by IT-MS–MS were different depending on the position of the methoxy group. For *ortho*-substituted MeO-PBDEs, the most intense fragments were $[M\text{-}Br_2]^+$ and $[M\text{-}Br\text{-}CH_3]^+$, while for *meta*-MeO-PBDEs, the most abundant fragment was $[M\text{-}Br_2]^+$. Finally, and despite the fact that for *para*-MeO-PBDEs there were also two significant fragments, $[M\text{-}Br_2]^+$ was selected as parent ion because the other fragment consisted of a loss of $—CH_3$ ($[M\text{-}CH_3]^+$) which is not specific of an MeO-PBDE.

In the case of halogenated norbornenes, the use of NCI-MS–MS clearly improves the LODs obtained by NCI-MS. There is only one published methodology for the analysis of halogenated norbornenes by GC–MS–MS [71]. This methodology allowed similar LODs than other methodologies developed using HRMS for halogenated norbornenes. Moreover, the analysis of halogenated

norbornenes by HRMS is not as strongly recommended as for BFRs. EI is the ionization source usually applied in HRMS, and with that ionization mode, the molecular ions of halogenated norbornenes have a low intensity due to a retro-Diels–Alder fragmentation. This fragmentation forms an intense ion at m/z 270 ($C_5Cl_6^+$) which is a common fragment of several organohalogen compounds. Thus, using that fragment leads to a loss of selectivity [116].

3.2.3 Time-of-Flight Mass Spectrometry

Similar to IT-MS, time-of-flight mass spectrometry (TOF-MS) avoids congener coelutions and the use of an isotopic dilution method, providing a high selective technique for a multiresidue analysis of several HFRs [117]. TOF-MS is the detector of choice when high acquisition scan rates are required [118], and it is the analyzer often used in high-speed GC/MS [119]. Ion ratios remain the same across the peaks since all ion fragments represent the same point on the chromatographic peak profile. This spectral continuity allows mass spectral deconvolution of overlapping peaks when the fragmentation patterns are different. Thus, coeluting compounds can be differentiated and other unknown compounds can also be identified [117]. Moreover, an accurate quantitative analysis is more difficult than using GC–MS because the data processing process is more complex.

In conclusion, even though the best option for the analysis of HFRs is GC–HRMS, its cost is too high for routine use at this time. Thus, GC–MS–MS, which provides a lower but similar sensitivity and selectivity, can be used as a good alternative to HRMS. If the only technique available is GC–MS, the selection of the ionization source (NCI or EI) is critical. If samples are highly contaminated, the best option would be EI, since it is more selective than NCI. On the other hand, if we suspect that samples may present low levels of contaminants, NCI should be used since it is more sensitive than EI. The pros and cons of these techniques are presented in Table 3.

4 LEVELS IN ENVIRONMENTAL SAMPLES

Levels of the "classical" HFRs such as PBDEs have been widely reported and are summarized in several reviews [1,10,14,120,121]. They have been detected in a wide range of matrices and almost everywhere in the world, proving that they are prone to long-range transport and can bioaccumulate in different trophic chains. There is less information about the emerging HFRs such as HBCD, PBEB, HBB, DBDPE, or halogenated norbornenes [13,15,46]. Regarding the halogenated norbornenes, most of the studies are focused in areas which are close to the production sources of these compounds. Obviously, the concentration levels found there are higher than the values found in other places of the world [116]. A recent review has summarized the different halogenated norbornene levels found in aquatic and

terrestrial biota samples, as well as in humans, showing the current research results on their bioaccumulation and biomagnification potential [116].

Table 5 summarized the recent reported levels of HFRs in different biological and environmental matrices. Only data from 2012 to 2013 are

TABLE 5 Recent Reported Levels of HFRs in Biological and Environmental Samples

Compounds	Matrix	Concentration Levels	References
Biological samples (ng/g lw)			
DP	Muscle and liver	10–810	[47]
PBDEs	Apple snail	3.04–16.3[a]	[122]
Emerging BFRs		0.17–16.8[a]	
PBDEs	Fish	9.40–36.7	[123]
DP		nd–0.47	
HNs	Fish	nd–92.24	[71]
Dec 602	Fish	1–100	[124]
HNs	Fish	2.1–110	[125]
PBDEs	Eels	22.1–140	[126]
Emerging HFRs		0.03–5.1	
PBDEs	Bullhead plasma	3.33–9.02[b]	[41]
MeO-PBDEs		nd–4.1	
PBDEs	Dolphin	6.3–119	[127]
Emerging HFRs		nd–20.1	
HNs		nd–20	
PBDEs	Dolphin bubbler	6–1797	[38]
Emerging HFRs		0.15–46	
MeO-PBDEs		61–2415	
PBDEs	Eagle tissues	10–24,000	[128]
MeO-PBDEs		1–590	
PBDEs	Bird eggs	1.5–117[b]	[129]
Emerging HFRs		nd–10[b]	

Continued

TABLE 5 Recent Reported Levels of HFRs in Biological and Environmental Samples—Cont'd

Compounds	Matrix	Concentration Levels	References
PBDEs	Breast milk	3–15	[78]
DP		nd–8	
PBDEs	Serum	0.07–10.5	[130]
Emerging HFRs		0.01–0.3	
MeO-PBDEs	Human plasma	0.38–52	[131]
OH-PBDEs		0.0053–0.49	
Environmental samples (ng/g dw)			
PBDEs	Sediment	nd–8.93	[132]
	Soil	0.21–4.02	
HNs	Urban sediment	nd–1.15	[54]
	Rural sediment	nd–0.58	
HNs	Sediment	nd–2	[71]
	Sewage sludge	nd–19.6	
PBDEs	Sewage sludge	20.7–2326	[70]
Emerging BFRs	Sewage sludge	5.17–203	
PBDEs		9.10–995	[48]
HNs		2.45–93.8	
HNs	Water	0.25–0.70 ng/L	[125]
	Sediment	nd–2.2	
	Air	0.12–0.37 ng/m^3	
DP	Indoor dust	2.9–42	[133]
	Soil	1.7	
	Sediment	17–140	
PBDEs	Indoor dust	60.4–82,400	[134]
PBDEs	Air	139–1693 fg/m^3	[135]
PBDEs	Air	nd–111 pg/m^3	[136]
Emerging BFRs		nd–0.31 pg/m^3	

HNs, halogenated norbornenes.
[a]*ng/g dw.*
[b]*ng/g ww.*

shown in this table since previous data have been already included in the various published reviews [116,137].

As not all HFRs have exactly the same properties, there are different distributions of the different congeners depending on the matrices. For example, it is well known that less brominated BDEs, such as BDE-47 or BDE-99, have more bioaccumulation potential than the higher brominated ones. On the other hand, BDE-209 or DBDPE are most likely found in sediment or sludge, as they do not have a high bioaccumulation capacity compared to low brominated BDEs due to its high molecular mass and bromination degree [27,138].

DP exists as two different isomers, the *syn*- and the *anti*-DP. Some studies analyzed the F_{anti}, defined as the concentration of the *anti*-DP divided by the total concentration of DP (sum of concentrations of *syn*- and *anti*-DP). Different values of F_{anti} show that the two isomers might not have the same bioaccumulation and/or biomagnification capacity. While the average value of F_{anti} in sediment does not change from the value of the commercial mixture (0.69 ± 0.5) [48], its value considerably decreases in biota samples. This might indicate either that *anti*-DP is more easily degraded or metabolized, or that *syn*-DP has more bioaccumulation potential [13].

The most interesting result is to compare the concentration levels of the classical HFRs against the emerging HFRs. Although PBDEs still dominate the HFRs profile, it is expected that this fact will change in the next years due to the recent restrictions over the three commercial PBDE mixtures. In fact, while the concentration of DBDPE reported in sediments or sludge has been increasing recently, the concentration of BDE-209 seems to be decreasing [139]. Thus, it is important to carry on monitoring studies for HFRs to assess, first, whether the PBDE levels declined upon their cessation of use and, second, whether there is an increase of emerging HFRs due to their increased use and production. It is also interesting to compare levels of naturally and anthropogenically produced halogenated compounds, as the total value should be considered for a complete evaluation of the environmental risk produced by halogenated compounds. Normally, MeO-PBDEs were found in marine environments and in top predators even at higher concentrations than those of PBDEs. Moreover, some studies suggested that the naturally produced BDEs have more biomagnification capacity than that of PBDEs [140].

ABBREVIATIONS

BFR	brominated flame retardant
DBDPE	decabromodiphenyl ethane
DCM	dichloromethane
DP	Dechlorane plus
dw	dry weight
EI	electron ionization

FR	flame retardant
GC	gas chromatography
GPC	gel-permeation chromatography
HBB	hexabromobenzene
HBCD	hexabromocyclododecane
HFR	halogenated flame retardant
HRMS	high-resolution mass spectrometry
iLOD	instrumental limit of detection
IT-MS	ion trap mass spectrometry
LC	liquid chromatography
LOD	limit of detection
lw	lipid weight
mLOD	method limit of detection
MS	mass spectrometry
MS–MS	tandem mass spectrometry
NCI	negative chemical ionization
PBDE	polybrominated diphenyl ether
PBEB	pentabromoethyl benzene
PCB	polychlorinated biphenyl
PLE	pressurized liquid extraction
POP	persistent organic pollutant
SPE	solid-phase extraction
TBBPA	tetrabromobisphenol A
TOF-MS	time-of-flight mass spectrometry
ww	wet weight

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Chapter 17

Industrial Applications of High-Resolution GC/MS

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1 INTRODUCTION: WHY GC/MS?

Today, gas chromatography/mass spectrometry (GC/MS) is a well-established technique used routinely in many industrial applications for the analysis of volatile and semivolatile materials. Early development of the technique owes a good deal to the pioneering efforts of Gohlke and McClafferty during the 1950s at The Dow Chemical Company (Dow) [1]. At that time, MS was primarily used for quantitative analysis of volatile hydrocarbon mixtures.

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Gas–liquid chromatography was introduced in the early 1950s [2], and by the end of that decade, homebuilt GCs constructed from packed stainless steel or copper tubing were used widely within Dow. In order to identify minor components in these complex mixtures, Gohlke and McClafferty coupled a homemade GC to a 1955 Bendix TOF-MS, as shown in Figure 1. The resulting instrument was capable of 10 kHz scan rate, and captured mass spectra by taking Polaroid photos of the auxiliary oscilloscope readout. Further advances in detection and electronics allowed Dow researchers to obtain quantitative profiles of eluting GC peaks on the mass spectrometer [3], and led to the introduction of an early "computerized" GC/MS identification system [4].

Although LC/MS has dominated modern literature for biological analyses; in many applications, GC/MS has advantages over LC/MS in peak capacity, chromatographic resolution, and ruggedness/reliability. The exceptional separation power of GC, often providing over 100,000 theoretical plates, is readily combined with universal ionization sources such as electron ionization (EI). This combination benefits from the reproducible fragmentation behavior of compounds across MS instruments, allowing EI mass spectra to be searched against commercial libraries for rapid compound identification. In cases where a molecular ion is not observed using EI, or the compound of interest is not present in the mass spectral library, "soft" ionization techniques such as chemical ionization (CI) may be applied to produce either a molecular ion, adduct ion, or one or several other characteristic peaks to facilitate

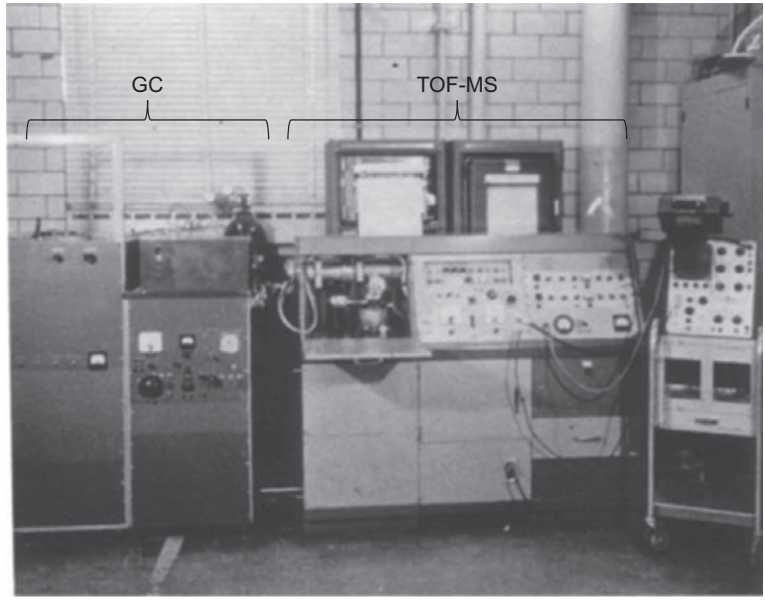


FIGURE 1 Dow gas chromatograph and Bendix TOF-mass spectrometer in Dow circa 1957. *Reprinted with permission from [1].*

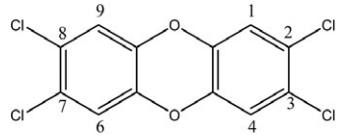
interpretation of the mass spectra. Additional structural information can then be obtained using in-source collision-induced dissociation or MS/MS. Several high resolution/accurate mass (HRAM) analyzers are compatible with GC/MS including time-of-flight, magnetic sector, orbitrap, and Fourier transform ion cyclotron resonance MS. These can provide valuable information on the elemental composition of the molecular ion, molecular adduct ion, and fragment ions. In addition, the coupling of GC with these HRAM instruments can improve the accuracy and reliability of both qualitative and quantitative measurements. These characteristics have allowed GC/MS to be used in a wide range of industrial applications including environmental analysis, toxicology, metabolomics, residue analysis, lipid analysis, and impurity profiling, several of which will be discussed in the following sections of this chapter.

2 INDUSTRIAL APPLICATIONS OF GC/MS

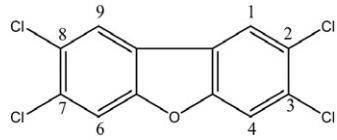
2.1 Environmental Analysis

*2.1.1 Polychlorinated Dibenzo-*p*-Dioxin and Polychlorinated Dibenzofuran Analysis*

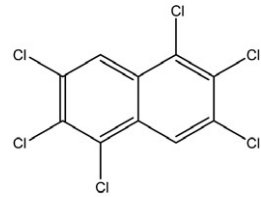
In the late 1970s, scientists Lamparski and Nestrick (Dow Chemical Co.) pioneered analytical work for the detection and quantification of polychlorinated dibenzo-*p*-dioxins (PCDD; example structure 2,3,7,8-TCDD, **1**) and polychlorinated dibenzofurans (PCDF; example structure 2,3,7,8-TCDF, **2**) in the environment. They published the original work on the "trace chemistries of fire" [5], which demonstrated that these compounds can be formed naturally as a result of forest fires, volcanic eruptions, etc., as well as through human activities, such as waste combustion, medical incineration, tire and wood combustion, power-generating facilities, and through combustion by gasoline and diesel-powered vehicles.



(1)



(2)



(**3**)

The Dow analytical laboratory developed methods to determine PCDDs and PCDFs at part per trillion (ppt) levels in fish, water, soil, dust, and industrial sludge using GC/MS. They also developed the first GC/MS methods to analyze water for dioxins at the part per quadrillion level, with sufficient reproducibility for use in the regulation of effluents from industrial sites. These methods, based upon "isotope dilution methodology" [6], have been adapted and modified by many other laboratories and been proven to be efficient to this day [7]. In fact, three current EPA methods for the determination of PCDDs and PCDFs are derived from this methodology [8–10]. The Dow trace dioxin laboratory currently provides analytical support for compliance, remediation, waste water treatment, and production plants.

The quantitative trace analysis of PCDDs and PCDFs in water, soil, organic, biological, and mixed media matrices using EPA methods 1613 and 8290 is still considered one of the most challenging of all EPA analytical methods. These GC/MS methods are based upon isotope dilution methodology, and typically utilize Soxhlet Dean Stark extraction followed by a series of up to four column chromatography clean up steps: (i) acid- and caustic-mixed silica; (ii) silver nitrate on silica; (iii) basic alumina; and either (iv) activated Florisil® or carbon. The removal of all possible isobaric interferences from complex environmental samples is often challenging, even when the extensive cleanup procedures are applied [7]. For that reason, high-resolution magnetic sector instrumentation GC/MS was selected for these quantitative analyses by the U.S. EPA and other global regulatory agencies [8,9]. Figure 2 shows an example comparison of selected ion monitoring (SIM) chromatograms obtained using low-resolution quadrupole (a) versus high-resolution magnetic sector GC/MS (b). Detection of the ^{13}C-2378-TCDD internal standard (traces A3-A4) using low-resolution GC/MS SIM was influenced by isobaric interferences from coeluting isomers of hexachloronaphthalenes (HxCNs) at *m/z* 332 and 334, making the accurate determination of total TCDD difficult. While GC can resolve most of the HxCNs, using high-resolution GC/MS (magnetic sector MS operated at a resolution >10,000 at 10% valley), all ten HxCN isomers, such as the 1,2,3,5,6,7-hexachloro isomer (**3**), were well resolved from the ^{13}C-2378-TCDD internal standard. This selective approach removed the interference in the measurement, and allowed

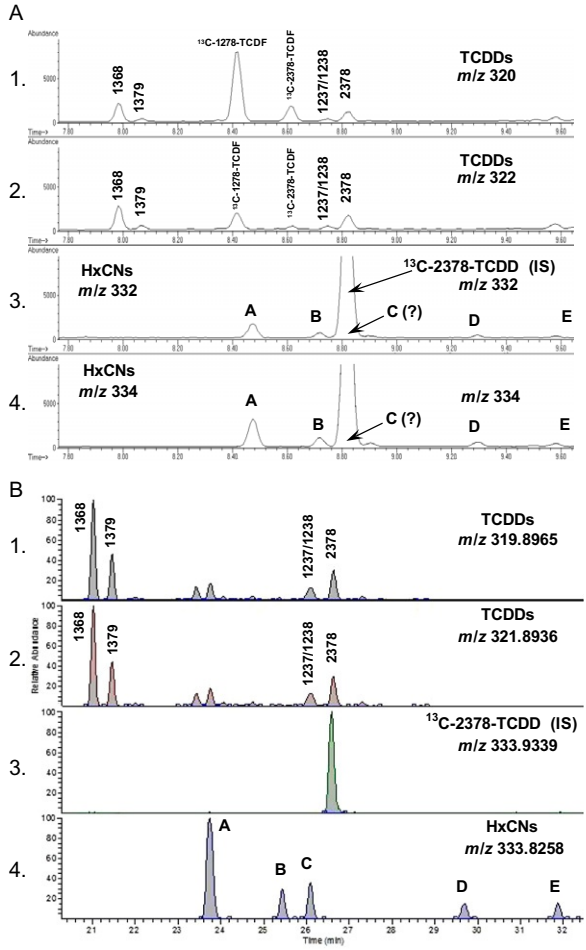


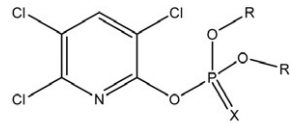
FIGURE 2 Examples of 2378-TCDD and homologue total TCDD detection using (A) quadrupole and (B) magnetic sector MS. *Note*: how ^{13}C-TCDF and HxCNs interferences complicate the low resolution extracted ion chromatograms.

the accurate quantitative measurement of each target compound, as is shown in traces B3 and B4 of Figure 2.

2.1.2 Determination of Chlorpyrifos in Waste Water

Organophosphate (OP) insecticides represent another class of compounds which is amenable to GC analysis. General OP quantitative methods often

use GC with flame photometric detection (GC/FPMD), which may encounter matrix interferences in complex environmental samples and is not a mass spectrometric technique. MS can provide additional specificity compared to conventional GC detectors, especially when operated at high resolution. One such example was encountered during the analysis of chlorpyrifos (CPF; **4**) in the environment as part of a pollution minimization program.



(**4**) X = S; R = $C_2{}^1H_5$
(**5**) X = S; R = $C_2{}^2H_5$
(**6**) X = O; R = $C_2{}^1H_5$

A modified method utilizing isotope dilution methodology combined with high-resolution (magnetic sector) GC/MS was developed to improve the precision, accuracy, and cost-efficiency of the procedure compared to standard EPA methodology.

Although OP insecticides are thermally stable, they may degrade during extensive cleanup procedures due to their chemical instability, resulting in poor method recoveries. The use of two adsorbents, activated silica and Florisil®, combined with the use of isotope-labeled internal standards, such as the decadeuterio- analog (**5**), was found to effectively account for potential compound losses during the sample preparation [11,12]. Quantitative measurement of CPF was achieved by monitoring the ratios of CPF chlorine isotope ions. If interferences were encountered in the low-resolution GC/MS measurements, the analysis was repeated using a GC-magnetic sector instrument to provide higher specificity as shown in Figure 3 (magnetic sector MS operated at a resolution >10,000 at 10% valley). This high-resolution GC/MS quantitative method enabled regulatory monitoring of CPF effluent concentrations in the low-ppt range, thus allowing engineers to effectively control sources of CPF.

2.2 Toxicology

2.2.1 Quantifying Xenobiotics in Biological Matrices

Although many of the GC/MS methods previously used to identify and quantify xenobiotics have recently been replaced by LC/MS methods, there are still many important applications for GC/MS in the field of toxicology. GC/MS has played an important role in a wide range of applications within

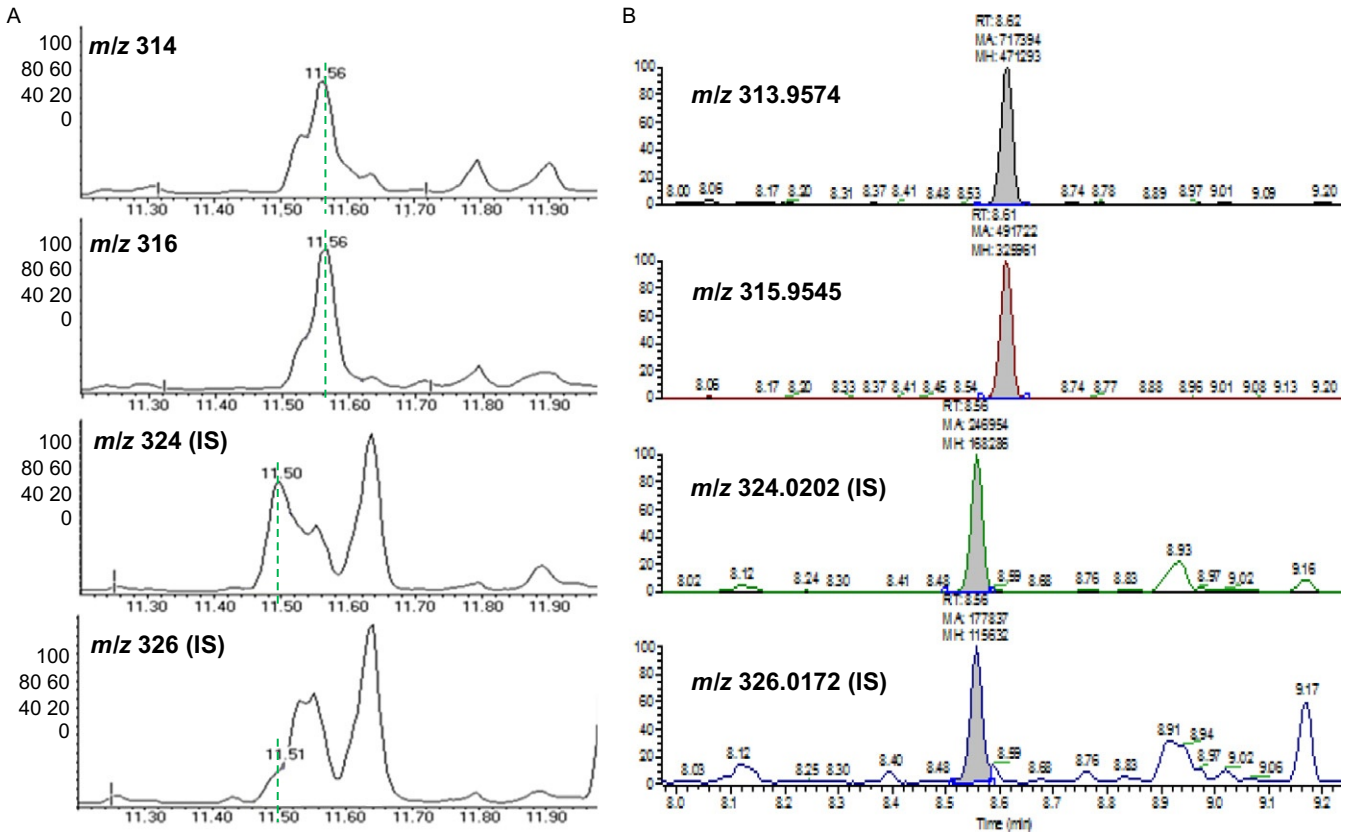


FIGURE 3 Example of chlorpyrifos identification in an environmental sample using (A) quadrupole and (B) magnetic sector GC/MS.

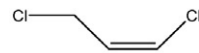
the Dow toxicology laboratory [13–15]. This is especially true in the safety evaluation of agricultural compounds, where GC/MS is particularly well suited for the detection of volatile fumigants, low-molecular weight agrochemicals, and their metabolites.

Chlorpyrifos (CPF; **4**) was first registered by Dow in 1965 under the trade name Dursban, and it remains one of the most effective OP insecticides for agricultural use. Inhibition of the cholinesterase enzyme in red blood cells is considered the relevant endpoint for the risk assessment of exposure to CPF. Blood concentrations of CPF and the CPF-Oxon metabolite (**6**) can provide information on the absorption and metabolism resulting from CPF exposure. Recent CPF toxicokinetic studies required the development of methods capable of measuring both CPF and CPF-Oxon at a lower level of quantification (LLQ) of 0.1 ppb. Existing methods for the measurement of CPF and CPF-Oxon could not achieve these sensitivities [16,17], so a new method was developed combining high-resolution GC/MS with isotope dilution to measure both CPF and CPF-Oxon in blood.

Duplicate control rat blood aliquots were fortified with CPF and CPF-Oxon and analyzed using both low-resolution and high-resolution (magnetic sector) GC/MS. The low-resolution GC/MS method could detect both analytes at an LOD of 1 ppb. The high-resolution GC/MS method was able to detect both target analytes at 0.1 ppb, achieving *S/N* ratios of 28 and 14 for CPF and CPF-Oxon, respectively, as shown in Figure 4. The accuracy and precision agreed well between the two methods; however, given the improved sensitivity and specificity achieved with high resolution GC/MS, this technique was selected to support the toxicokinetic studies. The resulting method combined the high resolution (>10,000 with 10% valley) of the magnetic sector MS with 1–2 μL injection volumes to achieve the required LLQ of 0.1 ppb per analyte while consuming only 0.5 mL of blood.

2.2.2 *Determining the Bioavailability of Volatile Materials*

In a second example, GC/MS played a key role in the study of the potential oral toxicity of 1,3-dichloropropene (1,3-D; *cis*-isomer shown as **7**) [18].



(**7**)

The relatively high vapor pressure of 1,3-D, its short half-life in drinking water, and its reactivity with constituents of feed required the use of a microencapsulated formulation (starch–sucrose shell) of 1,3-D in oral toxicology studies. In previous studies, dosing of individual rats with a single form of the test materials, either neat 1,3-D or microencapsulated 1,3-D, had resulted

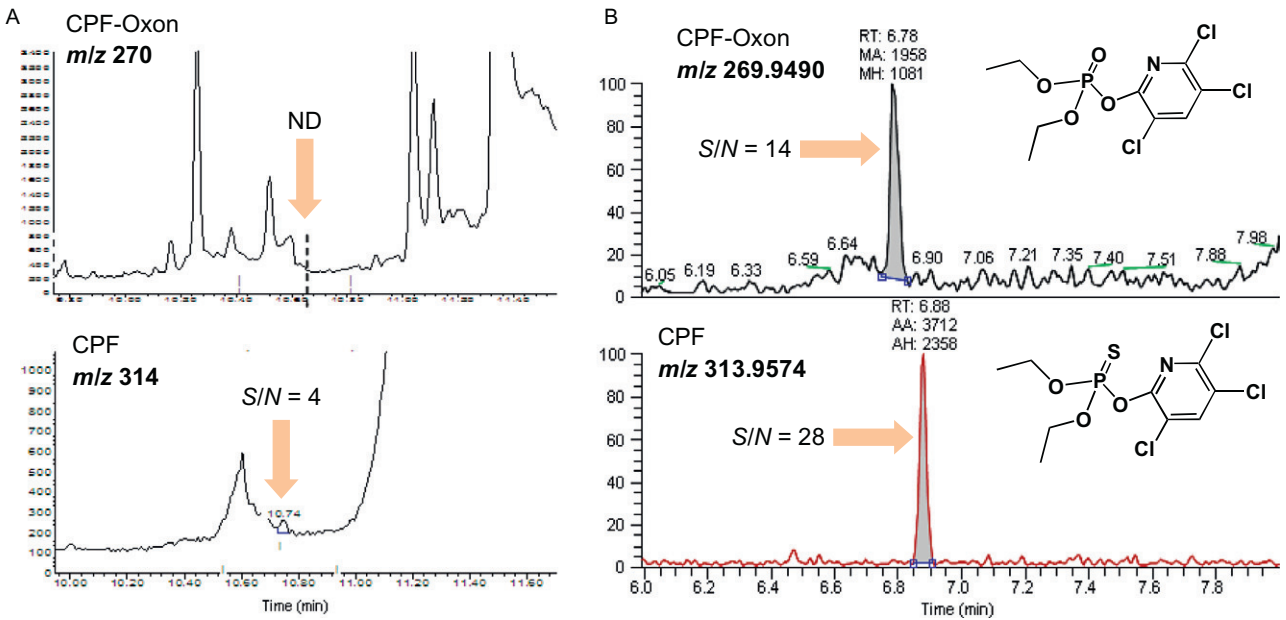


FIGURE 4 Example of chlorpyrifos (CPF) and chlorpyrifos-oxon (CPF-Oxon) detection in fortified rat blood at 0.1 ppb (or 100 fg on GC column) illustrating the selectivity and sensitivity advantages of magnetic sector (B) over quadrupole (A) GC–MS.

in a large degree of interanimal variability in measured 1,3-D blood levels. It also appeared that 1,3-D was rapidly absorbed upon ingestion, requiring that a relatively large number of blood samples be obtained within a short time of dosing. Therefore, when a more recent pharmacokinetic study was performed, the bioequivalence of ingested microencapsulated 1,3-D was simultaneously compared with that of ingested 1,3-D in corn oil gavage. This was accomplished by comparing the kinetics of 1,3-D in the blood of female F344 rats co-administered microencapsulated ^{12}C-1,3-D and neat ^{13}C-1,3-D (25 mg/kg each) via oral gavage. In this experiment, the ^{12}C-1,3-D was used to track the material originating from the microcapsules, and the ^{13}C-1,3-D was used to track that originating from the neat material in the corn oil. Blood concentrations of 1,3-D were measured using either GC/MS or *in situ* membrane introduction mass spectrometry (MIMS). The bioavailability of 1,3-D administered via oral gavage in corn oil was determined by quantifying the concentration of 1,3-D in the blood and calculating the maximum concentration attained, the time to peak blood concentration, and areas under the curve (AUCs) for each delivery method. Similar total blood AUCs of a compound administered via the two methods would indicate similar systemic bioavailability.

The study consisted of two phases: phase I where quantitation was performed using GC/MS, and phase II where the relative levels were determined by MIMS, as described below. In phase I, six fasted female Fischer 344 rats fitted with in-dwelling jugular cannulas were coadministered 25 mg/kg dosages each of neat ^{13}C-1,3-D and microencapsulated ^{12}C-1,3-D in a single corn oil solution/suspension via oral gavage. Blood samples were collected from the jugular cannula of dosed rats over the first hour postdosing, immediately injected into weighed septum-sealed vials, and the concentrations of *cis*- and *trans*-^{13}C-1,3-D and ^{12}C-1,3-D were then quantified using GC/MS. Due to the limited number of data points available using this approach, absorption and excretion constants, half-lives, and blood AUCs and absorption kinetics in phase I were calculated based upon the composite blood curves. Examples of the blood concentration GC/MS data for the *cis*-isomer of 1,3-D in rats coadministered 25 mg/kg of both neat ^{13}C-1,3-D and microencapsulated ^{12}C-1,3-D in phase I of the study are displayed in Figure 5A.

In phase II of the study, the blood level of ^{12}C-1,3-D and ^{13}C-1,3-D was monitored *in situ* using novel hollow fiber membrane probes implanted directly into the jugular vein. The design of these probes is shown schematically in Figure 6 [19]. Hollow fiber membrane probes were prepared using Silastic Rx65 medical grade tubing with a poly(dimethylsiloxane) elastomer as the semipermeable sensing membrane. Just prior to dosing, the probes were connected to the mass spectrometer, evacuated, and used to directly monitor the ^{12}C-1,3-D and ^{13}C-1,3-D at $m/z = 75$ and $m/z = 76$, respectively. The selective permeation of 1,3-D through the silastic membrane material afforded rapid and sensitive real-time detection on the mass spectrometer. The relative levels of 1,3-D were monitored continuously from 5 min predosing to approximately

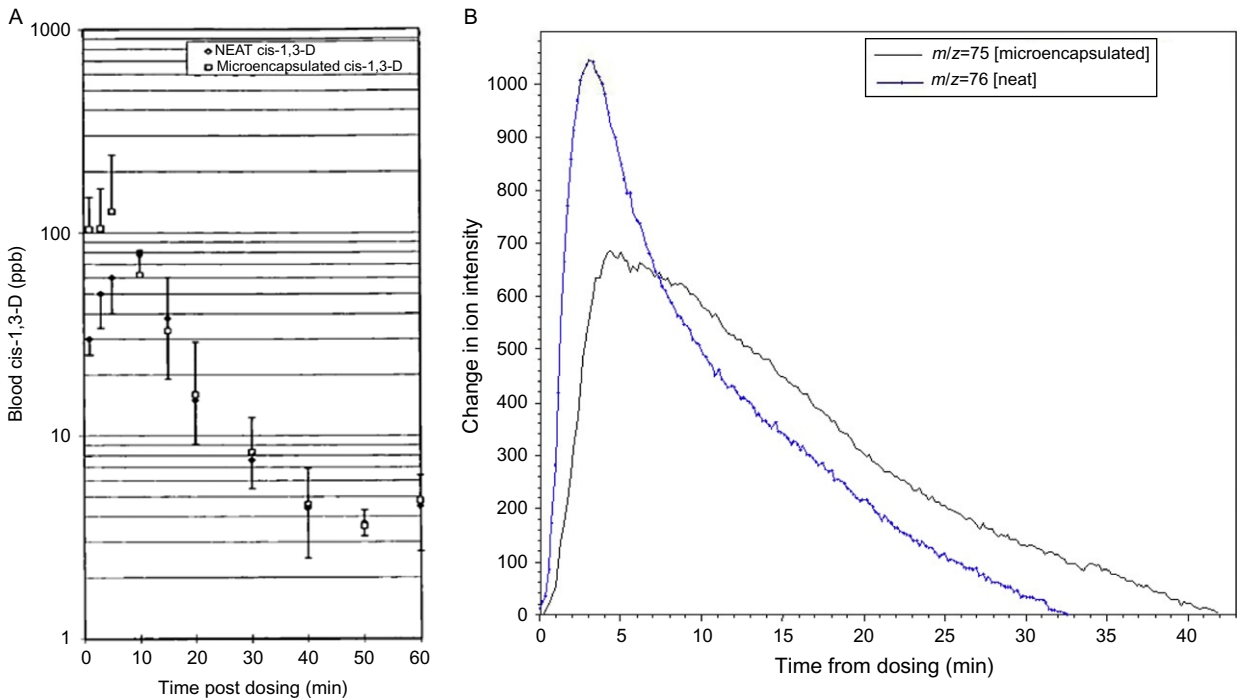


FIGURE 5 Blood concentration time course for the GC/MS analysis of both neat and microencapsulated 1,3-D using (A) quantitative GC/MS analysis and (B) monitoring relative levels in real time using MIMS.

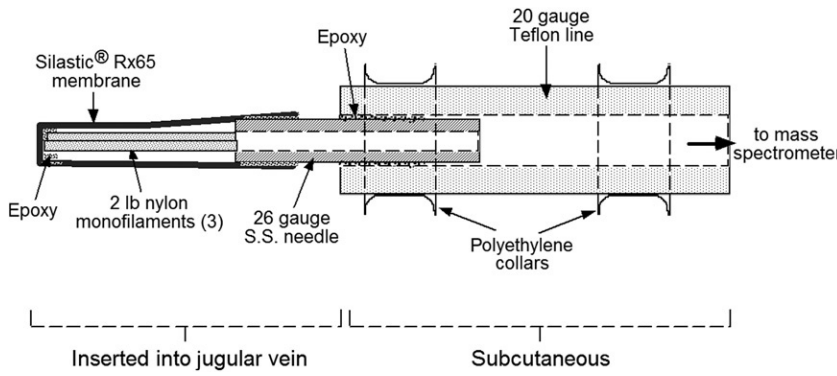


FIGURE 6 Schematic diagram of the membrane introduction mass spectrometry (MIMS) probes used to monitor blood kinetics of 1,3-D in real time.

30 min postdosing at a rate of approximately 60 measurements/min. These data were plotted, and the relative percentage of total AUC represented by each form of 1,3-D was subsequently calculated for each animal. A representative blood concentration curve based upon ion intensity data generated using this approach is shown in Figure 5B. The resulting blood concentration curves provided data suitable for calculating pharmacokinetic constants.

In conclusion, to address the unique characteristics of the 1,3-D molecule, several relatively novel experimental methods were employed in these studies. The coadministration of two isotopic forms of the test material, ^{12}C-1,3-D and ^{13}C-1,3-D, to the same animals in phase I of the study lessened the potential impact of interanimal physiological differences, and decreased potential experimental and analytical error. The use of an implanted, selectively permeable, hollow fiber probe to generate continuous, near real-time, blood concentration data in phase II of the study ensured that a complete concentration time curve could be examined postdosing, no matter how rapidly absorption occurred. Overall, the combined use of isotopic labeling with GC/MS and MIMS demonstrated the ready bioavailability of microencapsulated 1,3-D and the rapid elimination of 1,3-D from the blood of rats.

2.3 Metabolomics in Agriculture

2.3.1 Application of GC–MS/MS for Targeted Profiling of Common Classes of Endogenous Metabolites

The complement of endogenous metabolites in a biological tissue or fluid is a reflection of the current physiological status of the tissue. Changes in the tissue's physiological status (e.g., disease states, nutritional stress, developmental changes), environmental stresses (e.g., heat or cold, water stress) as well as

xenobiotic stresses (e.g., administration of pharmaceutical drugs, herbicides, fungicides, insecticides) can lead to rapid and either reversible or irreversible changes in the profile of endogenous metabolites present in the tissue.

The branch of analytical science used to generate an unbiased identification and quantification of as many endogenous metabolites as possible in a biological tissue is commonly referred to as metabolomics [20]. The goal of metabolomics, or global metabolic profiling experiments, is to gain insights into the underlying physiological status of the tissue through analytical characterization of its metabolites. These insights can include understanding the regulation of metabolic pathways, diagnosis of disease states, and characterization of the biochemical mode of action of biologically active xenobiotics. Metabolic profiling of endogenous metabolites in different biological tissues using hyphenated MS techniques such as GC/MS, LC/MS, and CE/MS has shown tremendous growth over the last 10 years [21].

One of the earliest applications of metabolic profiling in plant research was the use of GC with flame ionization detection to generate profiles of changes in the composition of silylated polar metabolites isolated from barley seedlings treated with several different herbicides [22]. Distinct changes in composition were found to occur in response to different herbicide treatments. The application of GC/MS with ionization by EI has resulted in tremendous growth in the field of global metabolic profiling because of the high chromatographic resolution, peak capacity, and the availability of standard mass spectral libraries for the identification of metabolites [23]. Metabolites involved in central carbon and nitrogen metabolism are shared across the animal, plant, and microbial kingdoms. However, in addition to these common metabolites, a wide variety of specialized natural products or secondary metabolites with limited taxonomic distribution can occur in plant and microbial systems [24,25]. The large volume of data generated by unbiased metabolic profiling experiments presents significant challenges for the efficient analysis and biological interpretation of the results [26]. In cases where experiments can be designed to target a class of metabolites, which share common structural features, the analysis of metabolic profiling data can be considerably simplified. One such structural feature shared by many metabolites in central carbon metabolism is the presence of one or more phosphorylated hydroxyl groups.

In order to profile many of the metabolites present in central carbon metabolism, a GC/MS/MS method was developed which took advantage of the observation that the trimethylsilyl (TMS) derivatives of sugar phosphate standards analyzed under positive chemical ionization (PCI) conditions showed prominent ions at either *m/z* 357 or 387 (Figure 7). Using these ions as precursors for MS/MS in an ion trap GC–MS (Thermo Polaris) showed virtually the same product ion spectra across a range of sugar phosphate standards. Thus, using *m/z* 387 as the precursor ion, the product ion spectra for ribose-5-phosphate, fructose-6-phosphate, glucose-6-phosphate, and

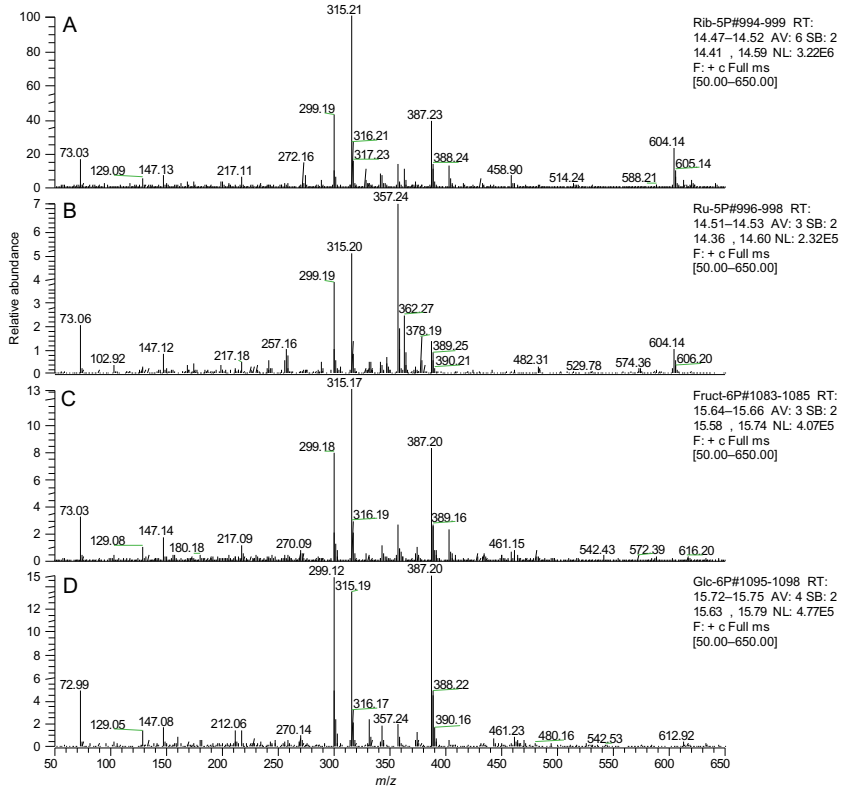
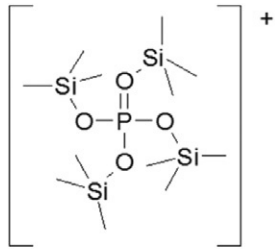
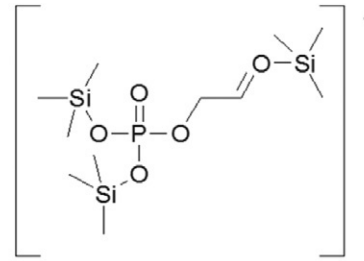


FIGURE 7 PCI mass spectra (methane reagent gas) of authentic standards: (A) ribose-5-phosphate(methoxyamine)$(TMS)_5$, (B) ribulose-5-phosphate(methoxyamine)$(TMS)_5$, (C) fructose-6-phosphate(methoxyamine)$(TMS)_6$, and (D) glucose-6-phosphate(methoxyamine)$(TMS)_6$.

sedoheptulose-7-phosphate all appear identical, with only their retention times or retention indices distinguishing them. This indicated that the MS/MS conditions developed are detecting a fragment common to sugar phosphates, regardless of how many carbons the sugar contains. Early work with both ^{13}C- and ^{2}H-labeling studies of the TMS derivatives of sugar phosphates under EI conditions indicated that either *m/z* 357 and 387 are commonly observed fragments with the likely structures 8 and 9 [27]. Thus, it is likely that the PCI MS/MS conditions developed in this study were detecting the same prominent fragments, as different sugar phosphates gave virtually the same fragmentation pattern, with only their retention time distinguishing them. The net result is that these conditions provide a selective monitoring of many different polyhydroxy phosphorylated metabolites, in addition to the specific standards analyzed.



(**8**) *m/z* 387



(**9**) *m/z* 357

Hydroponically grown seedlings of Arabidopsis were used as a model system for targeted profiling of sugar phosphates. Isopropanol/water extracts of seedlings were evaporated to dryness, derivatized with hydroxylamine to prevent cyclization of the sugars, and *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide followed by analysis with GC/MS using CI with methane as the reagent gas. As expected, the total ion chromatogram of the extracts (Figure 8A) showed a very complex mixture of metabolites. MS/MS, optimized for sugar phosphates (Figure 8B and C) using the precursor ions described earlier, showed a very simple chromatogram with easily quantified peaks. An example application of this technique is shown in Figure 8, which compares endogenous levels of sugar phosphates in hydroponically grown Arabidopsis seedlings grown in the presence of 0.8% sucrose (Figure 8C) as a carbon source with those present 20 h after transfer of the seedlings to sorbitol (Figure 8D), which is not metabolized as a carbon source. Of the metabolites monitored in this analysis, most showed less than a twofold change in levels, whereas the seven-carbon sugar phosphate sedoheptulose-7-phosphate showed a >98% decrease in intensity level after removal of sucrose as a carbon source.

Using this approach, many of the phosphorylated metabolites in central carbon metabolism were readily measured without interference from large amounts of chromatographically overlapping components. In addition, by monitoring fragment ions common to the TMS derivatives of phosphorylated metabolites, metabolites which were not originally included in the analysis were recognized as additional chromatographic peaks. This facilitated the identification of additional metabolites which were not included in the original analysis.

The recent introduction of the GC/atmospheric pressure chemical ionization interface (GC/APCI), discussed in more detail later, has opened up many new possibilities for GC/MS [28–31]. It allows a single instrument to be configured for both GC and LC analyses, providing a flexible cross-platform approach which has been applied to several areas of analytical chemistry including metabolomics [32]. Previously, metabolic profiling generally required the use of GC/MS to determine the volatile and semivolatile metabolites, and LC/MS to analyze the nonvolatile species. As a result, two or more instruments were typically required to cover the full range of compounds generally studied in

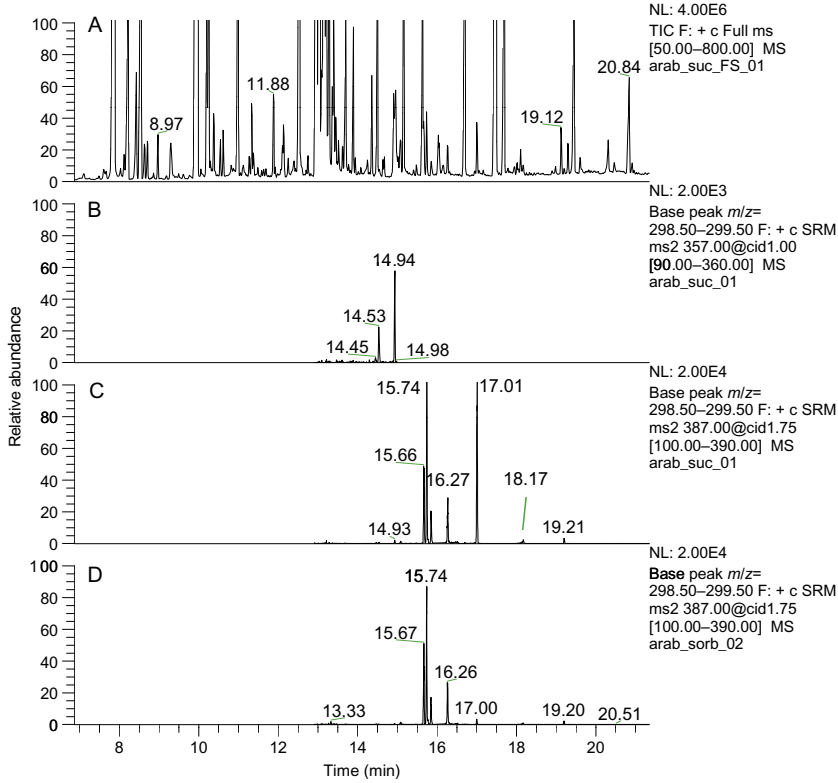


FIGURE 8 (A) Total ion chromatogram of hydroponically grown Arabidopsis seedlings grown in 0.8% sucrose. (B) Targeted MS/MS of ribulose-5-phosphate(methoxyamine)$(TMS)_5$. (C) Targeted MS/MS of glucose-6-phosphate(methoxyamine)$(TMS)_6$ (15.66 and 15.74 min), fructose-6-phosphate(methoxyamine)$(TMS)_6$ (15.85 min), inositol-phosphate$(TMS)_6$ (16.27 min), sedoheptulose-7-phosphate(methoxyamine)$(TMS)_6$ (17.01 min), and octulose-8-phosphate(methoxyamine)$(TMS)_6$ (18.17 min). (D) Arabidopsis grown in 0.8% sucrose, then transferred to 0.8% sorbitol for 20 h. *Note:* the >98% decrease in levels of sedoheptulose-7-phosphate(methoxyamine)$(TMS)_6$ (17.01 min).

comprehensive metabolomic analysis. With the introduction of GC/APCI, a *single instrument* can be configured for either GC or LC analyses, and also provides the added benefit of accessing the accurate MS, accurate MS/MS, and MS^n capabilities available on modern LC/MS instrumentation.

3 RECENT ADVANCES IN GC/MS TECHNOLOGY

3.1 Comprehensive Two-Dimensional Gas Chromatography Time-of-Flight Mass Spectrometry (GC × GC–TOF-MS)

Comprehensive two-dimensional gas chromatography (GC × GC) was first demonstrated by John Phillips in 1991 [33]. For the first 9–10 years, it was

practiced by only a select few research groups. In 1998, cryogenic modulation was introduced [34], which set the stage for reliable focusing of effluent from the first-dimension column into extremely narrow peaks (60 ms) for release onto the second-dimension column. The narrow peak widths necessitated using a time-of-flight mass spectrometer to maintain the mass spectral and chromatographic fidelity of the two-dimensional chromatograms. In 2001, LECO introduced the Pegasus 4D GC × GC–TOF-MS, which became the industry standard for the next 10 years. The majority of GC × GC publications cites the use of this instrument, and thus it has been largely responsible for the growth of GC × GC as a tool for the separation of complex mixtures. In 2002, Dalluge *et al*. published the first articles demonstrating the sensitivity and resolving power of GC × GC–TOF-MS [35–37]. Since that time, there have been nearly 700 publications in this field. These have been reviewed by Gorecki, Cortes, and Meinert [38–41].

GC × GC separations using cryogenic modulation are well suited for coupling to a mass spectrometer because the flow rate exiting the second-dimension column is typically 1 ml/min. Commonly, a 0.25 mm ID capillary column is used in the first dimension, and a 0.1 mm ID column with a length of 1–1.5 m is used in the second dimension. A minor consequence of this coupling is retention time shift due to the flow profile along the length of the first-dimension column being flatter than if it were coupled directly to vacuum at the outlet. A more significant consideration is that components at high concentrations can easily overload the second-dimension column. This is apparent in the two-dimensional plots as vertical streaks, making it impossible to detect components that coelute in the first dimension. To address this issue, some researchers have started using second-dimension columns with either 0.18 mm or 0.25 mm ID to provide greater sample capacity.

Alternatives to a conventional TOF-MS have been demonstrated with a supersonic beam MS developed by Amirav coupled with fast-scanning quadrupole mass spectrometers [42]. The former is ideally suited for flow modulation, developed by Seeley, where the flow from the second-dimension column is 20–30 ml/min. [43–45]. Fast-scanning quadrupole mass spectrometers, operated at 20–50 scans/s, do reasonably well at producing spectra that match library spectra [46]. Thus far, CI has not been applied in published research, which may result from the fact that the open source design of the LECO Pegasus 4D is not well suited for CI.

One of the biggest opportunities for the advancement of GC × GC–TOF-MS is in handling the data produced by the experiments. The number of components observed by a single GC × GC–TOF-MS analysis can be easily 5000–10,000 and, as a consequence, a typical unit resolution data file run with a TOF-MS is over 1 GB in size. This is due to both increased sensitivity and resolution compared with 1D GC–TOF-MS. Data files will become even larger for high-resolution TOF-MS analysis, and tools for analyzing complex data sets are still evolving. However, both LECO and Zoex currently have software that can facilitate the comparison of data files to identify the key differences.

In one example of the use of GC × GC–TOF-MS, polyacrylic acid was pyrolyzed, and the products were analyzed with the results shown in Figure 9. The sensitivity of the TOF-MS combined with the sensitivity gain using GC × GC revealed more components than previously reported by 1D GC, and also showed a number of unresolved components present in the first dimension. Some of these 1D resolution problems could be solved by peak deconvolution, but could not provide the same recognition of differences in chemical functionality that was readily seen in the 2D GC × GC plot.

For samples of complex mixtures that have many homologues, patterns may appear in the reconstructed GC × GC plots that aid in the interpretation of the sample composition. In this way, GC × GC is complementary to MS, as it reveals differences in chemical structure for components that are isobaric. Figure 10 illustrates a second example of the use of GC × GC–TOF-MS for the separation of diesel fuel, where a nonpolar column was used in the first dimension and a polar column was used in the second dimension. Regions of differing chemical functionality are labeled in the figure, which demonstrates that such a sample comprises several chemical families that are superimposed in the first dimension of separation. Even with this GC × GC separation, not all components are completely resolved. To provide

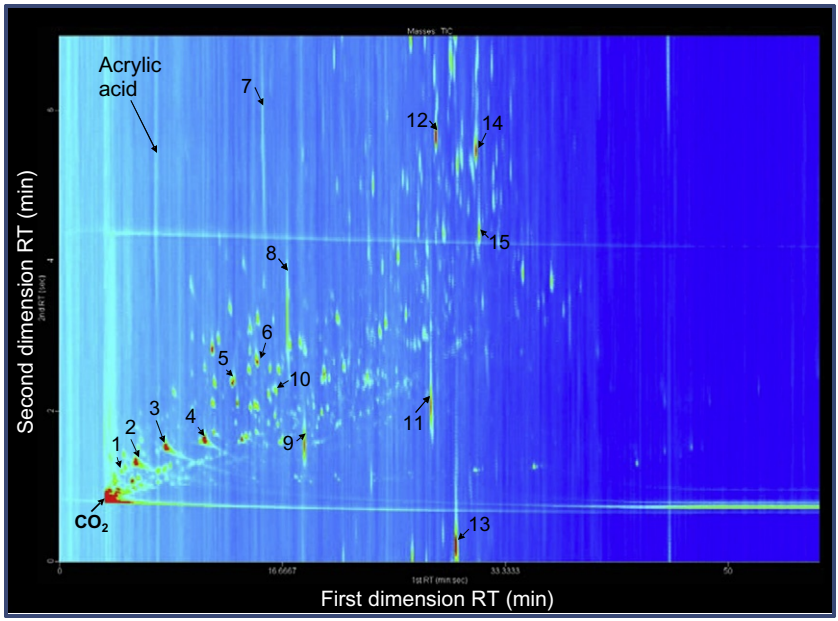


FIGURE 9 Pyrolysis GC × GC–TOF-MS of poly(acrylic acid). Component ID's 1, methacrylic aldehyde; 2, benzene; 3, toluene; 4, xylene; 5, 3-methyl-3-cyclohexen-1-one; 6, 3-methyl-2-cyclohexen-1-one; 7, phenol; 8, methyl phenol; 9, dimethyl phenol; 10, 2-3,5-dimethyl-cyclohexen-1-one; 11, MW 146; 12, MW 162; 13, MW 160; 14, MW 174; 15, MW 178.

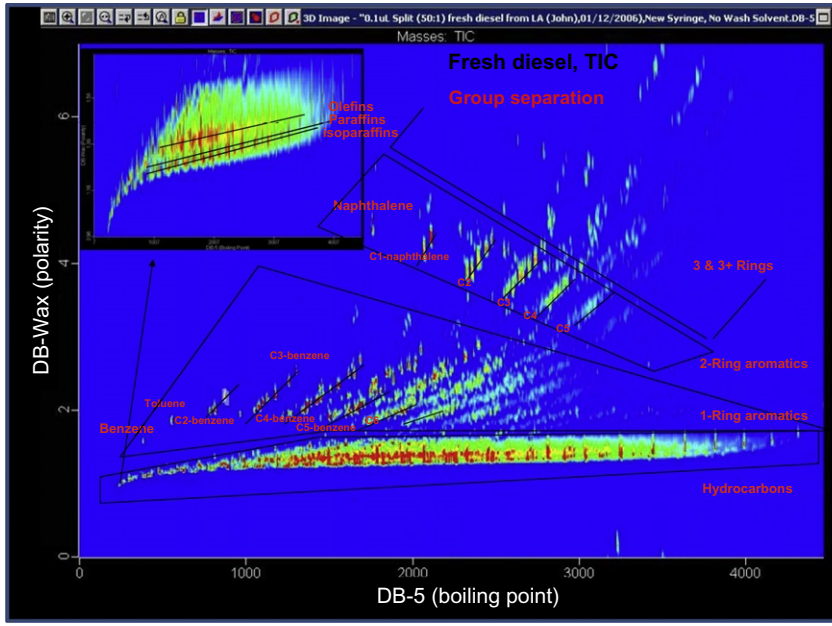


FIGURE 10 GC × GC–TOF-MS profile of diesel fuel, illustrating the complementary nature of separation of isobaric compounds using GC × GC and mass spectra.

improved separation for paraffins and olefins, the phase sequence can be reversed, with the polar phase in the first dimension and the nonpolar phase in the second dimension.

In summary, GC × GC–TOF-MS has been in general use for over 10 years, but it is far from mature as an experimental technique. The introduction of high-resolution mass spectrometers and CI is spawning a fresh wave of application development. GC × GC is complementary to MS in the information that it provides, especially in the case of isomers where the mass spectra do not provide sufficient differentiation for identification. Thus far, little has been done to explore the relationships of two-dimensional retention times and quantitative structure property relationships. There is incredible opportunity to further explore the rich data sets that are produced by GC × GC–TOF-MS. This will continue to drive the development of advanced data evaluation tools.

3.2 Accurate Mass GC/MS Instrumentation

During the period of 1995–2005, significant effort was focused on improving the mass accuracy, resolution, scan speed, and MS/MS experiments which could be performed using LC/MS, while over the same period fewer advances

were made in GC/MS instrumentation. This changed recently with the release of several new high-resolution GC/MS platforms. These commercial instruments are based on TOF and QqTOF mass analyzers that provide a combination of high resolution, mass accuracy, and scan speed which is compatible with GC/MS. Agilent recently introduced a GC/QqTOF instrument which can perform both EI and CI ionization providing high mass accuracy (low ppm) and high resolution (13,500 FWHM) in both MS and MS/MS modes [47,48]. LECO introduced a high-resolution GC-TOF instrument [49,50], which uses a folded-path TOF mass analyzer to provide high resolution (up to 50,000 FWHM) and scan rates (up to 200 scans/s) using either EI or CI ionization. This instrument, which can be configured in either GC or GC × GC modes, is now being widely utilized in metabolomics, and other fields [51,52].

Peterson *et al*. [53] recently demonstrated the first modification of a hybrid quadrupole linear ion trap (QLIT)-orbitrap for GC/MS. They successfully modified the electron transfer dissociation ion source to provide EI and CI ionization in combination with GC introduction. To accomplish this, a new "nested scan" type was developed which increased the duty cycle of the orbitrap to nearly 100%, while providing 4 Hz scan rates at 15,000 mass resolution (FWHM). The ability of this instrument to accurately measure isotopic distributions (spectral accuracy) was demonstrated in the analysis of a series of polycyclic hydrocarbons, which proved comparable to the instrument in LC/MS mode. Performance of this prototype instrument was demonstrated in the analysis of PCDDs and PCDFs. This prototype GC-orbitrap proved capable of resolving the isotopic envelope of both congeners simultaneously in full scan mode, while maintaining high mass accuracy (1 ppm mass accuracy at >31,000 resolution). Overall, using this prototype GC-QLIT-orbitrap instrument, they were able to show acquisition at GC/MS speeds (4 Hz), while maintaining high mass accuracy, high resolution, and preserving accurate isotopic profiles.

3.3 GC/APCI/MS

GC/APCI/MS was first introduced by Horning *et al*. [54] as an extension of their development of an atmospheric pressure ionization source utilizing ^{63}Ni β-decay ionization [55]. These techniques were subsequently applied to several areas of analysis [56–58]. In 2005, McEwen *et al*. [28] presented the modification of a commercial LC-APCI source to allow the coupling of a GC to an LC–MS instrument. In their design, a heated transfer line containing a capillary GC column was installed in the position normally occupied by the ESI or APCI probe, as shown in Figure 11. Using this approach, they were able to perform GC/APCI/MS analysis of several sample types, without significantly compromising the resolution of the GC separation. Results were

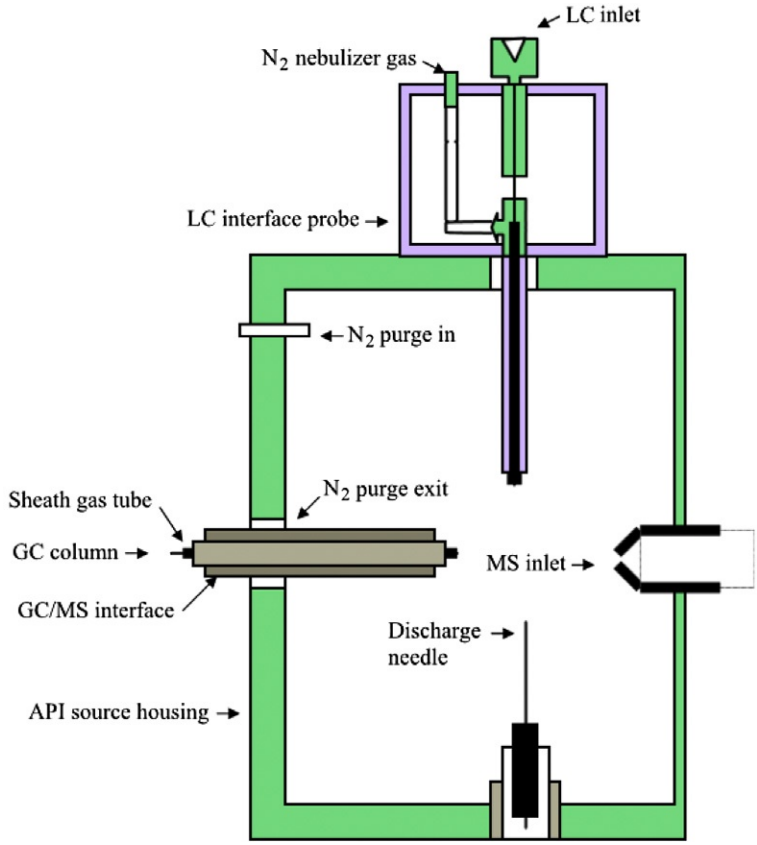


FIGURE 11 Schematic diagram of a GC/APCI interface. *Reprinted with permission from [28].*

shown for a complex pesticide mixture (EPA mix 8270) using both positive and negative APCI, where they observed that less polar compounds ionized with increased sensitivity under nitrogen purge (charge exchange) conditions. Overall, using this modified APCI source, they achieved comparable sensitivity to positive CI, without compromising the performance of LC/MS interface, indicating that GC/APCI was well suited for use in a wide range of labs.

More recently, Bristow *et al*. [59] presented an overview of the application of GC/APCI/MS for analysis of pharmaceutical impurities. They compared the performance of GC/APCI/MS to EI and CI for the analysis of reaction mixture impurities using a combination of accurate mass data for elemental composition and accurate isotopic pattern data. Pacchiarotta *et al*. [60] recently compared the use of a paired GC/APCI/MS and GC/FID to

comprehensive GC × GC approaches as a means to extend coverage of the "metabolome". Using online coupling of GC/APCI with GC/FID, they were able to study LOD and LOQs, as well as reproducibility for several target metabolites in cerebrospinal fluid (CSF). Overall, they found that MS produced lower LODs, while FID proved more robust, reproducible, and provided a wider dynamic range. This dual detector approach was especially useful for complex samples, where the wide dynamic range and predictable response of FID could be combined with the structural information and improved sensitivity of GC/APCI/MS. Carrasco-Pancorbo *et al*. [32] recently investigated the analytical variability associated with the use of GC/APCI/MS for metabolic profiling. The behavior of a series of metabolic standards including amines, amino acids, organic acids, alcohols, xanthenes, and dipeptides was studied in the analysis of pooled human CSF using GC/APCI/MS. Overall, the authors were able to detect over 300 compounds, and observed excellent intraday and interday reproducibility with this technique.

Another potential application area for GC/APCI/MS is the analysis of pesticide residues. Portolés *et al*. [61] recently evaluated the potential of GC/APCI/MS in this area. They analyzed a large set of GC-amenable pesticides using GC/APCI/MS. The response of these compounds was studied under both charge exchange and protonation conditions (using water as a protonation reagent). The pesticides were classified into six groups according to their ionization behavior in these conditions. Overall, over 90% of the compounds produced an observable molecular or molecular adduct ion, reinforcing the promise of GC/APCI/MS as a useful tool for environmental and residue analysis.

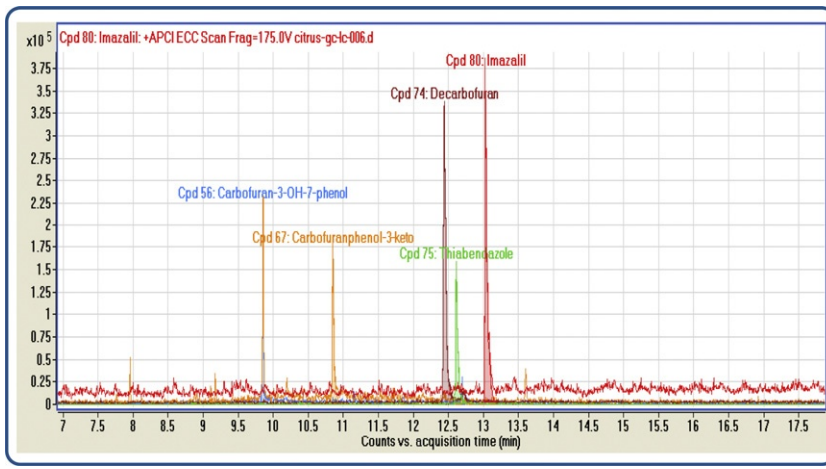


FIGURE 12 GC/APCI/MS analysis of an orange peel extract showing detection of several pesticides.

Currently, GC/APCI interfaces are commercially available from several instrument vendors including Waters, Bruker, and Agilent [62]. Many modern LC/MS mass analyzers can be used with GC/APCI; however, commercial TOF and QqTOF mass analyzers are particularly well suited for coupling with GC. The fast scan rate of TOF analyzers combines well with high-resolution capillary GC, where peak widths are typically in the order of 1–2 s. When GC/APCI is used with a QqTOF or TOF instrument, the high-resolution, accurate mass, and high-fidelity isotopic pattern data produced can augment the process of molecular formula determination.

In one example of pesticide analysis, GC/APCI/MS was applied to the determination of residues in an orange peel extract. Several fungicides, insecticides, and their metabolites were detected in the orange peel extract using an automated compound-finding and database-searching algorithm, as shown in Figures 12 and 13. These include thiabendazole, imazalil, carbofuran, decarbofuran, carbofuran phenol, and other carbofuran metabolites [30]. While the objective of these analyses was detection of contaminants, several naturally occurring compounds were also detected. Figure 14 shows some of these endogenous components including waxes, terpenoids, and organic acids. However, most of these compounds were present as complex mixtures of structural isomers, and their absolute structures proved difficult to assign without authentic standards.

In conclusion, the GC–APCI interface has great potential as a cross-platform analytical tool for solving complex analytical challenges. It is emerging as a new tool for samples requiring both GC and LC analyses, including degradation studies, impurity profiling, and metabolic profiling. Using the combination of LC/APCI and GC/APCI, these analyses can often utilize the same instrument, resulting in capital savings and improved utilization of sophisticated instruments such as TOF and QqTOF mass spectrometers.

4 CONCLUSIONS

GC/MS continues to solve a wide range of problems in industrial research and development, ultimately contributing to the discovery, development, registration, and stewardship of new products. The ability of GC/MS to address these problems has increased significantly in recent years due to improvements in both GC/MS and GC × GC instrumentation, providing advancements in the separation power, speed, sensitivity, resolution, and dynamic range of these instruments. In this chapter, we explored several industrial applications of GC/MS, and how they impacted several areas of industrial R&D including: (1) the analysis of trace level contaminants in the environment; (2) identification and quantification of xenobiotic compounds in toxicology; (3) quantification of endogenous metabolites of agricultural interest; and (4) applications of new advancements in both GC separations and high resolution accurate mass

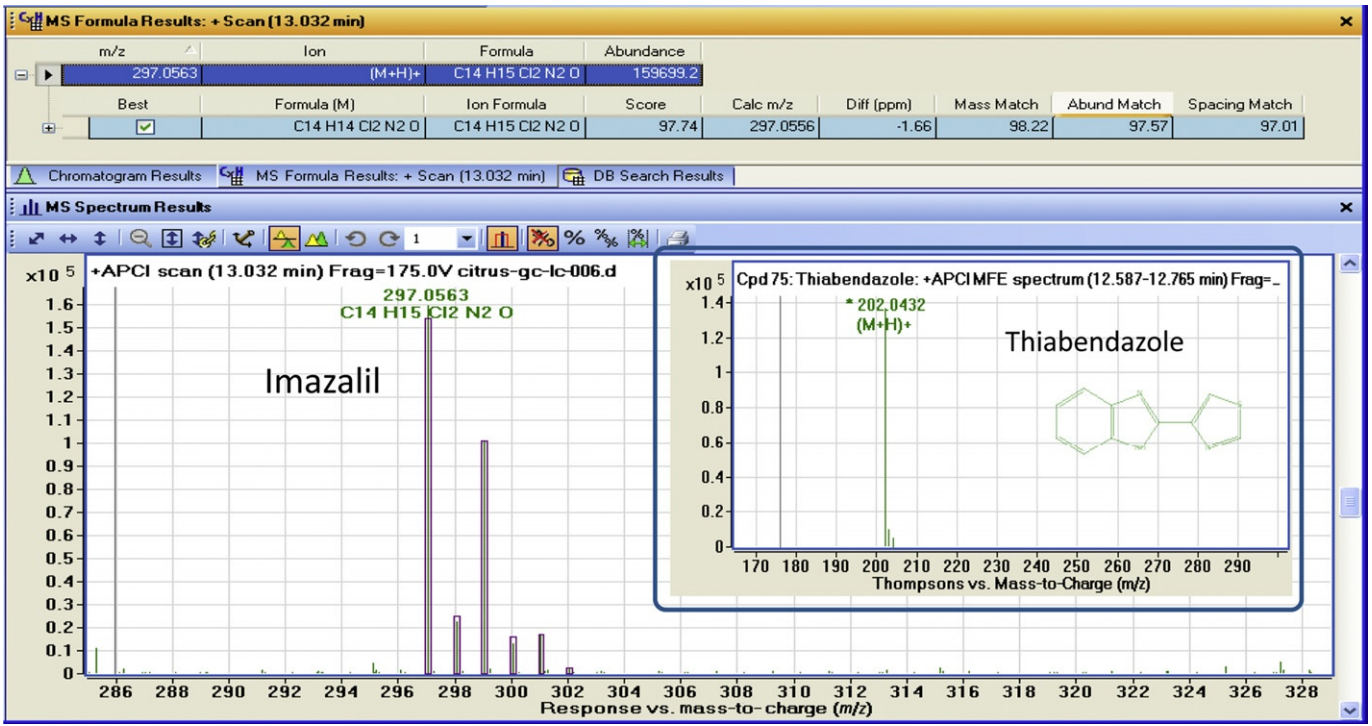


FIGURE 13 GC/APCI/MS identification of imazalil and thiabendazole (Inset) in an orange peel extract using a combination of accurate mass and isotopic fit.

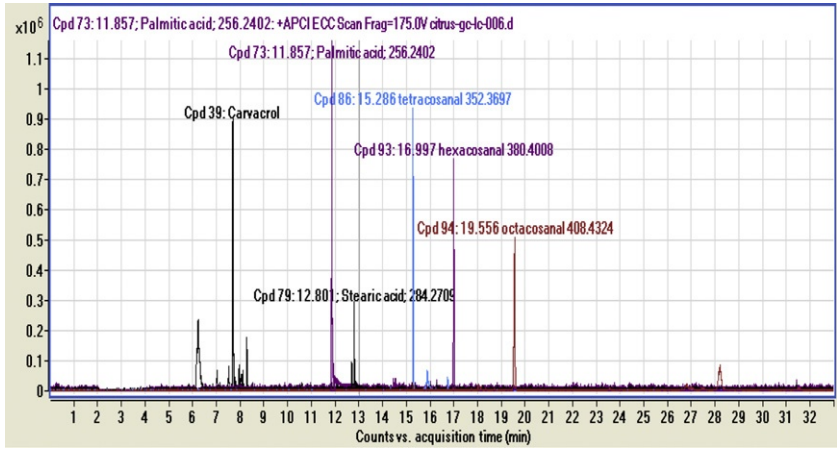


FIGURE 14 Natural compounds detected in an orange peel extract using GC/APCI/MS.

instrumentation. From these examples of state-of-the-art research in each of these areas, it seems clear that GC/MS will continue to serve as a critical tool in many areas of industrial research.

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Chapter 18

Current Applications of GC-(Q) TOF and GC–HRMS for the Determination of Persistent Organic Pollutants in Water and Sediments Samples

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1 INTRODUCTION

Current industrial and anthropogenic development has contributed to the use and production of chemicals, breakdown products, and by-products [1,2]. Many of these compounds often reach the marine environment [1], which unfortunately represents the final reservoir of many human/industrial wastes worldwide. This is the case of persistent organic pollutants (POPs), a heterogeneous group of toxic compounds which have been produced and introduced in the environment in large amounts since the 1950s [3,4]. More recently, an initial group of POPs, which were recognized under the Stockholm

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Convention [5,6] as causing adverse effects on humans and the ecosystem, has been divided into three main categories [6,7]: (i) organochlorine pesticides (OCPs, such as aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, hexachlorobenzene (HCB), mirex, toxaphene), (ii) industrial chemicals (HCB and polychlorinated biphenyls, (PCBs)), and (iii) by-products generated by industrial processes or incineration, such as polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), both also known as PCDD/Fs or named with the term "dioxins" [6–8]. Some examples of POPs are shown in Figure 1.

In general, POPs are characterized by a high lipophilicity and a remarkable physico-chemical and biological stability [1,3,9]. Therefore, they tend to persist in the environment and to bioaccumulate and biomagnificate throughout the food chain (notably in animal lipid tissue) over a wide range of trophic levels [1,9–11]. In addition, considering that POPs can be long-range transported via the atmosphere (they are semi-volatile), oceans, and other pathways, these chemicals may be present in regions far away from their source of origin, where they have never been used or produced [1,10,12]. In this sense, POPs can be particularly incorporated into marine and freshwater ecosystems through diverse means, such as atmospheric deposition, direct and indirect discharges, and riverine inputs [10,13]. Furthermore, as a consequence of their low aqueous solubility and their highly hydrophobic nature, POPs tend to associate with particulate matter and to accumulate in aquatic sediments [1,9,10,14,15], where they can remain for years due to their persistence and long half-lives [1]. As a result, riverine and estuarine sediments which present large amounts of organic matter may constitute significant reservoirs for POPs, acting as a long-term source of pollutants by release

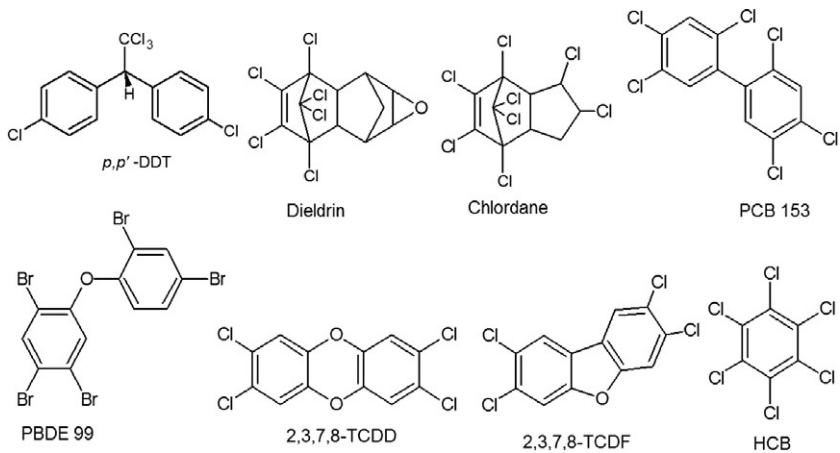

FIGURE 1 Structures of some examples of organochlorine pesticides (*p*,*p*′-DDT, dieldrin, chlordane, and HCB), polybrominated diphenylethers (PBDE 99), polychlorinated dibenzofurans (2,3,7,8-TCDF), and polychlorinated dibenzodioxins (2,3,7,8-TCDD).

and reintroduction into the ecosystem and food chain [1,9,10,16]. Therefore, the contamination of water and sediments represents a significant risk to aquatic organisms, which tend to bioaccumulate POPs, and to humans through the intake of contaminated seafood [1,15]. In fact, they have been shown to present potentially harmful effects in human health and animals, including cancer, nervous system damage, reproductive disorders, and disruption of the immune system [17–20]. As a consequence, the analysis of sediments is nowadays widely required to evaluate the spatial distribution, temporal trends, and possible source of pollutants [16,21,22].

Consequently, the study of the occurrence and bioaccumulation of POPs in the environment has increased in the past decades [3]. As aforementioned, POPs are officially registered by the United Nations Environmental Programme [12] under the Stockholm Convention (signed in 2001 and effective in 2004) [5,6,8,23]. This panel of experts has called for the establishment of a global monitoring plan for POP analysis [12,23]. Besides, other pollutants have been recently proposed as candidates for addition to the POP list, such as polybrominated diphenylethers (PBDEs) [4] and five new OCPs which were included in the POPs list in 2009 [6]. Thus, the Stockholm Convention together with Commission Regulation (EU) No. 756/2010 [24], 757/2010, and 519/2012 [25] on POPs [26] amending Regulation (EC) No. 850/2004 [27], have established measures to reduce and eliminate their emissions to minimize their presence in the environment. In addition, European Directive 2008/105/EC [28] on environmental quality standards in the field of water policy requires that Member States carry out the monitoring of sediments in relation to the Water Framework Directive to control a list of priority substances. Some POPs, such as PCBs and PCDD/Fs, are included for possible identification as priority hazardous substances [14].

Nonetheless, despite the established measures and the production of most POPs has ceased for over 20 years, considerable POP levels still remain in the environment [3]. Therefore, there is clearly a need to develop methods for the monitoring of POPs in marine aquatic systems. The monitoring of these pollutants in sediments is important priority in environmental analysis in order to evaluate the risk to wildlife and human health. Because liquid chromatography coupled to mass spectrometry (LC–MS) methods are not effective, gas chromatography coupled to mass spectrometry (GC–MS) has been widely utilized to investigate the presence of organic pollutants in environmental samples over the years. POPs are usually present at low concentrations in sediment and water samples, so analytical methods have to include extraction and enrichment steps prior to GC analysis [29]. The analysis of POPs in environmental matrices is complex because they can show different physicochemical properties and the presence of matrix interferences, and thus, highly selective, sensitive, accurate, and precise detection techniques are required, often together with tedious cleanup procedures, particularly in the case of PCDD/Fs, PCBs, and PBDEs analysis in sediments [4,30]. GC coupled to

high-resolution mass spectrometry (GC–HRMS) is a powerful tool for unequivocal determination of POPs (even at ultratrace levels) showing high specificity, high sensitivity in full-scan acquisition mode and high mass accuracy [31]. In fact, GC–HRMS based on the use of magnetic sector instruments is widely applied for the analysis of trace organic pollutants (mostly PCDD/Fs) in environmental studies. Nonetheless, in this chapter, a growing interest in HRMS with time-of-flight (TOF-MS) mass analysers has been observed in the past few years [31].

Briefly, this chapter shows an overview of the new trends, mainly since 2005, based on the current analysis of water and sediments samples specifically focusing on GC–TOF-MS [29,32] and HRMS [8,13,14,16,33] analysers. With respect to the extraction procedure, several techniques have been reviewed, such as traditional liquid–liquid extraction (LLE) and solid-phase extraction (SPE) for water samples or solid–liquid extraction (SLE) for sediments. In addition, miniaturized methods such as solid-phase microextraction (SPME) and stir bar sorptive extraction (SBSE) have been also described to determine trace amount of organic pollutants in water samples.

2 SAMPLE EXTRACTION

In general, the determination of trace organic pollutants in environmental samples requires the use of time-consuming and multistep sample preparation methods due to the extremely low levels that are found (from pg L^{-1} to ng L^{-1}) in sediments and water samples, and the presence of matrix interferences (often at higher concentrations than POPs) (Figure 2). Besides, low limits of detection (LODs) are required (from μg L^{-1} to pg L^{-1}). POPs analysis is also characterized by complex mixtures of congeners (e.g., 210 PCDD/Fs, 209 PCBs, or 209 PBDEs congeners) normally coexisting in the samples [3]. Thereby, a rigorous and exhaustive sample pretreatment is normally required to enable their adequate determination (Table 1, Table 2).

2.1 Extraction of Water Samples

In general, LLE has been widely employed for the extraction of water samples, although this technique is time consuming, tedious, labor intensive and requires large amounts of organic solvents [29,34]. It is used in reference methods such as EPA Method 1613 for the determination of PCDD/Fs [35]. Besides, a large sample volume (even 1000 mL in some cases) is also required [29,36] (Table 1). Alternatively, SPE permits to reduce solvent consumption; octadecylsilica (C18) is often used as stationary phase for the extraction of nonpolar and moderately polar compounds [36,37]. Nonetheless, SPE still requires large sample volumes and the enrichment factor can be limited; thus, either final concentration to small volume (<1 mL) or large volume injection (LVI) is often needed to reach LODs below the ng L^{-1} level [29]. POPs can also be extracted using disk SPE and

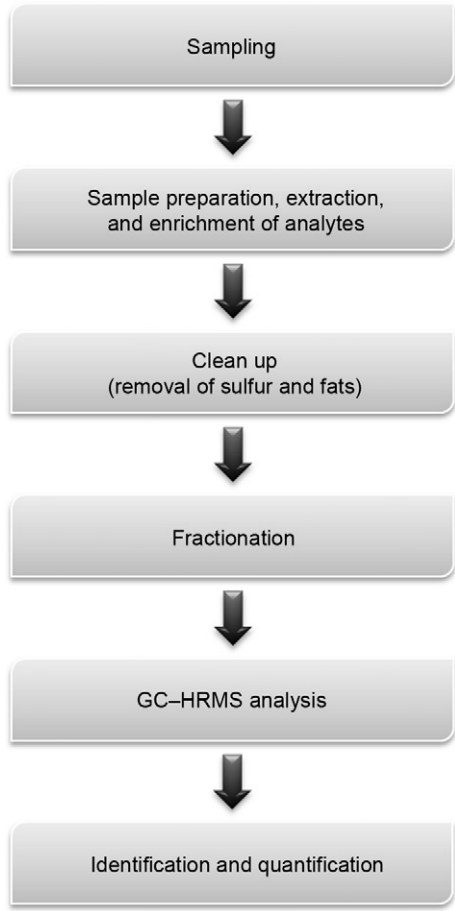

FIGURE 2 Scheme of the general procedure for determining POPs in environmental samples using GC–HRMS.

subjecting the solid phase and the residue obtained on the filter to a classical Soxhlet extraction process [38] (Table 1).

More recently, miniaturized methods such as SPME [39] and SBSE [29] have been also applied for the analysis of POPs in water samples. In these techniques, the analytes are extracted from the liquid matrix into a nonmiscible liquid phase, mainly polydimethylsiloxane (PDMS) [29,34], which is supported on a fiber (SPME) or a magnetic stir bar (SBSE). In the case of SPME, other phases have been successfully used, such as Carbowax/divinylbenzene (CW/DVB). Besides, NaCl may be added to the liquid matrix in order to modify ionic strength and increase the extraction yield [39]. Both SPME and SBSE are solvent-less techniques which allow the simultaneous concentration

and purification of the samples [29,39]. They provide a higher selectivity due to the extracted fraction (on a fiber or a stir bar), which may be introduced quantitatively into a GC system by thermal desorption (TD) [39]. In addition, combining TD with SPME and SBSE makes these techniques more suitable and efficient for trace analysis owing to less sample loss and contamination occurring; lower sample volumes are needed in comparison to SPE (e.g., 10–50 mL) and the extraction process can be fully automated [29]. On the other hand, liquid desorption can be also carried out performing a back-extraction step with an organic solvent. For the extraction of highly nonpolar POPs from water samples, often an organic modifier is added to minimize wall adsorption, such as methanol or acetone [29] (Table 1).

Water samples have also been analyzed using semi-permeable membrane devices (SPMDs). SPMD is a form of passive sampling [40], which provides representative samples and simultaneous analyte isolation and preconcentration. Thus, SPMD (a low-density polyethylene, LDPE, lay-flat tubing filled with synthetic triolein) has been used for the analysis of PCBs and OCPs in water [40] (Table 1).

2.2 Extraction of Sediments

POPs monitoring is widely focused on solid matrices, such as sediments, owing to its aforementioned hydrophobic character, and notably low concentration in water in comparison with wastewater and other solid samples. The traditional Soxhlet extraction normally followed by a multistep cleanup methodology is still widely applied to analyze POPs in sediments [11,14,22,30,41–43]. It is also used as reference technique in the EPA method 1613 for PCDD/Fs [35]. Nevertheless, Soxhlet presents well-known drawbacks such as consumption of large organic solvent volumes, high sample handling, and it is time consuming [34,44]. Alternative extraction techniques have been applied, including microwave-assisted extraction (MAE) [22,43] and pressurized liquid extraction (PLE) [13,43,45,46], which are widely used to replace Soxhlet extraction [34,44,45]. More recently, PLE, with an integrated carbon trap (PLE-C) using an available carbon (Norit SA 4PAH HF), has been employed for the analysis of PCDD/Fs in sediment certified reference materials (CRM), allowing higher sample throughputs than the conventional methods. More details and advantages of this technique may be found elsewhere [44] (Table 2).

With respect to the cleanup stage in sediment samples, the most widely used methodology is solid–liquid adsorption chromatography [13,30,41,45], which can be applied as a single multilayer column loaded with several combinations of all required sorbents [42,44]. Thereby, acidified, neutral, and basic silica gel, silica-based adsorbents and alumina can be used to remove nonvolatile coextractants [41], while an additional step for isolation of planar compounds (e.g., PCDD/Fs or dioxin-like PCBs (dl-PCBs)) from the nonplanar ones (e.g., PCBs) is often carried out including a carbon-based material

TABLE 1 Comparison of the Extraction and Separating Techniques for POPs in Water Samples

Analytes (number)	Sample Pretreatment	Separation/ Detection Technique	LOD (units)	Observations	References
OCPs (16) PCBs (3, nontarget analysis), other pollutants	(a) SBSE-TD: addition of acetone (5 mL); 10- and 20-mm bars coated with PDMS (0.5 mm), room temperature, 1500 rpm, 3 h. (b) addition of 5% NaCl; LLE (3×0.5 L, CH_2Cl_2); addition of *n*-hexane (500 mL); drying Na_2SO_4; concentration to 3 mL; cleanup: column chromatography (florisil and silica gel); concentration to 100 µL	(a) GC × GC – EI–HRTOF-MS (b) GC–EI–HRMS (SIM)	(a) 10–44 pg L^{-1}	(a) DB-5 fused silica capillary column (10 m × 0.18 mm × 0.18 µm) ^{2}D: BPX-50 fused silica capillary column (1.0 m × 0.10 mm × 0.10 µm); injection: splitless mode (b) DB-17HT fused silica capillary column (30 m × 0.32 mm × 0.15 µm); injection: splitless mode	[29]
PCBs (7), OCPs, HCB, PBDEs (11), other pollutants	SPME: CW/DVB fiber (65 µm) with 10% NaCl; stirring (45 min, room temperature); thermal desorption	GC–EI–TOF-MS	NA	Fused silica HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm)	[39]
PCBs, pesticides, PBDEs, other pollutants	SPE (Bond Elut C_{18}cartridges); elution with ethyl acetate:CH_2Cl_2 (1:1); evaporation to dryness; redissolution (hexane, 0.5 mL)	GC–EI–TOF-MS	NA	Fused silica HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm); injection: splitless mode	[37]
PCDD/Fs (17) and PCBs (12)	Suction filtration; drying of the solid phase and the residue on the filter; Soxhlet (1) acetone (8 h); (2) toluene (16 h); concentration; cleanup: multiplayer silica gel and porous graphitized carbon chromatography columns	GC–HRMS (SIM)	0.005 pg L^{-1} (PCDD/Fs) 0.01 pg L^{-1} (PCBs)	(a) SP-2331 (60 m × 0.25 mm × 0.2 µm) (TeCDD/Fs, PeCDD/Fs, HxCDD/Fs) (b) DB-17HT (30 m × 0.25 mm × 0.15 µm) (HpCDD/Fs, OCDD/F) (c) HT8-PCB (60 m × 0.25 mm × 0.15 µm) (dl-PCBs) Injection: splitless mode	[38]

Continued

TABLE 1 Comparison of the Extraction and Separating Techniques for POPs in Water Samples—Cont'd

Analytes (number)	Sample Pretreatment	Separation/ Detection Technique	LOD (units)	Observations	References
PCBs (18), OCPs (28) and PAHs (16)	SPMD (LDPE 65 μm); shaking with cyclohexane (100 mL) overnight; drying with Na_2SO_4 (organic phase); evaporation to dryness and redissolution (*n*-hexane: CH_2Cl_2, 1:1); cleanup: silica gel mixed column (silica + alumina with 3% $H_2O + Na_2SO_4$); SPE (C_{18}); concentration (to 20 μL)	GC–EI–HRMS (SIM)	NA	PCBs: Rtx-CL Pesticides2 (30 m × 0.25 mm × 0.2 μm) OCPs: Rtx-Dioxin2 (40 m × 0.18 mm × 0.18 μm) Injection: cold injection system	[40]
OCPs (21), HCB	Filtration (extraction disk); Soxhlet (acetone, 16 h)	GC–EI–HRMS (SIM)	NA	ENV-8MS column (30 m × 0.25 mm × 0.25 μm)	[11]

EI, electron ionization; NA, data not available; dl-PCBs, dioxin-like PCBs; PCBs, polychlorinated biphenyls; PCDD/Fs, dioxins and furans; SBSE-TD, stir bar sorptive extraction with thermal desorption; SIM, selected ion monitoring; SPE, solid-phase extraction; SPMD, semi-permeable membrane device; SPME, solid-phase microextraction.

[13,45], such as activated carbon [22], porous graphitic carbon (PGC), or 2-(1-pyrenyl)ethyldimethylsilyl column chromatography at the end of the cleanup step (Table 2). Using these carbon columns, the normal procedure consists in elution of nonpolar pollutants using a nonpolar solvent (e.g., *n*-hexane) and an elution in reverse direction of the target pollutants with toluene [4,13]. However, when simultaneously analysis of PBDEs, PCBs, and PCDD/Fs is carried out, total separation between these groups is considerably complicated [30], hindering GC analysis, regardless of the detector used. Consequently, separation of PBDEs, PCBs, and PCDD/Fs in three independent fractions was reported including $AgNO_3$ silica inside a multilayer silica column, followed by basic alumina and Florisil connected columns [30]. In addition, other treatments are carried out such as removal of elemental sulfur (the main coextractant normally present in sediments) using gel permeation chromatography (GPC) or reaction with copper (generally copper powder) and consequent precipitation as CuS [4,22,30] (Table 2). An acid treatment by LLE (normally with H_2SO_4) is also performed prior to adsorption chromatography to remove other remaining matrix interferences [41]. As an alternative to the commented cleanup methodologies, an automated multicolumn cleanup system (e.g., Power-Prep™ system from Fluid Management System™) consisting on disposable multilayer silica, alumina, and PGC columns has been widely employed over the last years in order to minimize sample handling and reduce analysis time (~3 h) [4,14,44] (Figure 3). However, this methodology presents some disadvantages, such as cost of the equipment and high consumption of ultrapure organic solvents [44].

On the other hand, saponification can also be applied for the analysis of some POPs in marine sediments. Simultaneous extraction and removal of elemental sulfur, as well as dissociation of humic substances (cleanup), is accomplished by the addition of ethanolic or methanolic KOH or NaOH [43]. Likewise, sonication represents another alternative method for extraction of sediment samples introducing the sample in an ultrasonic water bath in the presence of an adequate solvent, such as acetone [43]. Other cleanup strategies have been applied for POPs analysis in sediments samples, such as LLE (e.g., using *n*-hexane, water, and sulfuric acid) followed by SPE for OCPs and HCB [11] or PCBs analysis [43] (Table 2).

3 DETERMINATION BY GAS CHROMATOGRAPHY ANALYSIS COUPLED TO HIGH-RESOLUTION MASS SPECTROMETRY

As aforementioned, congener-specific analysis is required in order to differentiate between congeners with different toxicological properties. GC is normally used for the analysis of complex mixtures of halogenated pollutants. The development of capillary columns GC allows congener-specific determination providing the baseline peak separation required for most compounds [31]. Its coupling to MS is also necessary to overcome some of these analytical difficulties (Figures 4–6).

TABLE 2 Summary of Main Extraction, Cleanup and Separating Methodologies Used to Analyze POPs in Sediments

Analytes (number)	Sample Pretreatment	Separation/ Detection Technique	LOD (units)	Observations	References
PCDD/Fs, DL-PCBs, HCB	(a) PCDD/Fs and HCB: air-drying; Soxhlet extraction (toluene, 16 h); cleanup: H_2SO_4 treatment, multilayer silica gel column (Na_2SO_4, silica gel, 30% KOH-silica gel, silica gel, 44% H_2SO_4-silica gel, silica gel, 10% $AgNO_3$-silica gel, Na_2SO_4) and alumina column. (b) dl-PCBs: EPA method 1668; cleanup: multilayer silica gel column and alumina column.	GC–HRMS (SIM)	0.01–500 ng kg^{-1}	(a) PCDD/Fs: DB-5MS column (60 m × 0.32 mm × 0.25 μm); HCB: DB-5MS column (30 m × 0.25 mm × 0.25 μm) (b) dl-PCBs: DB-5MS column (60 m × 0.32 mm × 0.25 μm)	[41]
PCDD/Fs (17)	PLE (toluene:acetone, 70:30, v/v); cleanup: silica, Florisil and celite/carbon chromatographic columns	GC–HRMS	0.0009 pg g^{-1} of dry weight (PCDD/Fs)	DB-5MS column	[48]
dl-PCBs (12)			0.036 pg g^{-1} of dry weight (dl-PCBs)		
PCDD/Fs PCBs, other pollutants	Soxhlet (toluene, 24 h); evaporation to constant weight; cleanup: (1) multilayer silica column (anhydrous Na_2SO_4 + silica gel + 44% (w/w) H_2SO_4/silica gel + silica gel + 10% silver nitrate/silica + anhydrous Na_2SO_4); (2) activated alumina column; concentration	GC–EI–HRMS	NA	Rtx-5ms column (60 m × 0.25 mm × 0.1 μm)	[45]

PCDD/Fs (17), dl-PCBs	(a) Lyophilization; Soxhlet (toluene); cleanup: (1) Na_2SO_4 + silica gel column; (2) activated carbon and celite column; (3) aluminum oxide column; Precipitation of sulfur using activated copper powder. (b) Air-drying; Soxhlet (toluene); cleanup: column chromatography on silica gel/NaOH, silica gel/H_2SO_4, acidic Al_2O_3, active carbon AX-21/Celite. (c) Air-drying; MAE (toluene:methanol, 9:1); cleanup: column chromatography (silica column/alumina column/activated carbon/Celite column)	GC–HRMS	NA	DB-5MS column (30 m × 0.25 mm × 0.25 μm) DB-17 (30 m × 0.25 mm × 0.25 μm) (complementary chromatographic separation of PCDD/Fs)	[22]
PBDEs (20)	Freeze-drying and sieve; Soxhlet (10% acetone in toluene, 200 mL, 24 h); concentration and redissolution in *n*-hexane; cleanup: multilayer silica gel column (Na_2SO_4 + 10% $AgNO_3$-silica gel + 22% H_2SO_4-silica gel + 44% H_2SO_4-silica gel + silica gel + 2% KOH-silica); concentration and redissolution in *n*-nonane	GC–EI–HRMS (SIM)	0.1–0.5 pg g^{-1} (mono- to hepta-BDEs) 1 pg g^{-1} (deca-BDE)	DB-5MS column (30 m × 0.25 mm × 0.1 μm) (separation of mono- to hepta-BDE congeners) DB5-MS column (15 m × 0.25 mm × 0.1 μm) (separation of deca-BDE congeners)	[42]
OCPs (21), HCB	Sediments: air-drying; Soxhlet (300 mL acetone, 16 h); concentration; LLE (*n*-hexane); concentration; SPE (i) florisil; (ii) active carbon ENVI-Carb; concentration	GC–EI–HRMS (SIM)	NA	ENV-8MS column (30 m × 0.25 mm × 0.25 μm)	[11]

Continued

TABLE 2 Summary of Main Extraction, Cleanup and Separating Methodologies Used to Analyze POPs in Sediments—Cont'd

Analytes (number)	Sample Pretreatment	Separation/ Detection Technique	LOD (units)	Observations	References
PCBs (62)	Air-drying; (a) Saponification (50 mL ethanolic potassium hydroxide 1 M, 80 °C with reflux or at room temperature, shaking 1 h); addition of *n*-hexane (50 mL); filtration (glass fiber filter, 47 mm); LLE (water, 50 mL); LLE (*n*-hexane, 50 mL); shaking with H_2SO_4 (98%); washing (LLE, water); drying (Na_2SO_4); volume reduction to 1 mL; SPE (silica 500 mg) (b) PLE (addition of Na_2SO_4 in the extraction cell); *n*-hexane/acetone (1:1, v/v, 10 min, 80 °C); addition of activated copper powder; filtration (PTFE membrane filter (0.2 μm, diameter 20 mm); LLE (50 mL *n*-hexane + 250 mL water); LLE (50 mL *n*-hexane); cleanup: H_2SO_4 treatment; SPE (silica 500 mg) (c) MAE (20 mL *n*-hexane/acetone (1:1), 10 min, 100–145 °C); centrifugation; cleanup: activated copper powder treatment + Na_2SO_4; shaking; filtration (PTFE membrane filter, 0.2 μm); SPE (silica cartridge); normal phase liquid chromatography, HPLC (YMC-Pack NH_2 column)	GC–HRMS	NA	HT-8 column (50 m × 0.22 mm × 0.5 μm)	[43]

	(d) Addition of Na_2SO_4; Soxhlet (*n*-hexane/acetone, 1:1, v:v or CH_2Cl_2, 300 mL, 24 h); cleanup: copper treatment + SPE + HPLC (above described) (e) Addition of water and acetone (25 mL); shaking (10 min) and sonication (200 W, 10 min); centrifugation; solvent replaced with *n*-hexane; cleanup: silica gel packed glass column				
PCDD/Fs and PCBs	Drying; PLE (*n*-hexane:acetone, 75:25, v/v, 120 °C, 12 MPa, two cycles 10 min); cleanup: (1) column chromatography (activated silica gel + silica gel with 44% H_2SO_4 + silica gel + anhydrous Na_2SO_4) (2) reversible carbon column. PCB fraction: evaporation; redissolution in ACN; SPE (C_{18}-modified silica gel); reduction to 10 µL PCDD/Fs fraction: column chromatography (basic alumina); reduction to 10 µL	GC–HRMS (SIM)	PCBs: 0.1–0.2 pg g^{-1} dry weight PCDD/Fs: 0.3–1.1 pg g^{-1}	PCBs: Restek Rtx-2330 column (90% biscyanopropyl/10% phenyl cyanopropyl polysiloxane) (60 m × 0.25 mm × 0.1 µm) PCDD/Fs: Restek Rtx-Dioxin2 column (60 m × 0.25 mm × 0.2 µm). Injection: splitless (cold injection system)	[46]
PCDD/Fs (17), PCBs (18), PBDEs (16)	Freeze-drying; Soxhlet (CH_2Cl_2:*n*-hexane, 1:1, v/v, 150 mL, 24 h); cleanup: (1) addition copper powder; concentration and redissolution (*n*-hexane); (2) multilayer silica gel (activated, basic and acidic silica, $AgNO_3$ silica, and Na_2SO_4), basic alumina and Florisil chromatography columns; (3) GPC (only PCBs fraction)	GC–EI–HRMS (SIM)	NA	PBDEs: HP-5 (30 m × 0.25 mm × 0.25 µm) PCBs and PCDD/Fs: DB-5MS (60 m × 0.25 mm × 0.25 µm) Injector: splitless mode	[30]

Continued

TABLE 2 Summary of Main Extraction, Cleanup and Separating Methodologies Used to Analyze POPs in Sediments—Cont'd

Analytes (number)	Sample Pretreatment	Separation/ Detection Technique	LOD (units)	Observations	References
PCDD/Fs (17) and PCBs (18)	Soxhlet (toluene, 8 h); concentration; addition of *n*-hexane and filtration (glass fiber filter); cleanup: Power-Prep™ (multilayer silica column, basic alumina and PX-21 active carbon column); concentration to dryness and reconstitution (*n*-nonane)	GC–EI–HRMS (SIM)	NA	DB-5MS (5% phenyl–95% methylsiloxane) fused silica capillary column (60 m × 0.25 mm × 0.25 μm). Injection: splitless mode	[14]
PCDD/Fs (17) and PCBs (18)	PLE (*n*-hexane:acetone, 75:25, v/v, 120 °C, 12 MPa, two static cycles, 10 min); cleanup: (1) concentration; (2) sequential LC steps (Na_2SO_4, activated silica gel, silica gel–NaOH, silica gel, silica gel–NaOH, silica gel, silica gel with H_2SO_4, silica gel, and Na_2SO_4); (3) reversible carbon column: Fraction 1 (PCBs, *n*-hexane): evaporated and redissolved in ACN(0.2 mL); SPE (cartridge with 1g C18-modified silica gel); elution (ACN) and concentration to 0.1 mL (stream of N_2). Fraction 2 (PCDD/F, toluene): cleanedup (LC with basic alumina); concentration to 0.1 mL (stream of N_2)	GC–HRMS (SIM)	NA	PCBs: Restek Rtx-2330 column (90% biscyanopropyl/10% phenyl cyanopropyl polysiloxane) (60 m × 0.25 mm × 0.1 μm); PCDD/Fs: Retsek Rtx-Dioxin2 column (60 m × 0.25 mm × 0.2 μm) Injection: splitless mode (cold injection system)	[13]

ACN, acetonitrile; HPLC, high-performance liquid chromatography; GPC, gel permeation chromatography; LLE, liquid–liquid extraction; MAE, microwave-assisted extraction; NA, data not available; PLE-C, pressurized liquid extraction with an integrated carbon trap; SPE, solid-phase extraction.

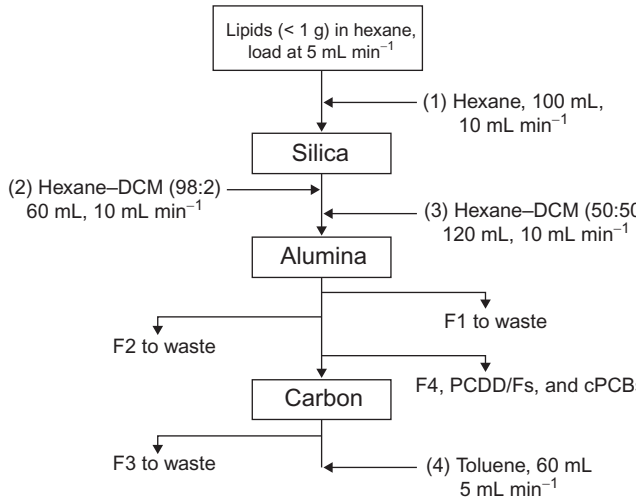

FIGURE 3 Flow chart showing the set of columns of the Power-Prep™ automated cleanup system applied for PCDD/Fs and PCBs. *Image courtesy of Elsevier from [47].*

As a consequence of the complex composition of most POP mixtures, the majority of studies have tried to enhance and optimize the chromatographic resolution [49]. With regards to stationary phases, nonpolar and slightly polar columns are frequently used (Table 1, Table 2), such as 100% dimethylpolysiloxane (e.g., DB-1), 5% phenyl–95% methylpolysiloxane (e.g., DB-5), with 0.25-μm film thickness and column lengths ranging from 30 to 60 m (Figures 5 and 6). Shorter columns (5–15 m) can be useful for certain applications (e.g., determination of thermolabile compounds such as BDE-209), providing a faster analysis without loss of resolution, although they are not widely employed [4]. Separation of PBDE mixtures can be performed in short columns which can efficiently separate nona- and deca-BDEs, while longer columns are adequate for separation of lower brominated BDEs [36].

Coelution of pollutants of interest and interferences has been mentioned as an important problem in GC separation [30,49]. For instance, the typical 5% phenyl columns exhibit multiple coelutions for PCBs and PCDD/Fs [50], and nonseparated peaks corresponding to DBE-47 and CB-180 can be observed in PBDEs analysis [36]. It is important to notice that a single analytical column capable of separating all PCBs, PBDEs, or PCDD/Fs congeners cannot be found in the market [4]. Alternatively, the use of more polar phases can be used to identify coeluting compounds (e.g., CP-Sil 88 [4]). Particularly, in the analysis of PCDD/Fs and dioxin-like PCBs (dl-PCBs) (a group of 12 PCBs congeners with similar toxicological properties than PCDD/Fs due to their coplanar structure), some columns have been developed, such

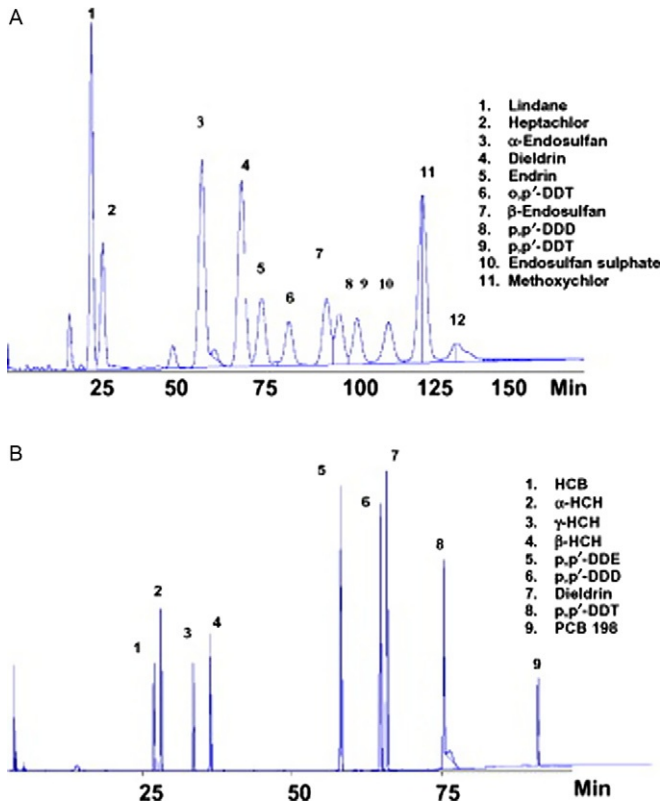

FIGURE 4 Chromatogram of a: (A) OCP mixture on a DB 1701 (15 m × 0.53 mm × 5 μm) column and (B) OCP standard mixture separated using a BPX35 (50 m × 0.25 mm × 0.25 μm). *Image courtesy of Elsevier from [23].*

as DB-Dioxin (44% methyl–28% phenyl–20% cyanopropylpolysiloxane), BPX-DXN, or Rtx-Dioxin2 (polysilphenylene phase). These columns are characterized by a more polar stationary phase and make possible the separation of critical pairs: (i) 2,3,4,6,7,8-TCDD and 2,3,4,7,8-pentaCDF and (ii) 2,3,4,6,7,8-TCDD and 2,3,4,6,7,8-hexaCDF [4]. It has been reported that Rtx-Dioxin2 column presents very few interfering compounds as well as BPX-DXN exhibits notably low bleed and improved separation in comparison with 5% phenyl columns [50]. Alternatively, liquid crystal columns have been used, providing exceptional separation for 2,3,7,8-substitued PCDDF/s. However, high bleed was observed; maximum allowable temperatures are low [4,50]; coelution problems of PCDD/Fs still remained, providing incomplete separation of all 17 most toxic PCDD/Fs, whose monitoring is recommended by the current legislation [4,49]. It has been discussed that to achieve complete separation of 17 PCDD/Fs, a combination of nonpolar and polar

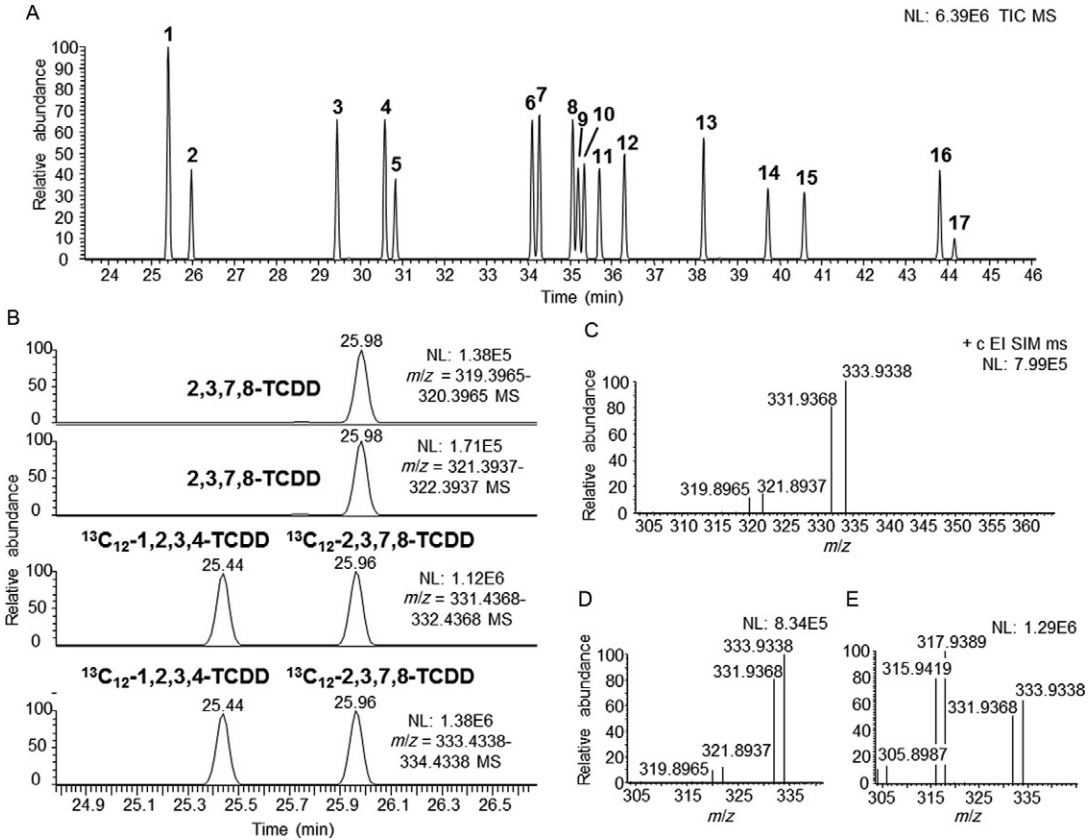

FIGURE 5 (A) Typical chromatographic separation (total ion chromatogram) of the 17 most toxic PCDD/Fs congeners at 0.5 ng mL^{-1} (1 ng mL^{-1} for OCDD and OCDF) by GC–HRMS (10,000 resolution, 10% valley; splitless injection, 1 μL; TR-DIOXIN-5MS column (60 m × 0.25 mm × 0.25 μm)). Peak identification: (1) 2,3,7,8-TCDF; (2) 2,3,7,8-TCDD; (3,4) penta-CDFs; (5) 1,2,3,7,8-penta-CDD; (6) hexa-CDFs (6,7,8,12); (9,10,11) hexa-CDDs; (13,15) hepta-CDFs; (14) 1,2,3,4,6,7,8-hepta-CDD; (16) OCDD; (17) OCDF; (B) extracted chromatograms of the native and labeled TCDD; (C) mass spectra of the native 2,3,7,8-TCDD congener; (D) mass spectra of ^{13}C labeled and (E) native 1,2,3,4-TCDD.

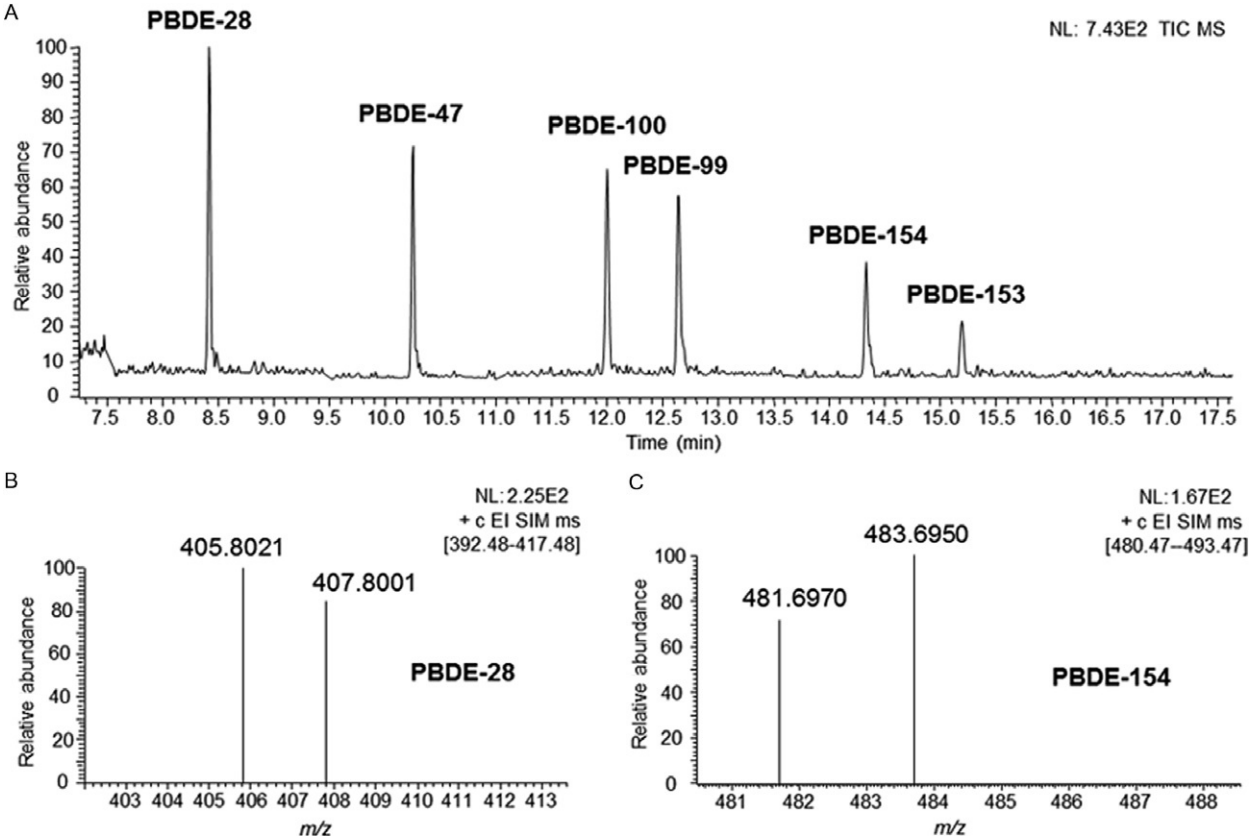

FIGURE 6 (A) Total ion chromatogram (TIC) of a sample containing PBDEs (0.1 μg L^{-1}) by GC–HRMS (10,000 resolution, 10% valley; splitless injection, 1 μL; TR-5MS column (30 m × 0.25 mm × 0.1 μm)); (B) mass spectra of PBDE-28 (low-brominated PBDE); and (C) PBDE-154 (high-brominated PBDE).

stationary phases (e.g., DB-5 and DB-225; DB-5MS and SP-2331(90% biscyanopropyl–10% phenyl cyanopropylsiloxane)) should be used [49].

Two-dimensional gas chromatography (GC × GC) [29,51] can also provide an improvement of chromatographic resolution, sensitivity, and selectivity and a reduction of analysis times in comparison with conventional GC [51]. Briefly, GC × GC consists of a first separation using a nonpolar column (separation based on volatility) and a second separation using a more polar column (separation based on polarity), allowing better separations between coeluting compounds [29,51]. However, the optimization of GC × GC separation is more laborious than traditional GC, and the obtained chromatograms are notably more complex, requiring powerful software tools. For example, GC × GC has been applied for the separation of complex mixtures of POPs such as PCDD/Fs and (dl-) PCBs, PBDEs in environmental samples [51] and OCPs in river water [29].

Concerning injection, splitless is the most commonly used injection technique [37,38] (Table 1, Table 2). Nevertheless, alternative techniques based on LVI, such as on-column injection or programmed temperature vaporization (PTV), have been applied to improve LODs [4,31]. In the specific case of PBDEs separation, PTV is recommended to avoid degradation of thermolabile PBDE congeners. When using the split/splitless injector, it is required to correctly adjust the temperature of injection to avoid discrimination of lower brominated BDEs and degradation of higher brominated BDEs [36]. Furthermore, a new dual data system which involves two GCs coupled to one HRMS able to analyze ions from both GCs simultaneously has been developed, although applications are scarce [31].

GC–HRMS is a powerful analytical tool characterized by mass resolution and accuracy, sensitivity, selectivity, specificity, and robustness, especially useful for determination of POPs, even at ultratrace levels [31]. It has been applied in both target analysis (e.g., PCDD/Fs) and alternative approaches for screening of nontarget contaminants or unknown pollutants [29,31].

Currently, GC–HRMS is undoubtedly considered as the reference instrumental technique for analysis of PCDD/Fs and dl-PCBs [49], although other detection techniques can be used for this purpose. In fact, determination of PCDD/Fs and dl-PCBs is the most important application of GC–HRMS. By contrast, in the case of the rest of POPs (especially in the case of OCPs and in some cases, PCBs), other techniques of detection are used frequently such as electron capture detection or low-resolution mass spectrometry, although HRMS is recommended when higher resolving power is required. In fact, several studies based on applications and strategies/trends for analysis of POPs by HRMS in the environmental field have been recently published [38,41,44,49].

GC–HRMS based on magnetic sector instruments is predominant in dioxin analysis [49] because it provides the necessary resolution and sensitivity, [31] and it is the reference technique for PCDD/Fs analysis in the EPA Method

1613 [35] (Figure 5). In general terms, POPs are analyzed by GC–HRMS at 10,000 resolution (10% valley definition), using electron ionization (EI) and selected ion monitoring (SIM) of the two most abundant ions of the molecular cluster (isotopically labeled ions included) [38,44,49]. However, potential interferences from chlorinated compounds (e.g., PCBs) may be found in PBDEs analysis using EI. For example, nominal masses corresponding to ions monitored for di-BDEs and penta-CBs are identical (m/z 326), so an extremely high resolving power of 24,000 would be needed to separate them [36]. Despite modern double-focusing mass spectrometers are able to reach higher resolution values, a remarkable loss of sensitivity is observed.

Because magnetic sector instruments require high investment, maintenance cost and highly qualified operators [31], alternative techniques have been used, such as TOF. Currently, two types of GC-TOF-MS instruments are available: (i) high-resolution TOF-MS (HRTOF-MS) instruments, which can reach mass resolution of 7000 (full width at a half maximum definition, FWHM), mass accuracy as low as 5 ppm, moderate acquisition speed (maximum acquisition rate 20 s^{-1}), and linearity range of approximately three orders of magnitude; and (ii) high-speed TOF-MS instruments (HSTOF-MS) showing maximum acquisition rate at 500 s^{-1}, an unit-mass resolution and linearity of approximately four orders of magnitude. HSTOF-MS instruments have been frequently employed together with fast and ultrafast GC or GC × GC [31]. On the other hand, GC-HRTOF-MS instruments have been applied in qualitative analysis, particularly for screening purposes and elucidation of unknowns [29,31] and for the analysis of POPs in water, including the target analysis of several POPs and a retrospective analysis to test the presence of PBDEs and other compounds by examination of full-scan accurate MS data [39].

Recently, atmospheric pressure chemical ionization source coupled to GC has been used for screening of organic contaminants in water samples. Less fragmented spectra are obtained, and the molecular ion can be normally observed. GC-HRTOF-MS equipped with a hybrid quadrupole-TOF-MS (QTOF-MS) was used in this study. The performance of tandem MS experiments and a reinjection of the sample using EI (to obtain fragment ions for confirmation of the structure) were carried out in order to perform unequivocal identification of a compound [31].

4 APPLICATIONS AND QUANTIFICATION STRATEGIES

With respect to quality assurance in POPs analysis, use of high-quality standards and internal standards, blank tests, replicate analysis, recovery experiments, and quality control charts are needed, as well as participation in proficiency tests and the analysis of CRM and laboratory reference materials are desirable [4]. In addition, the isotope dilution technique based on the use of internal standards labeled with ^{13}C provides the reliable quantification required for the accurate determination of POPs [35]. These standards are

added in known amounts prior to extraction or cleanup, allowing correction for recovery over the multistep cleanup methodologies, as well as they can also act as markers in order to perform identification of native analytes in samples [35,50]. For instance, in the case of PCDD/F and dl-PCBs, relative response factors were calculated for each individual congener with the corresponding calibration standard solutions containing both native and labeled toxic compounds [35].

Furthermore, in order to determine animal and human exposures due to intake of PCDD/F and dl-PCBs, individual toxic equivalency factors (TEFs) have been assigned based upon their relative potency in comparison with the most toxic dioxin, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD; TEF = 1) [52]. In this sense, the toxicity of a mixture is expressed in terms of International Toxic Equivalents, TEQ (TCDD equivalents), which is defined as the sum of the concentration of individual congeners multiplied by their TEF values [50].

As important environmental sinks of POPs and to evaluate their discharge to the environment, the spatial and temporal distributions of POPs in sediments have been reported in the past years. Several studies can be commented herein. PCBs and PCDD/Fs have been determined in sediments in the Nile river (Egypt), obtaining mean concentrations ranging from 1461 to 2244 (PCBs) and from 240 to 775 pg g^{-1} (PCDD/F), and revealing a diminish in the concentrations of these pollutants [45]. PBDEs were estimated in sediments from several coastal locations of Korea and 20 PBDE congeners were found in all samples at concentrations ranging from 0.45 to 494 ng g^{-1}, observing higher concentrations near industrial complexes and large harbors, demonstrating that human activities contributed to PBDEs pollution. Additionally, deca-BDE was mainly found (>90% of the total PBDE concentration) [42]. POPs levels were also evaluated in sediments of Han river (South Korea) [41]. DDTs, HCB, dl-PCBs, and PCDD/Fs were observed at 1.05–8.94 μg kg^{-1}, 0.49–3.73 μg kg^{-1}, 41.50–4.53 ng kg^{-1}, and 23.10–368.00 ng kg^{-1}, respectively. Similarly, PCDD/Fs and dl-PCBs have been analyzed in marine sediments from the Spanish Northern Atlantic Coast [14]. All the studied PCBs congeners were detected in all the analysed samples showing their ubiquity in coastal sediments. Marker PCBs levels were found at 385.5–4060.9 pg g^{-1}. PCDD/Fs and dl-PCBs were estimated in the range of 0.08–0.52 pg WHO-TEQ/g, and PCDDs were the predominant pollutants over PCDFs, being OCDD the most abundant congener [14].

On the other hand, studies about POPs levels in water samples have been also carried out but to a lesser extent. Thus, PCDD/Fs and PCBs were monitored in water samples collected from The Three George Reservoir (China) showing that the average WHO-TEQ of total PCDD/Fs + PCBs was 0.066 pg L^{-1}. The main dioxins found were hepta- and octa-chlorinated CDD/Fs [38]. Later, in 2008, PCBs and OCPs were also determined in water samples from the same reservoir obtaining a total PCB and OCP content that ranged from 0.08 to 0.51 ng L^{-1} and 2.33 to 3.60 ng L^{-1}, respectively [40].

5 CONCLUSIONS

Due to their potential risk for human health, persistence, and tendency to bioaccumulation, there has been an increasing concern over the presence of POPs in aquatic ecosystems, and determination of POPs in environmental samples has been a major subject for many years. Owing to its hydrophobic character, POPs tend to bind to the particle phase in water systems and then, deposition to the bottom via sedimentation processes occurs, and for this reason sediments are more frequently analyzed than water samples. Sediment and water samples belong to a group of environmental samples in which POPs are present at extremely low levels, making necessary the application of high efficient enrichment extraction procedures and rigorous cleanup methods for a reliable determination. In this context, the development of powerful, automated, and miniaturized sample preparation methods with low solvent consumption has been a continuous challenge. GC coupled to MS is normally applied for final determination, and more specifically, GC–HRMS. This technique using magnetic sector is considered the reference technique for determination of some POPs (e.g., PCDD/Fs). Additionally, the application of TOF analysers has attracted much attention, either HRTOF-MS (useful for screening purposes and elucidation of unknowns) or HSTOF-MS systems (useful in GC × GC).

In summary, remarkable developments in HR mass analysers have occurred over the last years, and it has allowed improving the analytical strategies used in environmental analysis. And undoubtedly, these instrumental improvements will continue into the future in order to achieve a promising combination between qualitative and quantitative analysis and structural elucidation of unknown compounds using a single instrument, as well as an increase in sensitivity.

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Chapter 19

A GC/MS–MS Versus GC/HRMS Dioxin Analysis Comparison. Some Critical Considerations for Low-Level Environmental Samples

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1 INTRODUCTION

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are a class of ubiquitous persistent organic pollutants (POPs) with high chemical stability and extremely poor water solubility [1–4]. These pollutants have 210 possible congeners with different numbers of chlorine atoms and position of substitutions. PCDDs consist of 75 congeners, while PCDFs consist of 135 congeners. Among these compounds, 17 congeners, those with chlorine substitution in the 2,3,7,8 positions, have a higher toxic potential. The most toxic congener is the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) evaluated from IARC (International Agency for Research on Cancer) as carcinogenic to humans (Group 1) [5].

One of the aims of the Stockholm convention, adopted in 2001 [6], is to protect humans and the environment against POPs, reducing, in particular, the unintentional release of PCDD/Fs. Human exposure has been associated

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with damage to the liver and immune system, adverse reproductive effects, and cancer [1–4]. Many countries have now compiled inventories of dioxin sources to better understand and quantify the amounts of PCDD/Fs emitted [6–8]. Once released in the environment, PCDD/Fs accumulate for years in environmental sinks such as soils and sediments, with a half life for 2,3,7,8-TCDD in soil of 10–12 years [9]. Many studies have investigated levels and sources of these pollutants in environmental samples [10–15].

The PCDD/F analytical determination in environmental samples is very complicated and requires highly sensitive and selective instrumentation [16]. Currently, gas chromatography coupled to high-resolution mass spectrometry (GC/HRMS) is recommended by conventional standard methods and is the reference technique for accurate and specific PCDD/Fs determination as described in U.S. EPA Method 1613 [17]. GC/HRMS provides high sensitivity and selectivity; for these reasons, it is recognized as a confirmatory method par excellence, but is an expensive technology, which requires extensive maintenance and highly trained staff.

Since the 1980s, alternative techniques such as triple quadrupoles or ion trap mass spectrometry working in MS–MS mode have been applied for the determination of PCDD/Fs and dioxin-like PCBs (DL-PCBs) in food and feed samples [18–20]. The difficulty of the measurement of these compounds is also related to the low levels at which they occur in environment, in food and feed matrices. Only in the past years, the new generation of triple quadrupole has shown a significant increase in their sensitivity. Kotz *et al.* [21] and Fürst *et al.* [22] reported the achievement of very low levels of quantification suggesting a potential for their application in food and feed analysis. GC/MS–MS triple quadrupole seems to be particularly suitable as screening technique in the multiple reaction monitoring (MRM) mode, as the selectivity of a triple quadrupole might be compared to that of an HRMS used in the selected ion monitoring (SIM) mode Current European Union regulations now permit the use of GC/MS–MS and bioassay techniques for screening of these compounds in food and feed analysis [23,24], but still requires the application of GC/HRMS as confirmatory method for their unequivocal identification and quantification.

In 2011, different studies evaluated the performance of GC/MS–MS systems for the PCDD/Fs and DL-PCBs in feed and food analysis. A good correlation between the results of GC/MS–MS and GC/HRMS was observed by Ingelido *et al.* using consensus-based sample [25]. Kotz *et al.* [21] for the concentrations above 1 pg WHO-PCDD/Fs toxicity equivalents (TEQ)/g fat for food and human milk. Fürst and coworkers [22] reported comparable results also for lower PCDD/Fs and DL-PCBs concentrations in the range of 0.5–3 pg WHO-PCDD/Fs TEQ/g, suggesting GC/MS–MS as a potential alternative confirmatory method to the classical HRMS [26]. As these rising techniques have not been applied to environmental samples yet, the aim of this

work is to provide a first critical comparison between the official GC/HRMS and the GC/MS–MS approach in the determination of PCDD/Fs in environmental samples. For this purpose, PCDD/F content in 22 Italian soil samples has been assessed in parallel using both GC/HRMS and GC/MS–MS methods.

2 MATERIALS AND METHODS

Approximately 10–30 g of soil samples were dried and spiked with $^{13}C_{12}$-labeled surrogate 2,3,7,8 PCDD/F congener standards and then extracted with organic solvents (*n*-hexane/acetone, 4:1 v/v), following EPA Method 1613: Tetra- through octa-chlorinated dioxins and furans by isotope dilution HRGC/HRMS. Extracts were cleaned up through multistage separation process to eliminate interfering components, which included a purification step by Extrelut®NT column (Merck Darsadt, Germany) with 15 ml of concentrated sulfuric acid (98%) overnight and further purification on neutral alumina columns (Merck) activated at 400 °C before use [27–30]. Purified samples were analyzed by HRMS first and subsequently by GC/MS–MS.

The GC/HRMS measurements were performed using a TRACE GC 2000, Thermo Finnigan (Thermo Fisher Scientific), coupled with a MAT 95 XP Mass Spectrometer, operating in the electron ionization (EI+) mode, at an electron energy of 48 eV and at a resolving power of 10,000. The ion source and the transfer line were set at 280 °C. The analysis was performed working in SIM mode and the monitored ions were M^+ and M^{2+} for tetra-CDD/Fs and M^{2+} and M^{4+} for penta-, hexa-, hepta-, and octa-CDDs/Fs.

For GC/MS–MS measurements, an Agilent 7000 Triple Quadrupole Mass Spectrometer interfaced to an Agilent 7890 GC (Palo Alto, CA, USA) in EI+ mode with MRM acquisition method was used. Source and transfer line temperatures were set at 280 °C.

For each congener, the ions monitored and their corresponding internal standards in HR-GCMS and GC/MS–MS acquisition are reported in Tables 1 and 2, respectively.

The chromatographic separation was performed using a BPX-DXN (60 m × 0.20 mm × 0.25 μm, SGE, Analytical Science, Melbourne, Australia) capillary column with splitless injection. Oven temperature program was 160 °C for 1 min, 2.5 °C/min increase until 300 °C, and 300 °C maintained for 6 min.

Peaks were accepted if the observed isotopic ratio of the molecular cluster was within 15% of calculated value both for native congeners and for internal standards. Limit of detections (LODs) were calculated individually for each sample on the basis of a signal-to-noise ratio of 3:1. Analytical blanks, covering the whole sampling and analytical procedure, were run. Blank samples showed no significant interfering peaks. Concentrations have been expressed

TABLE 1 GC/HRMS Parameters for PCDD/F Congeners and Their $^{13}C_{12}$-Labeled Internal Standards

Analyte	RT (min)	Q.I. (*m/z*)	C.I. (*m/z*)	Theoretical Ratio (C.I./Q.I.)
$^{13}C_{12}$-2,3,7,8-TCDF	34.22	317.9389	315.9419	0.77 (0.65–0.89)
2,3,7,8-TCDF	34.24	305.8987	303.8984	
$^{13}C_{12}$-1,2,3,4-TCDD	34.96	333.9338	331.9368	0.77 (0.65–0.89)
1,2,3,4-TCDD	34.99	321.8937	319.8965	
$^{13}C_{12}$-1,2,3,7,8-PnCDF	39.24	351.9000	349.9029	0.66 (0.53–0.71)
1,2,3,7,8-PnCDF	39.24	339.8597	337.8627	
$^{13}C_{12}$-2,3,4,7,8-PnCDF	40.68	351.9000	349.9029	
2,3,4,7,8-PnCDF	40.70	339.8597	337.8627	
$^{13}C_{12}$-1,2,3,7,8-PnCDD	41.01	367.8949	365.8978	0.66 (0.53–0.71)
1,2,3,7,8-PnCDD	41.03	355.8546	353.8576	
$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	44.98	385.8610	387.8580	0.82 (0.70–0.94)
1,2,3,4,7,8-HxCDF	44.96	373.8206	375.8178	
$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	44.98	385.8610	387.8580	
1,2,3,6,7,8-HxCDF	45.19	373.8206	375.8178	
$^{13}C_{12}$-2,3,4,6,7,8-HxCDF	46.15	385.8610	387.8580	
2,3,4,6,7,8-HxCDF	46.18	373.8206	375.8178	
$^{13}C_{12}$-1,2,3,7,8, 9-HxCDF	47.57	385.8610	387.8580	
1,2,3,7,8, 9-HxCDF	47.60	373.8206	375.8178	
$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	46.35	401.8560	403.8530	0.82 (0.70–0.94)
1,2,3,4,7,8-HxCDD	46.35	389.8156	391.8127	
$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	46.51	401.8560	403.8530	
1,2,3,6,7,8-HxCDD	46.54	389.8156	391.8127	
$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	46.95	401.8560	403.8530	
1,2,3,7,8,9-HxCDD	46.98	389.8156	391.8127	
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	49.93	419.8220	421.8191	0.98 (0.83–1.13)
1,2,3,4,6, 7,8-HpCDF	49.95	407.7817	409.7788	
$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	52.76	419.8220	421.8191	
1,2,3,4,7,8, 9-HpCDF	52.78	407.7817	409.7788	

TABLE 1 GC/HRMS Parameters for PCDD/F Congeners and Their $^{13}C_{12}$-Labeled Internal Standards—Cont'd

Analyte	RT (min)	Q.I. (*m/z*)	C.I. (*m/z*)	Theoretical Ratio (C.I./Q.I.)
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	51.81	435.8169	437.8140	0.98 (0.83–1.13)
1,2,3,4,6,7,8-HpCDD	51.82	423.7767	425.7737	
$^{13}C_{12}$-OCDD	56.73	471.7750	469.7780	0.88 (0.75–1.01)
OCDD	56.72	459.7348	457.7377	
$^{13}C_{12}$-OCDF	57.06	455.7801	453.7830	0.88 (0.75–1.01)
OCDF	57.08	443.7398	441.7428	

RT, retention time; Q.I., quantifier ion; C.I., qualifier confirmatory ion.

TABLE 2 GC/MS–MS MRM Parameters for PCDD/F Congeners and Their $^{13}C_{12}$-Labeled Internal Standards

Analyte	RT (min)	Quant. Precursor (*m/z*)	Product (*m/z*)	Qual. Precursor (*m/z*)	Product (*m/z*)
$^{13}C_{12}$-2,3,7,8-TCDF	34.22	315.9	252.0	317.9	254.0
2,3,7,8-TCDF	34.24	303.9	240.9	305.9	242.9
$^{13}C_{12}$-1,2,3,4-TCDD	34.96	331.9	268.0	333.9	270.0
1,2,3,4-TCDD	34.99	319.9	256.9	321.9	258.9
$^{13}C_{12}$-1,2,3,7,8-PnCDF	39.24	349.9	285.9	351.9	287.9
1,2,3,7,8-PnCDF	39.24	337.9	274.9	309.9	276.9
$^{13}C_{12}$-2,3,4,7,8-PnCDF	40.68	349.9	285.9	351.9	287.9
2,3,4,7,8-PnCDF	40.70	337.9	274.9	339.9	276.9
$^{13}C_{12}$-1,2,3,7,8-PnCDD	41.01	365.9	301.9	355.9	292.9
1,2,3,7,8-PnCDD	41.03	353.9	290.9	355.9	292.9
$^{13}C_{12}$-1,2,3,4,7, 8-HxCDF	44.98	383.9	319.9	385.9	321.9
1,2,3,4,7,8-HxCDF	44.96	371.8	308.9	373.8	310.9

Continued

TABLE 2 GC/MS–MS MRM Parameters for PCDD/F Congeners and Their $^{13}C_{12}$-Labeled Internal Standards—Cont'd

Analyte	RT (min)	Quant. Precursor (*m/z*)	Product (*m/z*)	Qual. Precursor (*m/z*)	Product (*m/z*)
$^{13}C_{12}$-1,2,3,6,7, 8-HxCDF	44.98	383.9	319.9	385.9	321.9
1,2,3,6,7,8-HxCDF	45.19	371.8	308.9	373.8	310.9
$^{13}C_{12}$-2,3,4,6,7, 8-HxCDF	46.15	383.9	319.9	385.9	321.9
2,3,4,6,7,8-HxCDF	46.18	371.8	308.9	373.8	310.9
$^{13}C_{12}$-1,2,3,7,8, 9-HxCDF	47.57	383.9	319.9	385.9	321.9
1,2,3,7,8,9-HxCDF	47.60	371.8	308.9	373.8	310.9
$^{13}C_{12}$-1,2,3,4,7, 8-HxCDD	46.35	399.9	335.9	401.9	337.9
1,2,3,4,7,8-HxCDD	46.38	387.8	324.9	389.8	326.9
$^{13}C_{12}$-1,2,3,6,7, 8-HxCDD	46.51	399.9	335.9	401.9	337.9
1,2,3,6,7,8-HxCDD	46.54	387.8	324.9	389.8	326.9
$^{13}C_{12}$-1,2,3,7,8, 9-HxCDD	46.95	399.9	335.9	401.9	337.9
1,2,3,7,8,9-HxCDD	46.98	378.8	324.9	389.8	326.9
$^{13}C_{12}$-1,2,3,4,6,7, 8-HpCDF	49.93	417.8	353.9	419.8	355.9
1,2,3,4,6,7,8-HpCDF	49.95	405.8	342.8	407.8	344.8
$^{13}C_{12}$-1,2,3,4,7,8, 9-HpCDF	52.76	417.8	353.9	419.8	355.9
1,2,3,4,7,8,9-HpCDF	52.78	405.8	342.8	407.8	344.8
$^{13}C_{12}$-1,2,3,4,6,7, 8-HpCDD	51.81	433.8	369.9	435.8	371.9
1,2,3,4,6,7,8-HpCDD	51.82	421.8	358.8	423.8	360.8
$^{13}C_{12}$-OCDD	56.73	467.8	403.8	469.8	405.8
OCDD	56.72	455.7	392.8	407.8	344.8
$^{13}C_{12}$-OCDF	57.06	451.8	387.8	453.8	389.8
OCDF	57.08	439.7	376.8	441.7	378.8

as TEQs in terms of pg TEQ/g calculated by multiplying the detected concentration by the corresponding toxic equivalent factors provided by the World Health Organization (WHO) [31]. For the TEQ calculations, concentrations below the LOD were considered as half the LOD (middle bound method, i.e., using half of the limit of detection when calculating the contribution of each nonquantified congener).

3 RESULTS

In Figure 1, a chromatogram of the 2,3,7,8-substituted hexachlororo dibenzo-*p*-dioxins (HxCDD) congeners is reported for the same environmental sample, analyzed by both methods, GC/HRMS and GC/MS–MS, showing the same chromatographic pattern for internal standards (^{13}C labeled) and native congeners for both instruments.

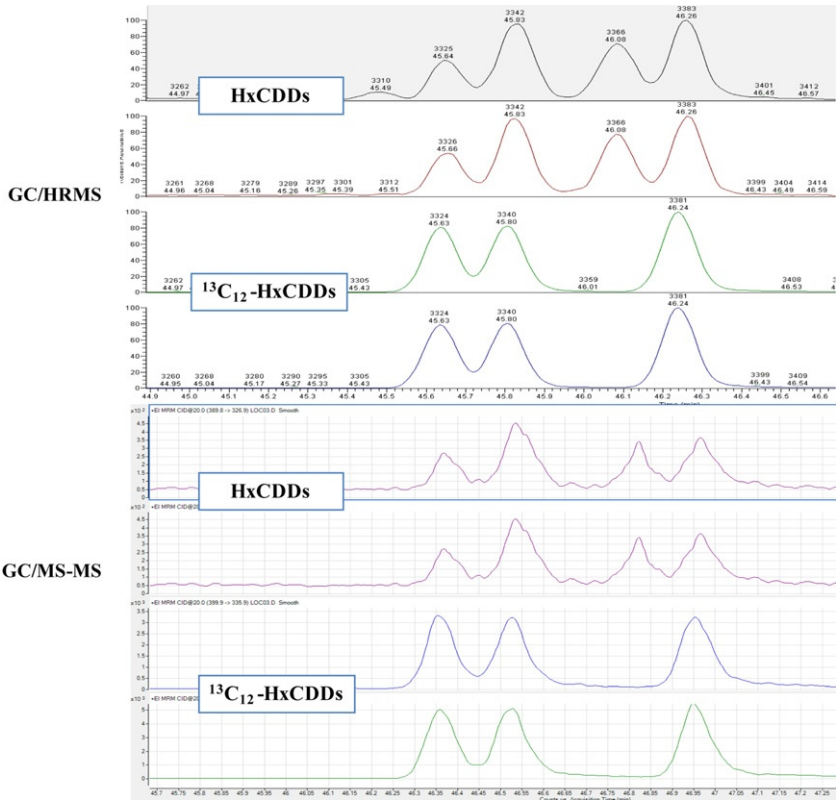

FIGURE 1 GC/HRMS and GC/MS–MS chromatograms of hexachlorodibenzodioxins (HxCDDs) in a soil sample. Upper chromatograms are profiles of native congeners and lower chromatograms are those from internal standards (^{13}C-HxcDDs).

Most of the samples analyzed had individual congeners below LODs as they were real, everyday, environmental samples. As mentioned before, in this work, middle bound values have been considered for the contribution of each nonquantified congener.

In Table 3, PCDD/Fs TEQ values obtained applying the two GC–MS methods are reported. As shown, PCDD/F soil concentrations ranged from 0.33 to 14.11 pg

TABLE 3 Results for PCDD/Fs TEQ Values Obtained by GC/MS–MS and GC/HRMS and Relative Differences |±Δ%| Between the Two Instrumental Methods

Sample	GC/HRMS (pg WHO-TEQ/g)	GC/MS–MS (pg WHO-TEQ/g)	|±Δ%|
1	0.33	0.56	69.51
2	0.51	0.53	3.06
3	0.52	0.93	80.63
4	0.54	1.13	108.08
5	0.58	0.74	28.40
6	0.60	0.61	1.71
7	0.62	0.78	25.61
8	0.65	1.29	98.68
9	0.67	1.48	121.47
10	0.70	0.89	26.20
11	0.91	2.12	132.17
12	0.99	1.35	37.36
13	1.24	0.85	31.47
14	1.29	1.54	19.19
15	1.75	1.98	13.20
16	1.96	1.48	24.37
17	2.17	2.34	7.91
18	2.43	1.48	39.04
19	2.77	2.77	0.24
20	2.85	2.94	3.46
21	5.76	5.37	6.91
22	14.10	14.11	0.12

WHO-TEQ/g. Only one sample exceeded the limit of 10 pg WHO-TEQ/g adopted in Italy in soils for green and residential uses, while none exceeded the threshold for commercial and industrial soils (100 pg WHO-TEQ/g) [32], indicating a low contamination level. This soil sample is the only sample that exceeded the more restrictive guidelines adopted in Germany, where a limit of 5 pg I-TEQ/g [33] was set to permit the use of soils for agricultural purposes, and in Canada, where a limit of 4 pg WHO-TEQ/g for agricultural, residential, commercial, and industrial purposes was established [34]. The international soil quality guidelines were previously summarized by Naile *et al.* [35] and are reported in Table 4.

TABLE 4 International Quality Guidelines for PCDD/Fs in Soils

Country	PCDD/F (pg WHO-TEQ/g)	Remarks
Canada [34]	<4	All land uses
Germany [33,36]	<5	Target concentration
	40–100	Children playgrounds
	100–1000	Residential soils
	1000–10,000	Industrial soils
Japan [37]	<1000	–
New Zealand [38]	<10	Agricultural
	10–1500	Residential
	1500–18,000	Industrial (unpaved)
	18,000–21,000	Industrial (maintenance)
	21,000–90,000	Industrial (paved)
Sweden [8]	<10	Sensitive uses
	10–50	Nonsensitive uses
The Netherlands [8]	<1	Agricultural farming
	1–10	Dairy farming
United Kingdom [39]	<8	Residential, allotment
	8–240	Commercial
United States [40]	<50	Screening level
	50–1000	Evaluation level
	>1000	Actionable level

Figure 2 shows the correlation obtained between GC/MS–MS and GC/HRMS for the PCDD/F soil concentrations, expressed as WHO-TEQ/g. Despite the low PCDD/F concentrations, the results obtained are in a good agreement showing an overall correlation expressed as R squared coefficient of correlation (R^2) around 0.97. The same correlation is observed if data are expressed as PCDD/F single congeners concentrations (Figure 3) with an R^2 value of 0.99, showing that no systematic errors in congeners' determination are present, due to the different ion selection (high resolution vs. MS–MS) and transport (magnetic sector vs. quadrupole analyzer) in the mass spectrometer. Figure 4 shows the relative difference percentage (%) between the two techniques compared. Nevertheless, this difference, as shown in Figure 4, is concentration dependent. The relative difference,

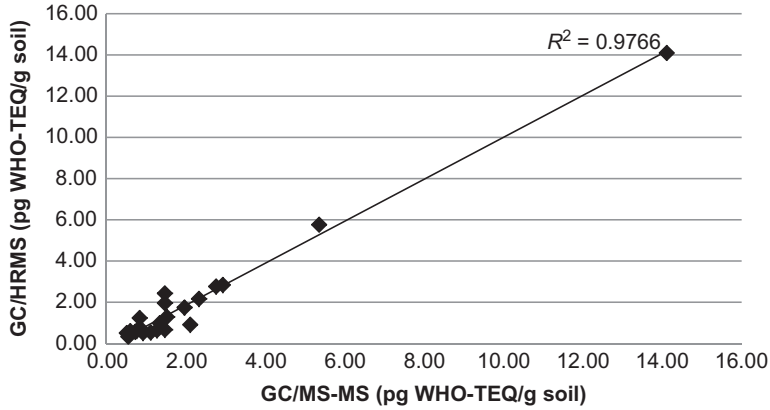

FIGURE 2 Correlation between GC/MS–MS and GC/HRMS methods for PCDD/Fs TEQ concentration values expressed as pg WHO-TEQ/g.

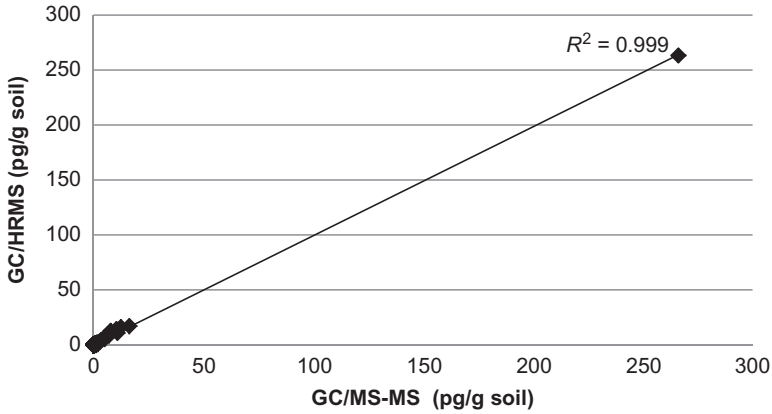

FIGURE 3 Correlation between GC/MS–MS and GC/HRMS methods for PCDD/Fs congeners concentrations in soil expressed as pg/g obtained.

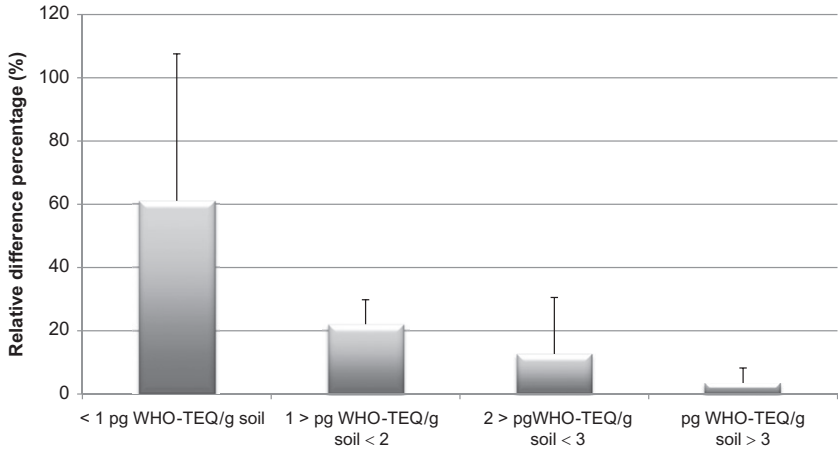

FIGURE 4 Relative difference percentage, expressed as $|\pm\Delta\%|$, between GC/MS–MS and GC/HRMS methods, at different TEQ ranges. It can be noted that samples with TEQ values higher than 1 pg/g showed differences of less than 20% compared to GC/HRMS data.

expressed as $|\pm\Delta\%|$, between the two measurements in soil samples with lower PCDD/Fs concentrations (<1 pg WHO-TEQ/g) was 61%. At PCDD/F concentrations higher than 3 pg WHO-TEQ/g, average relative difference is as low as 4%.

Therefore, for this range of application, this new generation of triple quadrupole GC/MS–MS is capable of quantitative analysis of PCDDs and PCDFs in environmental soil samples, offering a realistic alternative approach to the classical HRMS method, at least as a screening method, like the mentioned EC Regulations 1883/2006, 152/2009, and 252/2012 for certain foodstuffs and feed samples. Moreover, GC/MS–MS, due to the autotune routines, requires less training and provides higher robustness. Even after a severe contamination, as reported in Figure 5, instrument performances are not altered, and it is still possible to analyze environmental samples on the next injections.

4 CONCLUSIONS

The results of this study showed a good agreement between GC/MS–MS and GC/HRMS data for the analysis of soil samples containing at least 1 pg WHO-TEQ/g. Samples with TEQ values higher than 1 pg/g show differences lower than 20% compared to GC/HRMS data, reaching very small differences within 4% for samples with TEQ values higher than 3 pg WHO-TEQ/g.

Based on the data obtained, there is evidence that GC/MS–MS methods based on new generation instruments can be used for the quantitative analysis

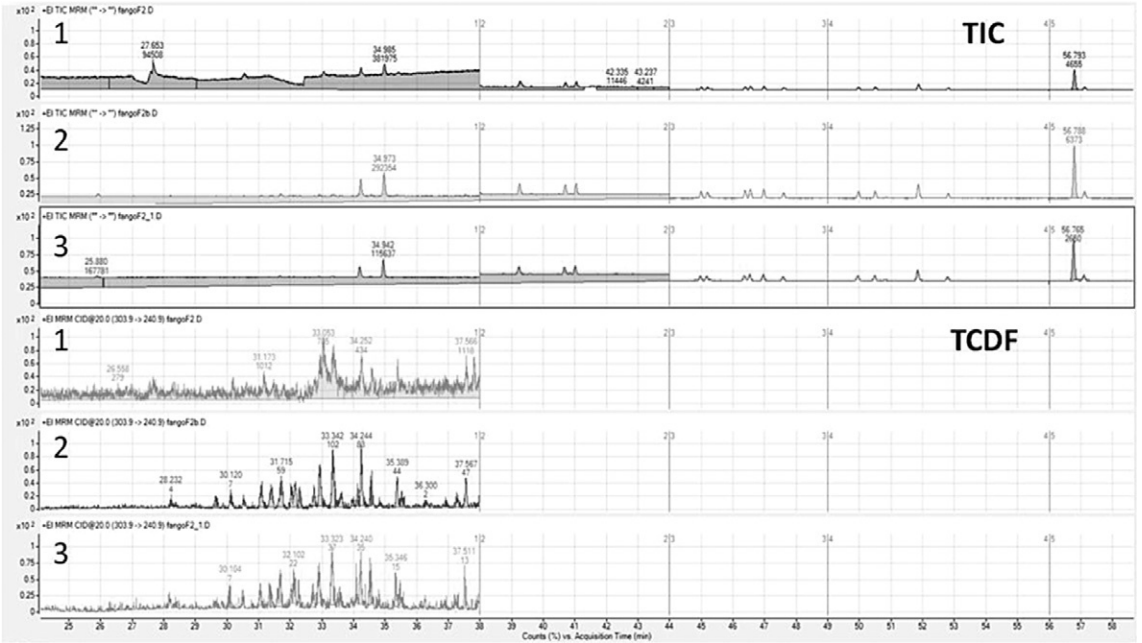

FIGURE 5 GC/MS–MS chromatograms of three different injections of a sludge sample after a severe contamination. The first injection shows residual contaminations in the column that mask peaks in total ion and in specific ion transition chromatograms. During the second injection, the column elutes contaminants, and peaks are well resolved. The third chromatogram is similar to the second, indicating that no residues are apparently present in the ion source that affect mass spectrometer response.

of PCDDs and PCDFs in environmental samples. A new monitoring strategy could be designed not only for food and feed samples but also for these type of samples offering a realistic, lower instrumental cost, and higher throughput alternative to the GC/HRMS method still requiring, for the sample extraction and purification step, trained personnel and dedicated laboratories. This approach could be used in the future as an official screening method, to classify a sample as compliant or suspected to be noncompliant, after the necessary interlaboratory method validation study.

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Chapter 20

The Future of GC/Q-TOF in Environmental Analysis

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1 INTRODUCTION

The need to continually measure and monitor the environment for its normal constituents and foreign or toxic substances discharged by anthropogenic and natural sources cannot be overstated. Empirical data suggest that entities such as personal care products, agricultural chemicals, pharmaceuticals, industrial chemicals, automobile emissions, and terrestrial contaminants such as volcanic emissions and biological decay have accumulated in the environment. It has further been determined that many of these compounds persist in the environment and bioaccumulate in the indigenous flora and fauna and are passed from generation to generation through the food chain. There is also evidence that human exposure to these compounds is the etiology of various pathologies. However, the aggregate effect on the health and wellbeing of the ecosystem and the morbidity and mortality of the human population from exposure to the total breadth of compounds released into the environment requires more investigation.

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In general, persistent chemicals that have been determined to have a negative effect on the environment and human health are labeled as persistent organic pollutants (POPs). Familiar POP chemotypes include polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (dioxins), polychlorinated dibenzofurans (furans), and agricultural chemicals such as dichlorophenyl trichloroethane (DDT) and other biologically relevant compounds used to manage and manipulate the environment including but not limited to insecticides, herbicides, pesticides, growth hormones, and antibiotics. For many of these compounds, wind and water transport them from one region to another, while for others their fate and transport are poorly understood. Because of the known and unknown transport mechanisms, the matrices that need to be examined for POP contamination are diverse and range from potable water, waste water, waste water sediment, soil, air, plasma serum, whole blood and urine, and a host of other biological and nonbiological sources. As noted earlier, however, the extensiveness of chemicals released into the environment, how they are transported across the globe, and their potential to have a negative impact on the environment and the health of its inhabitants is a matter of ongoing investigation.

Most industrialized nations have recognized the detrimental effects of POPs and implemented mitigating laws to control or negate their release into the environment. At the Stockholm Convention in May 2001, more than 90 nations agreed to reduce or eliminate the production of 12 compounds or families of compounds and created a mechanism for the continued review to add more POPs to the list. Since the 2001 Stockholm Convention, many international and national agencies such as the World Health Organization (WHO), the European Commission (EC), the Chinese Ministry of Environmental Protection, and the United States Environmental Protection Agency (EPA) have identified a multitude of new compounds and classes of compounds such as perfluorooctane sulfonic acids and polybrominated diphenyl ethers as POPs and worked to reduce or stop their production.

The inherent complexity of examining the environment for total POPs and correlating that to environmental or human health is a daunting task that will require an interdisciplinary approach including but not limited to epidemiology, toxicology, food safety monitoring, and analytical chemistry. The instrumentation required to examine these samples are just as diverse and include liquid chromatography, gas chromatography (GC), mass spectrometry (MS), inductively coupled plasma mass spectrometry, and nuclear magnetic resonance (NMR) spectroscopy techniques.

Notwithstanding the various analytical techniques, the primary methodology in today's environmental laboratory is GC or GC/MS. The reasons for GC and GC/MS being the analytical tools of choice in environmental analysis are to some extent determined by current governmental regulations and the historical precedence of GC and GC/MS in environmental science. However, it can be postulated that the primary reasons include a low cost of

operation, the ease of implementation and use, high chromatographic resolution, quantification over a large concentration range and with respect to GC/MS, identification and speciation of compounds in a sample through advanced postdata acquisition tools such as chromatographic deconvolution and subsequent searching of commercial and custom mass spectral libraries.

The breadth of mass spectrometers coupled to GC systems is also diverse and include ion trap, quadrupole, tandem quadrupole, time of flight (TOF), quadrupole-TOF, magnetic sector, and Fourier transform ion cyclotron resonance. Herein is reviewed the use of GC/MS technologies to examine the applicable chemical space and define a potential future for GC/TOF and GC/quadrupole-TOF mass spectrometry (GC/Q-TOF) in environmental analysis.

2 GC/MS AND ENVIRONMENTAL ANALYSIS

The application of GC techniques for the analysis of the environment dates back many years and even today GC/MS is the most commonly used technique for the analysis of volatile and semivolatile organic compounds found in environmental samples. In this section, we briefly review GC/MS technologies and define time-of-flight mass spectrometers in light of instrument resolving power, mass resolution, and mass accuracy.

2.1 A Brief Review of GC/MS Technologies

Single quadrupole and ion trap MS systems offer ease of use and excellent robustness at affordable prices and the ability to perform electron impact (EI) and soft ionization techniques such as positive or negative chemical ionization. The ion trap further offers MS^n. Tandem mass spectrometers, commonly referred to as "triple quadrupoles" because the early renditions used three quadrupoles on a linear axis, offer highly selective and sensitive methodologies and the ability to efficiently mitigate matrix interference in an easy to use and affordable bench top format. There are also more sophisticated MS technologies such as high-resolution magnetic sector MS, Fourier transform ion cyclotron resonance (FT-ICR) MS, linear quadrupole ion trap coupled to a Fourier transform ion cyclotron resonance (q-FT-ICR) MS that are increasingly more difficult to operate and expensive to own. For many applications where magnetic sector is not required by regulation, a potential alternative that offers relative ease of use, high mass resolving power, and accurate mass are TOF and Q-TOF mass spectrometers.

2.1.1 Why and When to use Single Quadrupole MS, MS/MS, TOF, or Q-TOF?

One of the most common applications for single quadrupole (GC/SQMS) in environmental monitoring is quantification of a target list of compounds

either from a single chemical class or a mixture of chemical classes as in multipesticide residue analysis wherein hundreds of analytes are measured in a single analytical run. The acquisition can be performed in single ion monitoring (SIM) mode or as full spectrum scan data. Another application for GC/SQMS is that of acquiring full-scan spectral data across a fixed mass range and searching commercial or custom spectral libraries for the identification of known–knowns or compounds that exist in a database and are found in the sample set. This approach can be coupled with spectral deconvolution algorithms and quantitative databases to provide accurate identification and quantification of known compounds in unknown samples.

Gas chromatography tandem mass spectrometry (GC/MS/MS) is best applied to targeted panels of analytes of a single chemical class or a mixture of chemical classes in heavy matrix such as biosolids from waste water treatment plants, soil extracts, petroleum sources, and biological matrices such as blood, urine, and tissue. The mechanism of matrix mitigation is SIM—collision induced dissociation—SIM on two quadrupoles situated adjacent to a radio frequency only (no mass filtering) collision cell on a linear or curved axis, that is, Q1-Collision Cell-Q2 (sometime referred to as Q3 for historical purposes). This reaction process, referred to as single or multiple reaction monitoring (SRM or MRM) wherein Q1 isolates precursor ions, fragmentation occurs in the collision cell and SIM of the fragment ions in the last quadrupole is performed, significantly reduces chemical interferences commonly observed in SIM only and vastly improves the signal-to-noise (*S/N*) ratio resulting in highly selective and sensitive methods.

TOF-MS always collects full-scan mass spectral data similar to that of a SQMS. The major differences between SQMS and TOF-MS are: (1) the inherent instrument resolving power, (2) the mass resolution, and (3) the mass accuracy are much greater on a TOF instrument. GC/TOF and GC/Q-TOF systems can be used to solve complex analytical problems such as identification of known–knowns and unknown–unknowns (compounds not present in any known database). The latter problem can arise in *de novo* metabolite identification after the administration of new drug entity to a biological model. The problem can be solved through accurate mass measurement and generation of empirical formulae followed by collecting accurate mass product ion spectra (MS/MS) for structure elucidation and confirmation.

2.1.2 Instrument Resolving Power and Mass Resolution

The terms resolving power and mass resolution are often used interchangeably but are truly different properties that define instrument and method-related performance. Resolving power refers to the instruments ability to distinguish two adjacent ions of equal intensity. On a TOF instrument, resolving power is fixed across the mass range while mass resolution is a function of both ion width and the mass being measured.

The IUPAC definition of mass resolving power is [1]:

"For two peaks of equal height with masses m_1 and m_2, when there is overlap between the two peaks to a stated percentage of either peak height (10% is recommended), then the resolving power is defined as

$$\frac{m_1}{m_1 - m_2}. \tag{1}$$

The percentage overlap (or "valley") concerned must always be stated."

IUPAC further offers a peak-width definition for mass resolution:

"For a single peak made up of singly charged ions at mass m in a mass spectrum, the resolution may be expressed as

$$\frac{m}{\Delta m} \tag{2}$$

where Δm is the width of the peak at a height which is a specified fraction of the maximum peak height (e.g., 50%)."

That is to say if a monoisotopic, singly charged ion measured on a TOF system has a profile width of 0.05 amu at a mass of 500.0000 amu, the mass resolution is 10,000. For comparison, the same 500 amu measured on a SQMS or quadrupole MS/MS system with typical ion widths of 0.6 amu has a mass resolution of approximately 833. It is evident therefore that the more narrow the ion width, the better the ability to distinguish similar masses.

2.1.2.1 How Much Resolution is Enough?

In his article *Debating Resolution and Mass Accuracy*, Balogh [2] noted that sensitivity is reduced as mass resolving power is increased and the argument for higher mass resolution does not become persuasive until the molecular weights being measured become significant. Another trade-off for very high resolution (100,000 or greater) is the spectral acquisition rate. As Balogh further notes, the resolution of instruments such as FT-ICR is undeniable but potentially impractical and references a conclusion:

FT-ICR MS provides unequalled resolving power and mass accuracy as long as sufficient time is available to acquire the necessary degree of information . . . the problem is (the) required time is too long to allow sufficient spectra to be obtained across a high-resolution (chromatographic) peak for the peak to be properly delineated (sic) [3].

Kellman *et al.* [4] suggest a required mass resolving power of 25,000 on a LC/FT-ICR instrument to measure drugs, pesticides, mycotoxins, and plant toxins at levels down to 10 ppb in feed matrix samples. Upon inspection of the chromatographic peak widths in Kellman's work, they appear to range from 6 to 24 s with acquisition rates of 2 and 4 Hz and resolving powers of 50,000 and 25,000, respectively. As a reference, a resolution of 50,000 can distinguish ions differing by about 50 ppm (e.g., 292.0403 and 292.0266 amu, $\Delta\text{ppm} = 47$. *Note*: see Section 2.1.3 for ppm definition). In addition to resolution, one must

consider the molecular mass being measured and the chromatographic speed. The acquisition rates and peak widths noted earlier are far too slow and wide for most GC analyses.

An argument is made in subsequent sections that the relevant small molecule chemical space is predominantly in the mass range of 500 amu or less, and it is not uncommon for capillary GC to generate chromatographic peaks widths on the order of 1 s at the baseline. With the comments by Balogh and Kellman and the above principles in mind, it appears that to sacrifice sensitivity and acquisition speed for resolution is not tenable for fast GC/TOF (Q-TOF).These instruments will require acquisition rates of 5–10 Hz for 1 s chromatographic peak widths. Moreover, a resolution of 10,000 can distinguish masses differing by about 200 ppm or more in the mass range of 500 amu or less, and this should be adequate for most small molecule analyses while still maintaining good mass accuracy. Still, there will be occasions where higher resolution will be required. For example, to distinguish ions differing by about 100 ppm, mass resolution will need to be about 20,000 and as noted earlier, for about 50 ppm mass differences a resolution of 50,000 is required.

2.1.3 Mass Accuracy

Mass accuracy refers to the experimental error in the mass assignment compared to the true mass being measured. It is typically reported in parts per million (ppm) and is defined in Equation (3):

$$\Delta\text{ppm} = \left(\frac{\text{true}_{\text{amu}} - \text{observed}_{\text{amu}}}{\text{true}_{\text{amu}}}\right) 10^6 \tag{3}$$

As mass accuracy increases, so does the confidence of the molecular identification as it reduces the number of possible molecular formulae. Mass resolution works in conjunction with mass accuracy and enables narrow extracted ion windows at the expected mass. It should be pointed out that without high mass accuracy, high resolution is virtually meaningless.

An example of how mass accuracy and resolution can be used for the identification and quantification of analytes in heavy matrix is the analysis of fluorotelomer alcohols (FTOH) in biosolids from waste water treatment plants. These compounds are polyfluorinated alkanes with the generic formula $CF_3(CF_2)_{N-1}(CH_2)_M OH$ and have the shorthand notation N:M FTOH, where N refers to the perfluorinated alkyl segment and M refers the nonfluorinated alcohol segment of the carbon backbone. Figure 1 illustrates the extracted ion chromatogram (EIC) and the extracted mass spectrum for three replicate injections of 11:1 FTOH spiked into biosolids from waste water treatment plants at 50 pg/g. The data were collected in positive chemical ionization mode (methane, 1 mL/min) at 10 Hz and a chromatographic peak width of 2.1 s. Table 1 illustrates the uncorrected mass accuracy and resolution for the three replicate injections.

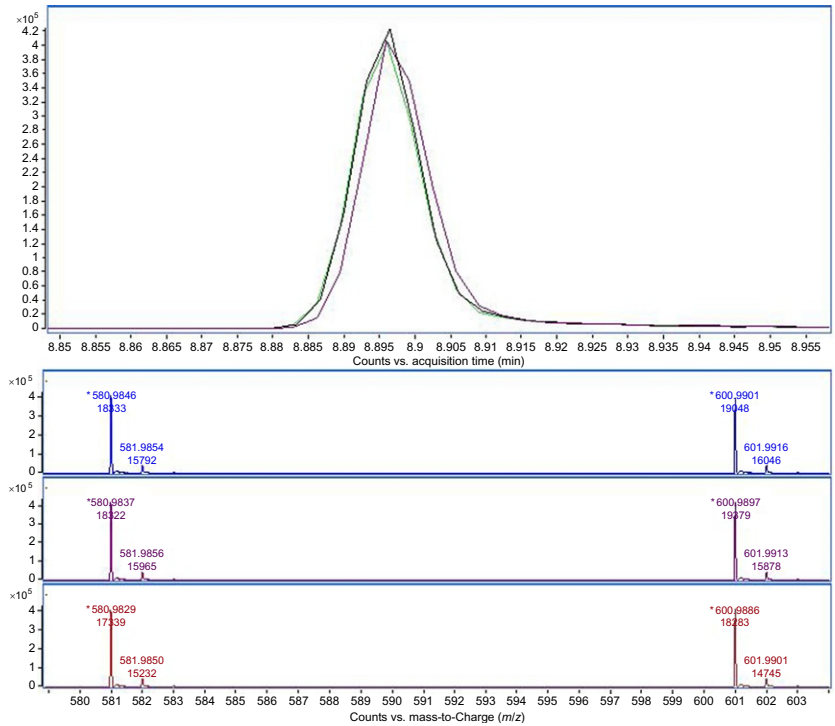

FIGURE 1 Chromatogram and extracted mass spectrum for 11:1 FTOH [5].

TABLE 1 Uncorrected Mass Accuracy and Resolution for Three Replicate Injections of 11:1 FTOH in Biosolid Matrix

Compound Name	Replicate	Observed amu	Δppm	Resolution
11:1 FTOH	1	600.9901	1.93	19,048
11:1 FTOH	2	600.9897	1.26	19,379
11:1 FTOH	3	600.9886	0.57	18,283

The absolute mass accuracy in the absence of a lock or reference mass is consistently better than 2 ppm, and the average mass resolution is 18,903.
Data taken from Ref. [5].

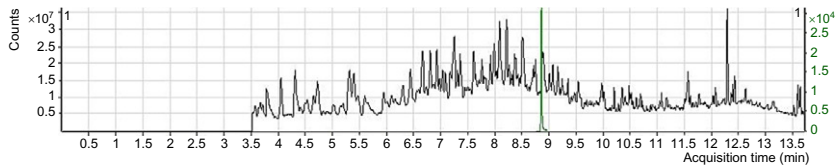

FIGURE 2 TIC of FTOH analysis.

Figure 1 also illustrates a molecular fragment ion with an average mass of 580.9837 amu for the three replicate injections. Insertion of this value into an accurate mass molecular formula calculator results in an empirical formula of $C_{12}H_2F_{22}O$. This differs from the parent empirical formula by a loss of HF with an absolute uncorrected mass error of only 1.7 ppm.

Figure 2 provides a frame of reference of how high mass resolution and mass accuracy work concomitantly to identify and quantify analytes in heavy matrix. Therein, the total ion current (TIC) is on the order of 10^7 counts over the range of 3.5–13.5 min while the analyte, 11:1 FTOH eluting at approximately 8.9 min, is three orders of magnitude less intense. Note the *y*-axes on the left (TIC) and on the right (11:1 FTOH EIC). Without high mass accuracy and resolution, the analyst would be unable to extract the expected mass from the intense matrix background.

2.1.4 GC/Q-TOF

Even high mass resolution and accurate mass may not be enough to definitively identify a novel compound, especially if the relative abundance of an analyte is low enough that wider extraction windows are necessary and confirmation is not effective. In these cases, the ability to perform accurate mass product ion MS/MS experiments on a quadrupole time of flight (GC/Q-TOF) system can be implemented to provide structural identification of fragment ions for retrosynthetic reconstruction of the molecule.

The example given in Figures 1 and 2 illustrates a rather simplistic loss of HF from 11:1 FTOH. For all FTOH with $N=5$ through 11 and $M=1$, this loss was observed. However, for FTOH with $N=4$, 6, 8, or 10 and $M=2$, a nominal loss of 38 was observed. With a unit mass instrument such as a SQ or MS/MS system, one might deduce the loss is F_2 (37.9968 amu). However, with accurate mass and high resolution, it can be determined that the neutral loss mass was 38.0168 amu differing by more than 500 ppm from F_2. Figure 3 illustrates that the molecular fragment at 227.0104 amu for 4:2 FTOH is the result of a bimolecular loss of H_20 and HF, and there are two plausible molecular structures that may exist as a tautomer in the gas phase.

As another example, a measurement of pentakis(trimethylsilyl)-*O*-methyloxime-D-galactose in human serum using EI mode on a GC/Q-TOF system reveals that the spectrum is comprised of structural fragments of the parent molecule. Replicate injections using an internal lock mass generated an average of 319.1576 amu as the highest mass fragment. The most likely empirical formula for this mass is $C_{13}H_{31}O_3Si_3$. With this information, a plausible molecular structure can be seen in Figure 4.

The nominal ion at 319 amu can be used as a precursor in accurate mass MS/MS mode to validate the postulated structure. Figure 5 illustrates the product ion (MS/MS) spectrum resulting for the 319 amu precursor.

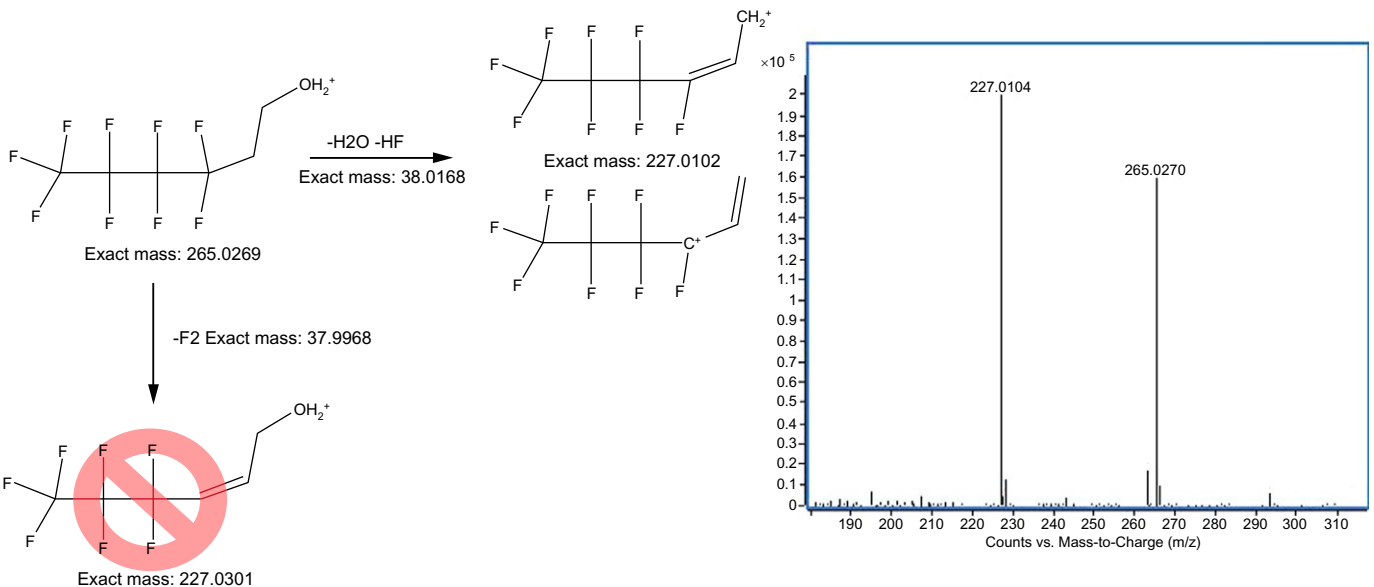

FIGURE 3 Molecular structure elucidation using accurate mass.

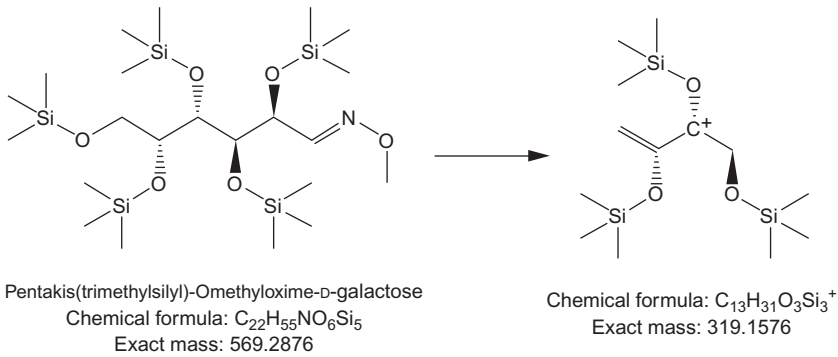

FIGURE 4 Plausible molecular structure for galactose fragment [6].

3 BIOLOGICALLY RELEVANT CHEMICAL SPACE

The universe of potential chemical entities or the total chemical space has been estimated at more than 10^{60} [7]. The predicted chemical space includes all potential molecules that can be created via known elements and chemical principles and spans a vast range of molecular weights and chemotypes. With the exception of chromosomes, proteins, and other macromolecules, most compounds in the known and measured chemical space have molecular weights less than 500 amu. A quick method to potentially validate this assumption can be done through filtering the NIST Standard Reference Database 1A [8] to reveal that of its 243,893 constituents, more than 96% have molecular weights less than 500 amu. Of course, many of the compounds in the NIST database may not have biological relevance. Nonetheless, this is a practical exercise because it reflects many years of analyses and cataloging of compounds across a host of disciplines not necessarily related to environmental chemistry. With this in mind, if one applies several rudimentary restrictions on the possible enumerations of the total chemical space to predict biological relevance of small molecules, the number of compounds in the resulting space is vastly smaller. For example, filtering by molecular weight less than 500 Da, restricting the atoms to C, H, N, O, S and applying basic chemical principles of covalent bonding would significantly reduce the number of viable compounds in the resulting region of chemical space to a more manageable number that can be realistically tested and measured.

Attempts to determine how many small molecules abide by known physicochemical rules and are stable and synthetically viable are made in the Chemical Universe Databases GDB-11, GDB-13, and GDB-17 [9–11] (GDB: Generated Data Base. The number represents the highest number of atoms in each molecule. Professor Dr. Jean-Louis Reymond personal communication, April 25, 2013). The largest of these is GDB-17 (17 or less atoms in each molecule) includes 166 billion unique entities and yet still only represents a small fraction

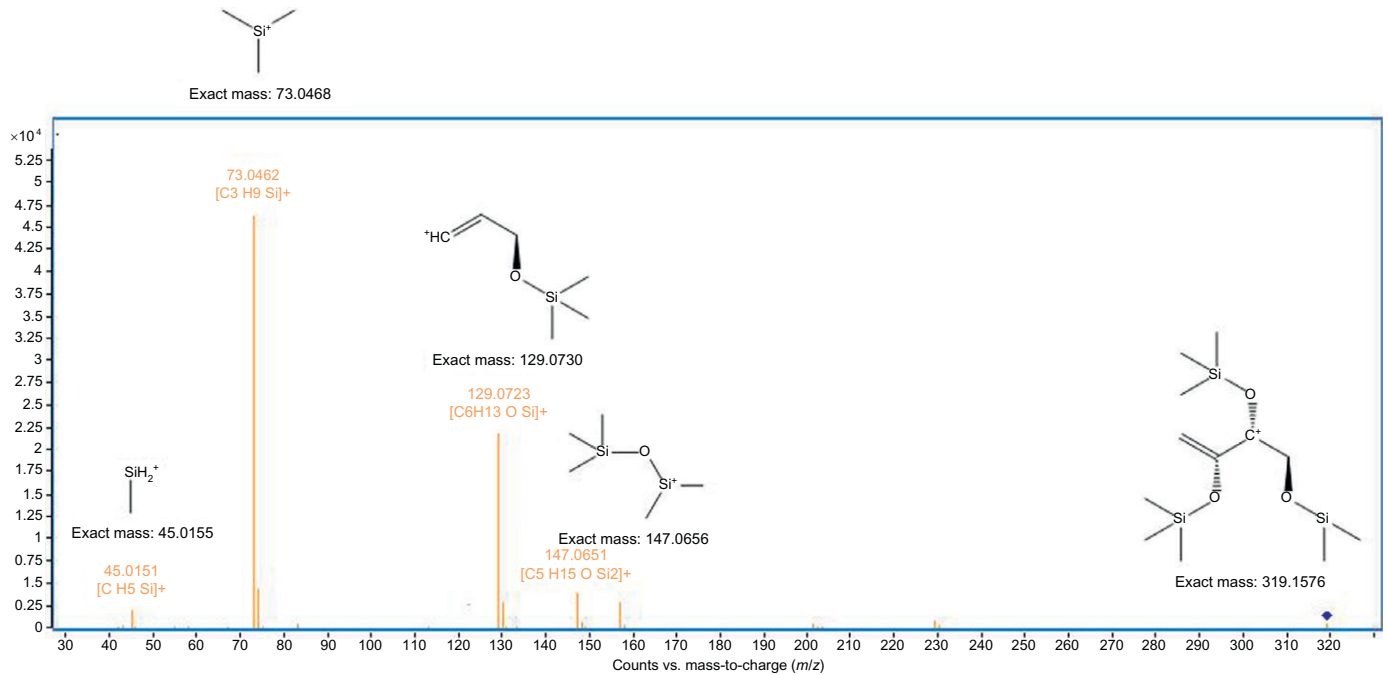

FIGURE 5 MS/MS product ion spectrum and structure elucidation for the nominal 319 fragment of derivatized galactose.

of the total chemical space. GDB-13 enumerates a virtual library of greater than 910 million compounds deemed to be drug-like based on descriptors defined by Lipinski [12] and Vieth [13]. Other databases with biological relevance include the Human Metabolome Database (HMDB) with approximately 8500 entries [14], Drug Bank with approximately 1000 entries [15], the Toxin and Toxin-Target Database (T3DB) with approximately 3100 entries [16], and Metlin: Metabolite and Tandem MS Database with more than 64,000 entries [17]. Thus, it appears that using current methodologies, the measurable space that is biologically relevant is quite manageable and those compounds that have not been identified can be done so through techniques such as GC/TOF (Q-TOF).

It stands to reason that there are many as of yet unidentified chemical entities that are affecting or will affect the environment and the health of its constituents. If one assumes that only drug-like compounds can modulate biological systems and pursues the biologically relevant chemical space with respect to these rudimentary rules, a vast portion of pertinent data will be overlooked. The reason for this is that many synthetic compounds derived from anthropogenic sources do not fit the traditional paradigm of a drug-like entity yet still have the ability to modulate *in vivo* chemical processes. In light of the reality that nondrug-like molecules can modulate a living system, other chemical entities not generally considered to have biological properties must be evaluated. These compounds may be found in other databases such as NIST 11 or the commercially combined Wiley 09/NIST 11 which contains more than 800,000 compounds [18] or may be true unknowns meaning that they have not been observed previously and do not exist in any known database. The latter represents the truly challenging nature of molecular

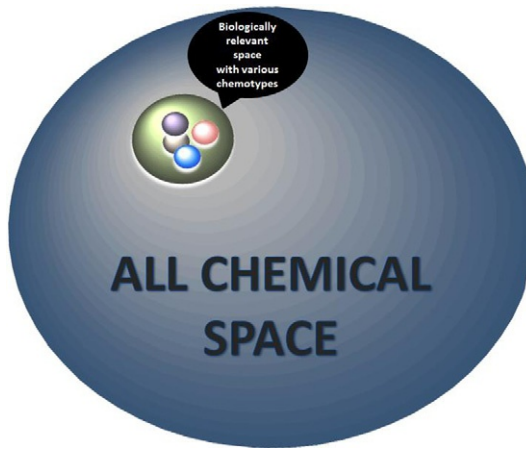

FIGURE 6 Illustration of chemical space.

identification and quantification and typically requires advanced technologies. Figure 6 is a representation of the total chemical space. The larger inset sphere represents the potential biologically relevant space, and the smaller subspheres represent various measureable chemotypes in the biologically relevant space. This illustrates that the total space needed to be measured is much smaller than the total chemical space.

Of the chemical space suspected to contain biologically relevant compounds, most can be readily measured using mass spectrometric techniques in either the gas or liquid phase and the optimum tool to use is Q-TOF in nature and, with respect to environmental analysis, more specifically GC/Q-TOF in nature.

4 THE EXPOSOME, EXPOSOMICS, AND THE FUTURE OF GC/Q-TOF

In 2011, Macherone and Nakayama [5] commented that the traditional approach to environmental monitoring is through literature search, newspaper articles, and whistle-blowing or simply by chance and generally follows this series of events:

This chemical may not be safe; let's test it on laboratory animals, cell lines, or other assays; yes, this one is bad; need to test the environment and measure exposure to the chemical; risk management; OK, it's toxic and it's there. We've got a risk. We need to handle it.

The problem with this approach is that the list of known contaminants is far shorter than that of unknowns, and it will never catch up with industry and the hundreds of thousands of chemicals on the market. Realistically, chemicals are not solitary in the environment but exist as a mixture of chemicals that may or may not interact with one another in a vast array of matrices. One needs to apply sophisticated nontargeted (agnostic) analytical methodologies using tools such as GC/Q-TOF to measure and confirm the total array of compounds presented in the total array of environmental and biological samples. The measurement of all environmental compounds and correlating biological exposure to these compounds to their effect on health has only recently been described as the exposome and exposomics.

4.1 The Exposome

CP Wild [19] defined the exposome as the sum of all exogenous environmental exposures over a complete lifetime. The United States Centers for Disease Control and Prevention (CDC) offers the following definition [20]:

Success in mapping the human genome has fostered the complementary concept of the "exposome". The exposome can be defined as the measure of all the exposures of an individual in a lifetime and how those exposures relate to health. An individual's exposure begins before birth and includes insults from environmental and occupational sources. Understanding how exposures from our environment, diet, lifestyle, etc. interact with our own unique characteristics such as genetics, physiology, and epigenetics impact our health is how the exposome will be articulated.

4.2 Exposomics

Wild [19] questioned using "omics" tools for measurement of the exposome. It is multidisciplinary and integrates the analytical procedures within those disciplines. Exposomics will require academia, industry and government to engage in a freely open, scientific milieu. The purpose of exposomics is to incorporate environmental health sciences into genetic research and determine the etiology of disease.

4.3 GC/TOF and Exposomics

Although there is a vast understanding of the genome, the influence of the environment and the exposome on the genotype is still poorly understood. Quite frankly, more is known about genetics and genotyping then is known about how the exposome influences the genome [19]. The role of small molecules in genetic perturbation *and* as biomarkers and induction factors for disease needs to be determined. For example, using *in vitro* DNA micro-arrays, endocrine disruption by 17β-estradiol was determined to alter genetic expression for similar genes [21]. 17β-estradiol has also been monitored in water sources as a persistent environmental contaminate and in human serum as a biomarker for breast cancer [22, 23]. This example illustrates how a single compound or a class of compounds can be measured from a genetic, an environmental and biomarker perspective and offers evidence of the synergy between the genome, the metabolome, and the exposome.

There is a general consensus among researchers that the majority of disease results from the interaction of the exposome and the genome and note that diseases such as cancer, heart disease, obesity, and type II diabetes among others are all influenced by the environment [24]. The mechanisms of disease modulation by small molecules are diverse and include but are not limited to genetic damage, transcriptase interferences, RNA damage, protein anomalies, enzyme inhibition, and endocrine disruption. Many small molecules found in the environment have been determined to have an impact on one or more of these systems in one way or another.

Evaluating the exposome via exposomics is analogous to Genome-Wide Associated Studies in that the study of the exposome is the comparative

search for unique markers or signals resulting from exposure [25]. The approach to measuring the exposome should implement global discovery methodologies that begin agnostically to simultaneously measure multiple classes of small molecules using an Exposome-Wide Association Study model and become more knowledge driven as data are collected and defined [26]. To be successful, exposomics will require tools such as GC/Q-TOF in conjunction with others like LC methodologies. The compounds that need to be examined include but are not limited to POPs, endocrine disruptors, carcinogens, and teratogens found in the air, land, water, and biological matrices. These environmental compounds are absorbed, digested, metabolized, and excreted by the indigenous inhabitants and are exhibiting a negative impact on the quality of life across the globe. It is for these reasons that exposure and its effect on the ecosystem and human health should be examined within the exposomics paradigm.

In Refs. [19] and [25], Wild noted that if exposure–disease (dose–response) relationships are to be made, identification and validation of biomarkers and integration of these into environmental monitoring are the seminal features of exposomics. Biomarkers are small molecules arising from metabolic processes and the exposome and should be subclassified into markers of exposure and markers of disease recognizing that these are not always one and the same. Some are polar and drug-like while others are polycyclic and lipophilic and most have molecular weights below 500 amu. These small molecules either modulate biological processes or are the result of them. The sum of all biomarkers resulting from metabolic processes is referred to as the metabolome and metabolomics is the application of omics tools to measure these compounds. Metabolomics is a comparative paradigm that contrasts populations: on drug versus off drug, disease versus healthy, knockout versus wild type, etc., and provides an excellent model for monitoring the exposome via exposomics.

Currently, there are three primary technologies for metabolomics: NMR, LC/MS, and GC/MS. Each of these techniques provides orthogonally corroborative and complimentary data about the specimens being examined and each has its own analytical space and limits of detection. NMR can measure a broad range of chemotypes but is the least sensitive of the three and, in the absence of chromatography, cannot fully evaluate complex mixtures. GC/MS covers an even broader range of chemotypes and is generally the technique of choice when measuring organic acids, fatty acids, amino acids, sugars, and steroids among other chemical classes. LC/MS using electrospray ionization can arguably measure the broadest range of drug-like and biological chemotypes and can identify thousands of "features" in a typical serum sample.

Figure 7 illustrates the relative analytical space for NMR, GC/MS, and LC/MS and the estimated limits of detection (x-axis), and the typical number of metabolites identified (y-axis) by each method taken from a recent manuscript [27]. It should be noted that the author does not differentiate modes

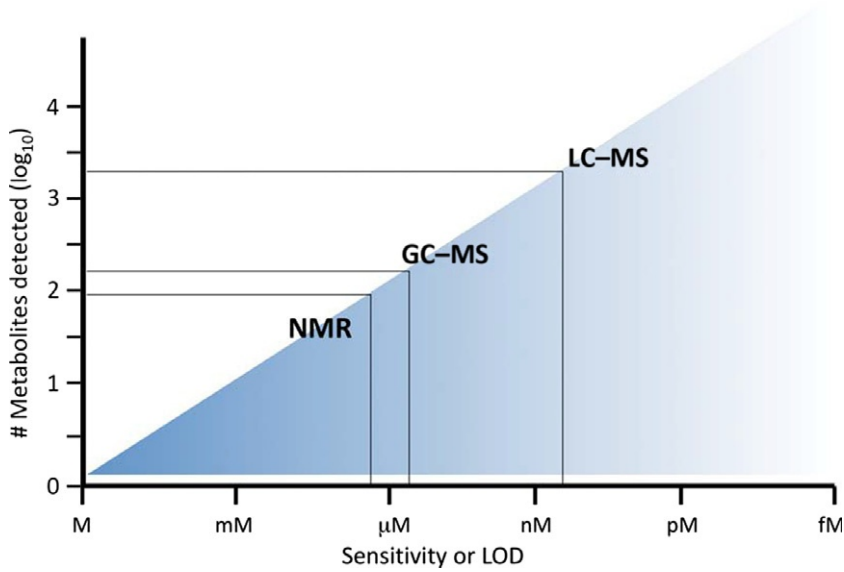

FIGURE 7 Estimated limits of detection (*x*-axis) and the typical number of metabolites identified (*y*-axis) by NMR, GC/MS, and LC/MS techniques. *Reprinted with the permission of Cambridge University Press and David S. Wishart.*

TABLE 2 Human Serum Metabolite Identification by LC/TOF in ESI Positive and Negatives Modes and by GC/TOF in EI Mode [28].

	LC/TOF (ESI+)	LC/TOF (ESI−)	GC/TOF (EI) RTL[a] Fiehn.L	GC/TOF (EI) NIST11.L
Total features	4403	2867	312	312
Annotated by database	602	541	30	68
Annotated by formula	567	336	43	22

As noted in Figure 7, LC/TOF using electrospray (ESI) elicits more than 23-fold more features than GC/TOF in EI mode. However, of the total features annotated by database and formula both methods have a 25–30% success rate in identifying compounds form the total number of features found.
[a]*RTL, retention time locked to myristic acid-d_{27}.*

of analysis (e.g., ESI± or EI/CI for LC/MS or GC/MS, respectively) and MS, MS/MS, or TOF are not distinctly contrasted. Table 2 illustrates a real example of LC/TOF and GC/TOF from a nontargeted metabolomics analysis wherein many more features are identified via LC/TOF versus GC/TOF. For completeness, it should be stated that the GC/MS experimental design incorporated sample derivatization using methoxyamine followed by

TABLE 3 Correlation of GC/TOF and LC/TOF Data for Acids, Phthalates, and Naphthylamine

Compound	Formula
2-Naphthylamine	$C_{10}H_9N$
Benzylbutylphthalate	$C_{19}H_{20}O_4$
Dioctylphthalate	$C_{24}H_{38}O_4$
Naphthalene dihydrodiol	$C_{10}H_{10}O_2$
Mono-octylphthlate	$C_{16}H_{22}O_4$
Docosanedioic acid	$C_{22}H_{42}O_4$
9,10-Dioxo-octadecanoic acid	$C_{18}H_{32}O_4$
3-Butyrl propionic acid	$C_7H_{12}O_3$
O-Linolenic acid	$C_{18}H_{30}O_2$
N-stearoyl glutamic acid	$C_{23}H_{43}NO_5$

All compounds in this table were observed by LC/TOF and GC/TOF
Confirmation of metabolites such as naphthalene dihydrodiol and mono-otcylphthalate and glucuronides by LC/TOF corroborates the observation of parent molecules in GC/TOF data and confirms exposure to these compounds is real and not artifacts of the system or sample preparation methodologies.
Data taken from Ref. [27].

N,*O*-bis(trimethylsilyl)trifluoroacetamide and this modification narrows the analysis space for the GC application. The LC/MS samples were not chemically modified. Table 3 demonstrates corroborative information for both the LC/TOF and the GC/TOF data wherein parent molecules and metabolites are observed in both datasets.

Tables 2 and 3 demonstrate the results from a global metabolomics screening paradigm using both GC/Q-TOF and LC/Q-TOF. This model illustrates the interdisciplinary approach that is postulated for exposomics. For example, the GC/Q-TOF can be used for simultaneous global screening of multiple chemical classes found in environmental samples. This is akin to current environmental methodologies but with the added benefit of targeted and nontargeted screening using high resolution, high mass accuracy for extraction of knowns and unknowns out of heavy matrix, and MS/MS for structure elucidation and confirmation. This information could be used for chemical cartography to map geographical regions with contaminate information. GC/Q-TOF and LC/Q-TOF methodologies could then be designed to monitor the population exposome from inhabitants of the mapped regions. The data can then be correlated to public health information for epidemiological studies and determination of disease-exposure and genome-environment associations. The

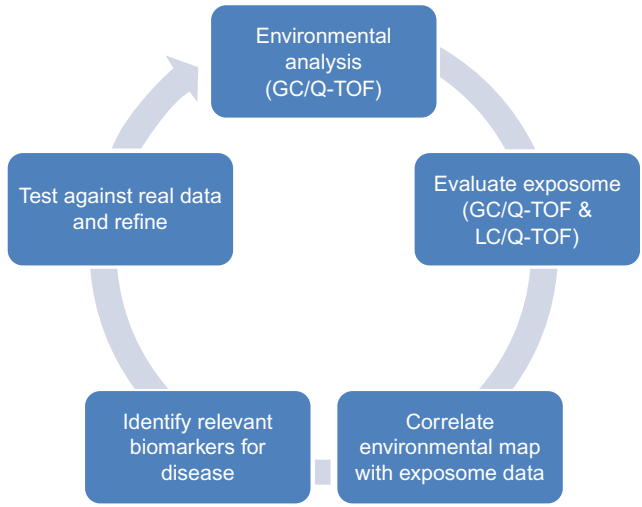

FIGURE 8 Composite loop of bottom up and top down exposomics to identify biomarkers.

experimental design can be refined with each iteration of this loop (environmental exposure, exposome measurement, epidemiology, and correlation with disease) to determine better and better biomarkers for various disease states. Figure 8 illustrates this model as a composite loop of bottom up and top down exposomics that combines the features of both to generate lead biomarkers and refine experimental design. The cycle typically begins agnostically and becomes more knowledge driven with each iteration.

5 SUMMARY

The future of GC/Q-TOF may very well be as a central tool in exposomics. The sections herein are meant to build a picture of why GC/Q-TOF should be a tool of choice to measure the biologically relevant chemical space in the environment and the exposome. GC/Q-TOF offers the ability to perform targeted analysis of any chemotype in any matrix. It further offers hybrid techniques targeting specific compounds with subsequent mining of the same data for nontargeted compounds in the sample set. Because GC/MS has such a strong history in environmental analysis, current methods are easily ported to GC/Q-TOF resulting in robust exposure assessment methodologies to accurately quantitate currently identified POPs and biomarkers and differentiate markers of exposure and disease.

The interdisciplinary exposomics model given in Section 4 illustrates the tandem application of GC/Q-TOF and other tools and researchers working

together in a cycle that combines a bottom up and top down approach. This approach will require metabolomics, toxicology, ADME, epidemiology, food safety, biostatisticians among others, and very large cohort studies over long terms. The goal of these investigations is to develop an understanding of how exposure influences disease and the genome environment relationship. Questions that exposomics needs to answer include: can exposomics determine a relationship between past exposures and current disease, determine which exposures are safe, deal with multiple exposures at the community level, and integrate cumulative exposures to assess health risk [25].

Monitoring the exposome may seem like an insurmountable endeavor but consider that less than two decades ago, the expected human genome was on the order of 100,000 genes with >10 million single nucleotide polymorphisms (SNP), and the human genome project ultimately determined the genome to be on the order of 30,000–40,000 genes and perhaps only 1–2 million SNPs [29]. Contrast this with the size of the human metabolome which is anticipated to be on the order of 200,000 or more entitles of which at least 64,000 have already been identified. The number of environmental compounds with biological activity may also be on the order of hundreds of thousands and again, many of these have already been identified and agencies such as the WHO, the Stockholm convention, and other national entities are consistently identifying new candidates every year. Even with the expected size and length of exposome studies, mega-cohorts are already funded or in the planning stages [19], and it can be predicted that GC/Q-TOF will be a primary tool in these efforts.

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