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REVIEW

Ognjen Panić · Tadeusz Górecki

Comprehensive two-dimensional gas chromatography (GC×GC) in environmental analysis and monitoring

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Abstract Compared to conventional one-dimensional gas chromatography (1D-GC), comprehensive two-dimensional gas chromatography (GC×GC) offers increased peak capacity, improved resolution and enhanced mass sensitivity. In addition, it generates structured two-dimensional (2-D) chromatograms, which aids in the identification of compound classes. Sample preparation procedures can often be minimized, or even eliminated in some cases, due to the superior separating power offered by the technique. All of these advantages make GC×GC a very powerful tool in environmental analysis involving the determination of trace levels of toxic compounds in complex matrices. This review paper summarizes and examines the historical and recent GC×GC applications in environmental analysis and monitoring.

Keywords Comprehensive two-dimensional gas chromatography (GC×GC) · Environmental analysis · PCBs/PCDDs/PCDFs · Pesticides · Air analysis

Introduction

Many years of industrialization and urbanization have resulted in the worldwide distribution of numerous chemicals throughout the atmosphere, hydrosphere and lithosphere. Many of these compounds can be hazardous to the world's ecosystems and to humans. Environmental analytical chemists have the task of analyzing these compounds in the environment. Whenever the analytes have reasonably high vapor pressures, gas chromatography is the method of choice.

The main challenge in environmental analysis is that the analytes are usually present in trace amounts in very complex matrices. As a result, tremendous research effort goes into the analysis of major environmental pollutants, including polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), organochlorines, pesticides and endocrine disruptors [1]. More information on the analysis of such compounds can be found in literature reviews published elsewhere, e.g., [1, 2].

The approach utilized in environmental analysis is generally the same as in any analytical procedure. It consists of sampling, sample preparation, separation and detection. All of these steps could benefit from improvements, yet it is usually the separation step that imposes the biggest limitations. In the case of gas chromatography (GC), most environmental samples contain so many closely eluting peaks of analytes and matrix components that altogether the peak capacity in one chromatographic dimension (1D) is greatly exceeded, and numerous coelutions and/or entire unresolved regions are observed during the separation. This leads to poor identification and quantification of the analytes of interest [1].

Poor chromatographic resolution places high demands on both sample preparation and detection instrumentation. Sample preparation can be expensive and labor-intensive, and can generate large amounts of environmentally harmful solvent waste. The development of microextraction approaches, such as liquid–liquid microextraction (LLME) and solid-phase microextraction (SPME), and solvent-free sample introduction systems (e.g., direct thermal desorption), has the potential to vastly simplify the sample preparation process without sacrificing the sensitivity and/or selectivity [3–6]. With respect to detection, insufficient GC resolution usually means that the use of mass spectrometry (MS), including high-resolution mass spectrometry (HRMS) in some cases, is mandatory. Figure 1 illustrates the common problem encountered in GC–MS [52]. In a 1D-GC analysis of a food extract spiked with pesticides (Fig. 1b), trace amounts of the analytes of interest (in this case chlorfenvinphos) commonly coelute with more abundant components of the sample matrix. As a result, the mass spectra obtained for such compounds (Fig. 1e) frequently

O. Panić · T. Górecki (✉)
Department of Chemistry, University of Waterloo,
200 University Ave. W.,
Waterloo, ON, N2L 3G1, Canada
e-mail: tgorecki@uwaterloo.ca
Tel.: +1-519-888-4567
Fax: +1-519-746-0435

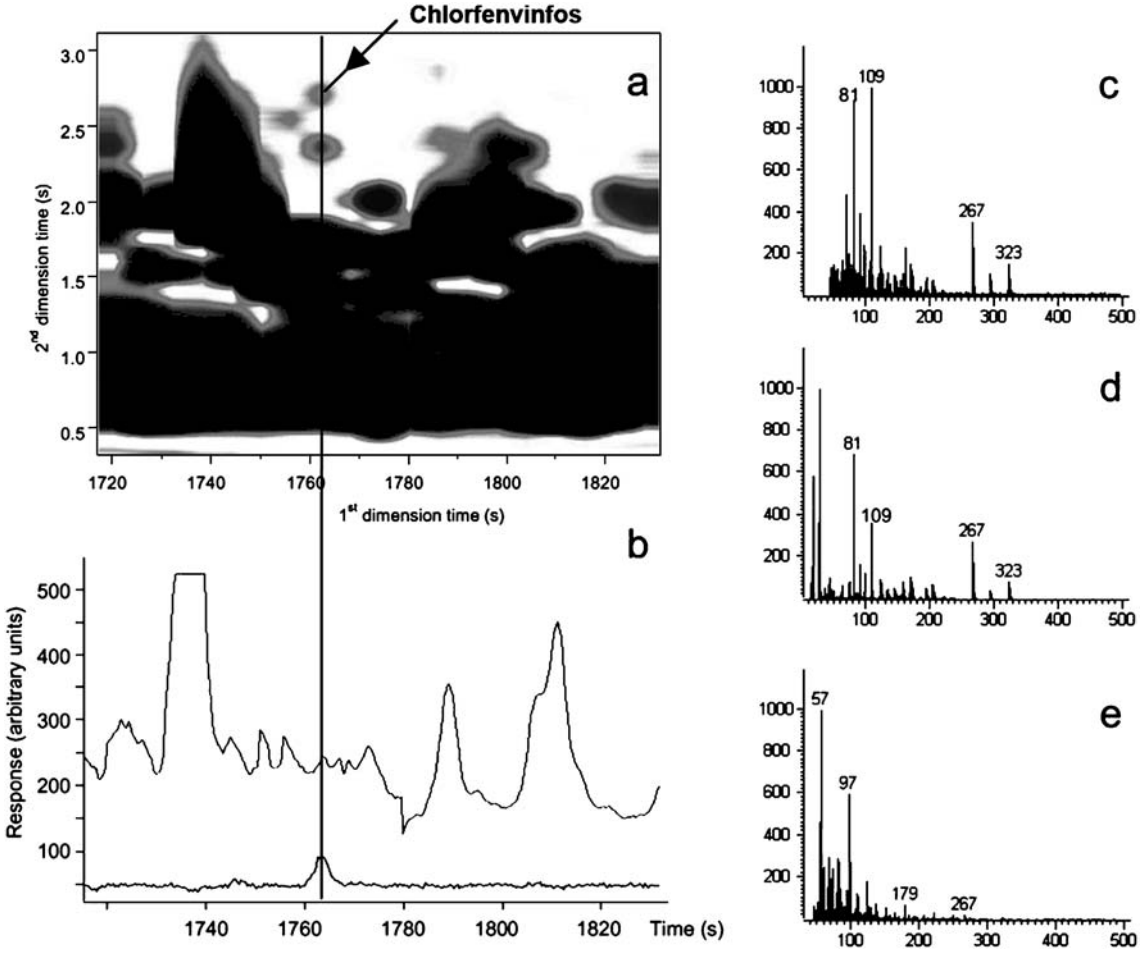

Fig. 1a–e GC×GC–TOF MS versus 1D-GC–TOF MS for the analysis of a carrot extract. **a** GC×GC–TOF MS contour plot; **b** 1D-GC–TOF MS chromatogram of the same region: *upper trace*, TIC scaled to 1%; *lower trace*, *m/z* 323 ion trace; **c** Mass spectrum obtained after GC×GC separation showing the characteristic *m/z* values of chlorfenvinphos (*m/z* 81, 109, 267, 295, 323). **d** Library spectrum of chlorfenvinphos and **e** spectrum obtained at the retention time of chlorfenvinphos after 1D-GC separation. Reprinted with permission from [52]

contain fragments originating from the interfering compounds, causing poor matching with the library mass spectra (Fig. 1d). MS deconvolution algorithms can significantly improve the quality of spectral information for coeluting peaks, but they are not always successful when the number of coelutions is high. As shown in Fig. 1a, comprehensive 2D-GC (GC×GC) increases the separation space and improves the chromatographic resolution, leading to separation of the analyte of interest (chlorfenvinphos) from the coeluting compounds and/or matrix components. As a result, the quality of the mass spectra of the analytes is improved (Fig. 1c), allowing for more confident analyte identification (Fig. 1d). It is still possible that some coelutions will be present; these can usually be efficiently resolved with MS deconvolution, which produces better results when the number of coeluting components is reduced. The increased separation power of GC×GC thus leads to successful analyte identification and/or quantification.

Historically, the peak capacity problem in conventional gas chromatography was dealt with through the implementation of multidimensional gas chromatography (MDGC). In this method, also referred to as "heart-cutting," a complex and unresolved portion of a one-dimensional (1D) chromatogram is subjected to additional separation on a second column coated with a stationary phase of different selectivity [1]. Although this approach does increase the resolution of the selected portion(s) of the 1D chromatogram, automation of this method is challenging, and only some sample components can be fully resolved. Nevertheless, numerous applications dealing with the analysis of PCBs, pesticides and toxaphene, among others, have been reported with varying degrees of success [7–13]. Overall, however, it is clear that many separations would benefit if the entire sample was subjected to separation in two dimensions. This became possible with the introduction of comprehensive two-dimensional gas chromatography (GC×GC).

Comprehensive two-dimensional gas chromatography (GC×GC)

Comprehensive 2D-GC is the ultimate solution to the peak capacity problem encountered in 1D-GC. A block diagram

of a typical GC×GC set-up is illustrated in Fig. 2. Most instrumental components utilized in GC×GC are in fact the same as in 1D-GC. These include the injector, the oven, the columns and the detectors. In a typical GC×GC system, a long column coated with a thick film of a nonpolar stationary phase is installed as the primary column. Its outlet is connected through a special interface, or modulator, to the inlet of a second dimension column, coated with a stationary phase of different selectivity. The modulator not only physically connects the primary and the secondary columns; its main role is to repeatedly trap the components of the effluent from the first dimension and periodically inject them in the form of narrow pulses into the secondary column for further chromatographic separation. Since the second dimension operates under fast GC conditions, detector choices in GC×GC are limited to those capable of fast data acquisition rates. Examples of detectors that were found suitable for GC×GC include a flame ionization detector (FID), an electron capture detector (ECD), an atomic emission detector (AED), a sulfur chemiluminescence detector (SCD), a nitrogen chemiluminescence detector (NCD), and a time-of-flight mass spectrometer (TOF MS). Numerous reviews dealing with GC×GC instrumentation have been published, and readers interested in this topic should consult them [14–16].

The modulator is the heart of the instrument because it ensures that the separation is both comprehensive (the entire sample is subjected to both separation dimensions) and multidimensional (separation accomplished in one dimension is not lost in the other dimension) [17]. Since the first practical implementation of GC×GC in 1991, the field has witnessed numerous modulator designs [18]. Initially, thermal modulators utilizing heating to modulate were implemented; however, modulation with cryogenic liquids (liquid CO_2 or N_2) is presently predominant. Within the cryogenic modulator family, each design has its own distinct advantages and limitations, making it suitable for specific types of analyses. For example, Beens et al. developed an interface for the analysis of surface water contaminants [23], while Hyötyläinen et al. described the

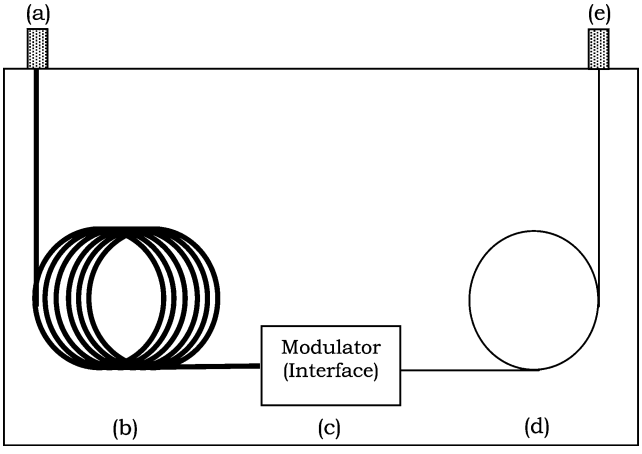

Fig. 2 A block diagram of a GC×GC system. **a** injector; **b** primary column; **c** modulator or interface; **d** secondary column; and **e** detector

construction and application of an in-house modulator for the quantitative analysis of PAHs and PCBs [19]. Shortly thereafter, Kristenson et al. evaluated different types of modulators in the analysis of organohalogenated analytes [20]. More information about the evolution of GC×GC interfaces in general can be found in various review papers (e.g., by Górecki et al. [15]).

The implementation of GC×GC offers the following advantages over 1D separation methods: enhanced separation power; improved mass sensitivity (observed only with thermal modulators due to the focusing effect); and structured, or highly ordered, chromatograms. In environmental analysis, GC×GC has the potential to improve separation of the toxic compounds from the coeluting analytes and matrix components, to increase the detection limits of such chemicals, and to provide structured two-dimensional chromatograms ideal for monitoring applications. Consequently, this can lead to minimized sample preparation procedures, and hence decreased analysis time. Other applications are also possible. For example, most recently, GC×GC has been used for the estimation of environmental partitioning properties of diesel fuel hydrocarbons, which are important in oil spills affecting many ecosystems [21]. This review does not intend to cover all applications of GC×GC in environmental analysis. The potential of the technique will be illustrated instead through selected representative examples.

GC×GC in environmental analysis and monitoring

Water and sediment analysis

Drinking water is the most essential substance to life on this planet. In order to assess the safety of potable water for humans, there is a need for rapid, precise and accurate methods for its monitoring and analysis. River and lake sediments also play an important role in the health of aquatic ecosystems; therefore, they must be analyzed as well. Typical methods for the analysis of water pollutants include time-consuming sample preparation, followed by GC–MS analysis. It was realized early on that GC×GC has a great potential to improve the analysis of water and sediment pollutants.

In one of their earliest research papers in the area, Gaines et al. illustrated that GC×GC can fully separate the BTEX fraction (benzene, toluene, ethyl benzene and xylenes) and methyl *tert*-butyl ether (MTBE) from common matrix interferences in a single run when combined with SPME [22]. MTBE is an additive to gasoline and an indicator of an approaching gasoline plume in groundwater. Even after selective sample preparation methods, such as static headspace and purge-and-trap extraction, multiple injections onto 1D-GC of the water samples are required to resolve MTBE from common hydrocarbon coeluters [22]. However, due to the improved separation power of GC×GC, both MTBE and benzene were baseline-resolved in the 2-D chromatographic space. This study illustrated the great potential of GC×GC used in combination with a

microextraction technique (headspace SPME) for rapid identification and monitoring of aqueous pollutants.

Beens et al. investigated the effect of the type of GC×GC modulator on the separation of a mixture of 80 surface water contaminants [23]. The two modulators investigated were the thermal "sweeper" modulator (originally designed by Liu and Phillips [18]) and a cryogenic modulator constructed in-house. Although the separation utilizing both modulators was superior to that obtained with 1D-GC, modulation with cryogenic liquids (liquid CO_2) did not impose additional maximum oven temperature limitations, and was more efficient in trapping the more volatile analytes. This is particularly important in trace analysis of toxic water contaminants with high Henry's law constants.

Some of the Earth's freshwater bodies are subject to daily petroleum and oil contamination from numerous sources. As a result, there is a high demand for the quick and reliable analysis of water and sediments from the affected sites. In the 1970s, it was noticed that chromatograms of petroleum samples exhibited a characteristic, unresolved, raised baseline "hump" [24]. This complex part of the chromatogram, consisting of many different classes of compounds, is presently referred to as an "unresolved complex mixture" (UCM) [25]. Standard methods for the exploration of the chemical composition of the UCM depend primarily on sample clean-up procedures. For example, open-column silica gel chromatography, silver-impregnated silica gel chromatography and thin-layer chromatography (TLC) are utilized to fractionate the sample into specific classes of compounds [25]. However, even when such fractions are analyzed with GC–HRMS, the peak capacity in one chromatographic dimension is exceeded, leading to many coelutions, poor analyte identification, and costly analysis. Frysinger et al. used GC×GC–FID to resolve the UCM in two different freshwater sediments [25]. Adhering to conventional sample preparation procedures, the authors took advantage of the superior resolving power and structured chromatograms generated by GC×GC to study the different fractions of the sediment's UCM. The chromatograms obtained for both samples provided the researchers with clues pointing to the main source of contamination. More importantly, the research illustrated the potential of GC×GC in environmental forensics, an essential tool for environmental chemistry, environmental law and environmental audits.

In the 1990s, it was realized that surfactants such as nonylphenol (NP) isomers, degradation products of nonylphenol polyethoxylate, are possible estrogen disruptors [26]. Adding to the concern, NPs are presently found in water and sediments from urban areas [27]. Toxicological studies are limited due to the analytical inability to separate, identify and structurally elucidate the different NP isomers. For example, only 22 NP components could be identified by GC/tandem mass spectrometry combined with cluster analysis [28]. Moeder et al. [29] used GC×GC–TOF MS for the separation of NP isomers from a technical mixture. Forty-one components were identified. Figure 3 illustrates the application of GC×GC–TOF MS in the analysis of individual ion traces of NP isomers from the same study. Two NP fragmentation products are illustrated, *m/z* 135 (Fig. 3a) and *m/z* 149 (Fig. 3b). Both chromatograms exhibit group-type separations, emphasized by sloped lines connecting peak maxima of compounds within the same homologous family. It is evident from this figure that because of the structural similarity of various NP isomers, complete separation is very difficult. Nevertheless, the additional resolution power provided by GC×GC provided "cleaner" mass spectra, which made analyte identification much easier.

Ieda et al. analyzed NP isomers from a technical mixture and river samples (Japan) with GC×GC–MS [27]. A total of 102 NPs were separated from the technical mixture, allowing the structural identification of 13 isomers. In addition, two NP isomers were quantified in the water samples with minimal sample preparation. The authors predicted that purer technical mixtures and nuclear magnetic resonance (NMR) analysis of the eluting peaks could further enhance isomer characterization.

GC×GC was recently applied for the analysis of environmental pollutants present in marine sediments [30]. Morales-Munoz et al. developed a method for qualitative, fast and high-resolution analysis of complex samples based on dynamic ultrasound-assisted extraction (UAE) coupled to GC×GC–TOF MS. UAE offers a fast and efficient alternative to sample pretreatment procedures applied to solid samples [30]. Conventional 1D-GC lacks the resolving power to separate and identify all the components of marine sediment samples, especially those collected near urban wastewater treatment plants. A combination of an efficient and selective sample preparation method, UAE, with a powerful separation method, GC×GC, led to the resolution of 1500 compounds and identification of several polycyclic aromatic hydrocarbons (PAHs), NPs and dialkylated benzenes. Once again, the ability of GC×GC to not only separate analytes from each other but also from the sample matrix proved invaluable.

Suspected carcinogens and mutagens, PAHs are by-products of many industrial activities and are ubiquitously distributed in the environment. Their trace determination in sediment samples is difficult because it requires laborious and selective sample preparation. In an effort to improve trace analysis of PAHs in complex matrices, Cavagnino et al. utilized a large-volume splitless injection (LVSI) technique in conjunction with GC×GC–FID [31]. It should be noted that no analysis of actual sediment samples was reported in this study; however, the complexity of the samples analyzed was representative of many sediment samples obtained from rivers and lakes. Separation and detection of seven PAHs diluted in composite diesel fuel at low ppb levels demonstrated the potential of LVSI–GC×GC-FID as a powerful and rapid tool for the analysis of trace amounts of PAHs in complex matrices.

Around the same time, Ong et al. developed a method for rapid monitoring of PAHs in soil samples, utilizing pressurized liquid extraction (PLE)–GC×GC–FID [32]. The study compared qualitative, quantitative and reproducibility aspects of GC–MS and GC×GC. The currently published work is merely an indicator of the potential

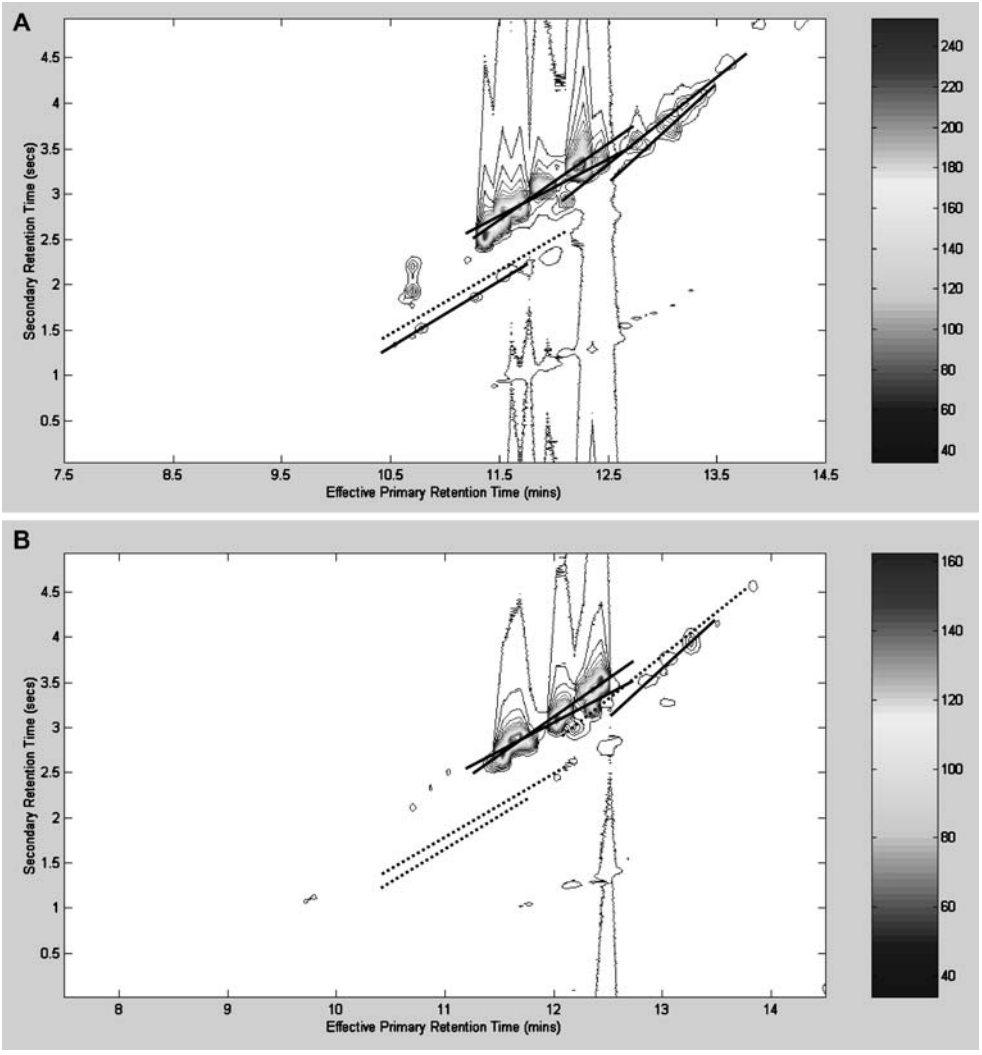

Fig. 3a, b Extracted ion GC×GC–TOF MS chromatograms of a technical nonylphenyl (NP) mixture; **a** *m/z* 135; **b** *m/z* 149. The sloping lines connect peak maxima for a homologous series (i.e. m/z 135 and m/z 149). Both chromatograms illustrate 6 different lines (solid and dashed), indicating the presence of 6 different families of NP isomers

applicability of GC×GC in the analysis of PAH in sediment samples.

Overall, with improved resolution, enhanced mass sensitivity and ordered chromatograms, GC×GC can be coupled with effective and rapid sample preparation methods to yield results unachievable by conventional analytical procedures.

Analysis of PCBs, PCDDs and PCDFs

Polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and some polychlorinated biphenyl (PCB) congeners are subject to bioaccumulation and biomagnification in the environment and are thus dangerous to wildlife and humans. Many of them are suspected carcinogens and mutagens [33]. It should not then be surprising that a tremendous amount of research has been conducted on these compounds, not only by toxicologists but also by chromatographers. Confident assessment of PCBs, dioxins and furans in the environment requires a method capable of isolating and quantifying them in complex samples such as foods, soil and water.

PCBs exist as a mixture of 209 congeners, differing in the number of chlorine substituents and their substitution pattern on the benzene rings [33]. Although not all congeners are hazardous, the toxic ones are present in trace amounts and frequently coelute with the more abundant and "less-dangerous" congeners in 1D-GC. In an effort to create a reliable method for PCB toxicity assessment, the World Health Organization (WHO) has identified 12 non- and mono-*ortho* PCB congeners (77, 81, 105, 114, 118, 123, 126, 156, 157, 169 and 189) as indicators of toxicity, due to their dioxin-like chemical behavior [34]. Similarly, the European Union (EU) has chosen seven PCB congeners (28, 52, 101, 118, 138, 153, and 180) to act as markers of harmful contamination [35]. Thus, depending on the analysis regulations and standard operating procedures (SOPs) followed, the separation method applied must be able to identify and quantify at least the specific set of PCB congeners.

Presently, standard methods for the analysis of PCBs, PCDDs and PCDFs commonly require liquid chromatography (LC) pretreatment in order to eliminate interferences from matrix components [1]. Following this, most samples require multiple gas chromatography–mass spectrometry

(GC–MS) analyses for the identification of the specific groups of toxic congeners. In fact, the most efficient columns can only separate seven out of the 12 WHO-recommended PCB congeners [1]. Additionally, in the presence of dioxins and furans, HRMS is required for confident analyte identification and quantification. Taken as a whole, the entire process is expensive, time-consuming and labor-intensive.

GC×GC offers an alternative and advantageous approach to the analysis of PCBs, PCDDs and PCDFs in complex matrices. In one of their early experiments, Haglund et al. used a liquid crystal primary column (separation based on planarity) and a nonpolar secondary column (separation based on vapor pressure) to separate mono - and non-*ortho* PCB congeners from a technical mixture [36]. The group was successful in baseline separation of the 12 marker PCBs and the seven EU indicator PCBs from a technical mixture in 15 minutes; however, they did not obtain highly structured chromatograms due to long secondary retention times and the resultant numerous "wraparound" peaks. Korytár et al. applied GC×GC linked to microelectron-capture detection (μECD) for the determination of toxic PCBs, PCDDs and PCDFs in cod liver samples [34]. The results of the analysis illustrated full separation and identification of all 12 priority PCB congeners as well as the most toxic dioxins and furans from liver samples spiked with 90 PCBs and 17 toxic PCDDs and PCDFs. Additionally, when compared to standard sample preparation procedures, the liver sample pretreatment was nonselective and minimized. It consisted of cell lysis, centrifugation and fractionation followed by direct injection into the GC×GC system. Figure 4 illustrates the 2-D chromatogram obtained from the analysis of the cod liver sample. In addition to fully resolving compounds that would coelute in 1D chromatographic space, highly ordered chromatograms were generated, which helped when classifying the PCBs into homologous groups based on the number of chlorine substituents. This is ideal for monitoring applications, as the analyst can make a rapid identification of the specific PCB based on two retention times. Most recently, Danielsson et al. performed a multilaboratory study, analyzing PCDD/Fs and WHO PCBs in food samples by comparing GC×GC–μECD with GC–HRMS, and illustrating once again the great potential of GC×GC in rapid monitoring applications [37].

In an effort to compare GC×GC to standard methods of analysis, Focant et al. evaluated the performance of GC×GC coupled to ^{13}C-label isotope dilution (ID) TOF MS (GC×GC–ID-TOF MS) with conventional GC–HRMS [38]. Quantification of 17 PCDD/Fs and four PCBs spiked onto soil and sediment samples was comparable for both methods. However, GC×GC implementation required only minimal sample preparation and resulted in signal enhancement (factor of 5–10), superior resolution, lower instrumentation costs, and improved spectral deconvolution of the TOF MS data [38]. As a consequence of the increased peak capacity and resolution, identification of unknown compounds was possible. In addition to dioxins, furans and PCBs, the following compounds were identified during the analysis: polychlorinated naphthalenes (PCNs), PAHs, polybrominated diphenyl ethers (PBDEs), phenols and phthalates. Following this, Focant et al. utilized GC×GC–ID-TOF MS for the separation and quantification of seven toxic PCDDs, ten toxic PCDFs and 18 toxic PCBs in food samples such as pork, fish and milk [39]. Quantification, quality of analysis and costs (instrumental and operational) were evaluated for GC×GC–ID-TOF MS, GC–HRMS, and GC–ID-tandem-in-time quadrupole ion storage mass spectrometry (QUIST–MS/MS). The authors stated that data processing times were limiting the speed of analysis in GC×GC–TOF MS, yet they also reported that the method could be considered complimentary to GC–HRMS, rather than being just a screening method. Fortunately, as data processing times are decreasing rapidly with software and hardware improvements, the GC×GC–TOF MS should soon become a cheaper alternative not only for screening, but also for quantitative determination of PCBs, PCDFs and PCDDs in the environment.

In addition to monitoring environmental samples for toxic PCB markers, there has recently been a growing interest in the application of GC×GC for the determination of chiral PCBs. Out of the 209 PCB congeners, 19 tri- and tetra-*ortho* congeners exist as atropisomers at room temperature; these chiral PCB congeners are released into the environment as racemic mixtures [40, 41]. Confident determination of nonracemic chiral PCBs distribution in animal tissues and food extracts could provide crucial information relevant to biotransformations and selective bioaccumulation [42]. It was recently shown that with rigorous sample preparation and multiple injections, 1D-GC equipped with cyclodextrin columns can partially resolve 15 out of 19 atropisomeric PCBs [42]. However, in real-life samples, such as food extracts, this cannot be achieved because 1D-GC cannot separate the atropisomeric PCBs from both regular PCB congeners and from the remaining matrix components. This leads to poor resolution and poor identification of enantiomeric chiral PCBs. In one of the early experiments, Harju et al. utilized a narrow-bore β-cyclodextrin primary column and a liquid crystal secondary column to separate atropisomeric PCBs [43]. The group illustrated the separation and identification of nine atropoisomers by GC×GC in a single run, which is not possible with conventional methods. Later, Harju et al. analyzed chiral PCBs in complex real-life samples, such as gray seal blubber, liver, brain and muscle tissues [44]. Although not all atropoisomers were resolved, the GC×GC method was capable of separating nine chiral PCBs from common 1D-GC coeluters and the sample matrix. More recently, Bordajandi et al. developed a GC×GC method for the simultaneous analysis of chiral PCBs and toxicity indicator PCBs [42]. The group was successful in separating and identifying nine atropisomeric PCBs and all 12 WHO and seven EU toxicity marker PCB congeners from food samples (salmon and dairy products). When compared to conventional 1D-GC analyses, GC×GC decreased analysis time (due to minimized sample preparation procedures) and provided the higher resolution chromatograms required when analyzing complex food samples.

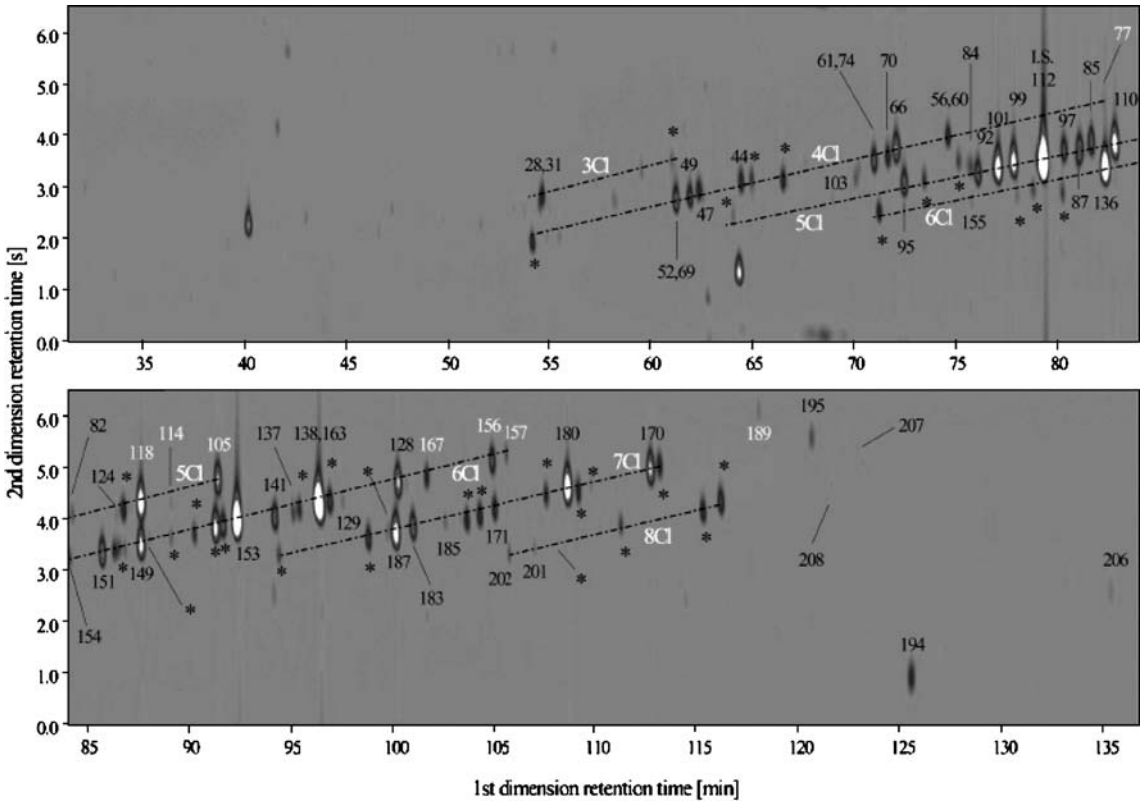

Fig. 4 GC×GC–ECD chromatogram of a cod liver sample spiked with 90 PCBs. Reprinted with permission from [34]

Shortly thereafter, Bordajandi et al. evaluated several primary and secondary column sets in an effort to improve chiral PCB analysis by GC×GC [45]. Although the group was not successful in separating more atropisomers than previously reported, they did develop several methods suitable for the determination of specific enantiomeric pairs.

Last year, GC×GC provided researchers with more insights into conventional sample preparation processes. In a study primarily designed to evaluate the separation of twelve classes of organohalogen compounds from a marine sediment sample, Korytár et al. discovered that even after the application of SPE prior to GC×GC analysis, matrix components persisted in relatively large concentrations with respect to the fully resolved trace amounts of PCDDs and PCDFs in the second dimension [53]. This is illustrated in Fig. 5, where matrix components are present in high concentrations (abundant and unresolved diagonal bands in the GC×GC chromatogram) with respect to the resolved PCDDs and PCDFs [53]. Closer examination of this abundant sample matrix revealed that it was composed of polychlorinated alkanes (PCAs). This discovery was interesting, as it indicates that previous assumptions about the efficiency of SPE as a pretreatment method for sediment samples might have been overconfident.

Presently, no single column can separate all 209 PCB congeners in one run. Frame organized an interlaboratory study utilizing 27 different high-resolution GC (HRGC) systems, including 20 different columns, for the separation of all PCB congeners [46, 47]. The most optimal HRGC–MS system in this study exhibited coelutions of 34 PCBs. Although this is a significant achievement in 1D-GC, it is unsatisfactory in routine analysis where certain target congeners belong to the coeluted groups of PCBs. In addition, the number of observed coelutions would certainly rise when analyzing PCBs in natural samples. Recently, the increased peak capacity and separation power offered by GC×GC instrumentation has been exploited in efforts to enhance the separation of complex PCB technical mixtures. Harju et al. utilized GC×GC–μECD to separate 194 PCB congeners in 240 minutes [48]. Focant et al. recently separated 192 PCB congeners with GC×GC–TOF MS in 146 minutes [35]. Among the baseline-separated PCBs were the 12 WHO-classified toxic congeners and the seven EU marker PCBs. The latter approach is more desirable due to the structural elucidation obtained from MS detection, as well as the decreased analysis time. Unfortunately, the separation generated very large data files (up to 500 MB each) and at times took up to two hours to process [35]. The most recent version of the commercially available GC×GC software minimizes this limitation by significantly shortening data processing times.

Pesticide analysis

Analysis of pesticides poses challenges to analytical chemists with respect to both sample preparation procedures and chromatography. Similar to other toxic compounds, pesticides are usually distributed throughout the

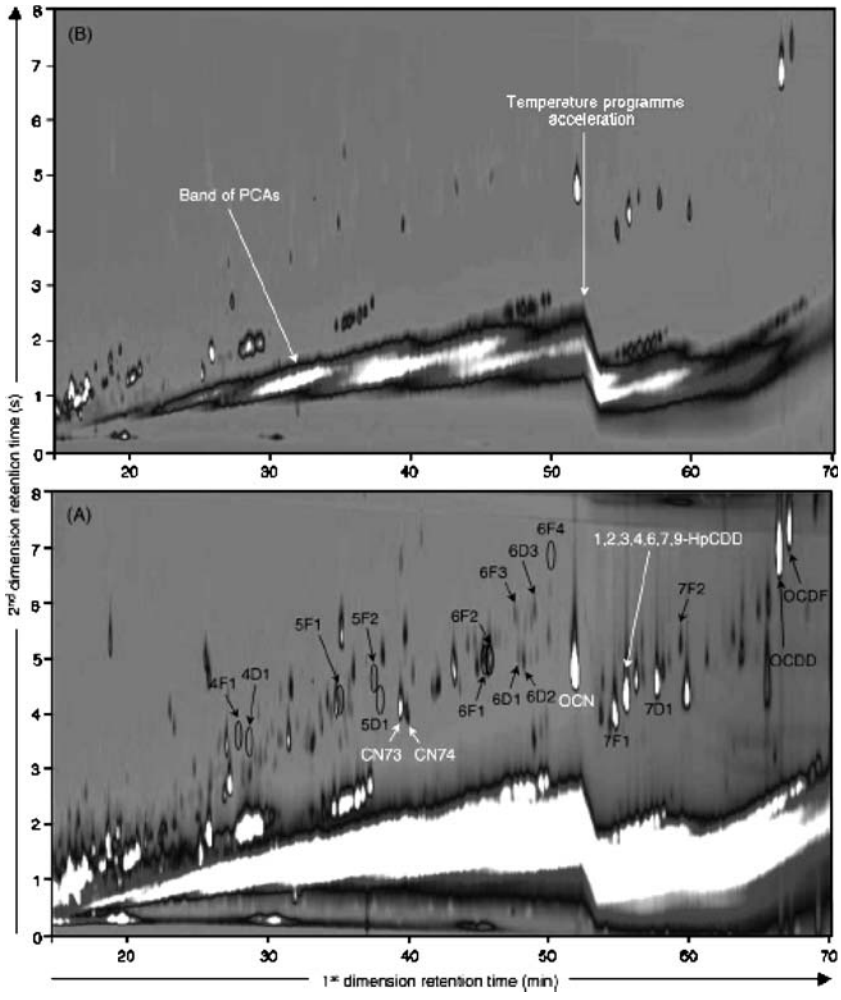

Fig. 5a, b GC×GC–ECD chromatogram of a marine sediment extract, illustrating the determination of the PCDDs/Fs fractions in two intensity scales (**a** and **b**). Reprinted with permission from [53]

environment in trace amounts. Additionally, they are part of extremely complex matrices such as food, soil and water samples. The need for rapid high-resolution methods of analysis is as pressing today as it ever was. A review article by Geerdink et al. dealing with trace analysis of pesticides using GC and LC is available to interested readers [2].

Standard methods for pesticide analysis from food extracts require rigorous and time-consuming sample preparation methods, often generating large amounts of waste solvents. In an effort to eliminate matrix components as efficiently as possible, multiple solid-phase extractions (SPEs), LC pretreatment, or liquid–liquid extractions (LLEs) are routinely applied [2]. These pretreatment procedures might lead to analyte loss unless they are integrated on-line. GC–MS is most often utilized for pesticide identification and quantification. However, as in the applications described above, the main limitation of 1D-GC in pesticide analysis is the lack of resolution required to separate analytes from each other, as well as from the sample matrix.

Although the amount of published work dealing with the applications of GC×GC to pesticide analysis is relatively small, it is sufficient to illustrate the method's superiority over conventional 1D-GC approaches. Early applications of GC×GC to pesticide analysis in human tissues illustrated the potential of the method for routine implementation in the future. Liu et al. utilized supercritical fluid extraction (SFE) in conjunction with GC×GC–FID to analyze pesticides in human serum [49]. The group achieved baseline separation of 15 pesticides extracted from spiked human serum in less than four minutes. Later, Dimandja et al. utilized GC×GC–FID for assessing pesticide exposure of children by using small volumes of urine and serum [50]. This particular example illustrated complete separation of 16 pesticides in less than four minutes. In fact, when compared to GC–TOFMS, GC×GC obtained better separation in four minutes than the one-dimensional method after one hour of analysis time [50]. More recently, Focant et al. [51] demonstrated the identification and quantification of 59 tissue contaminants, including PCBs and organochlorine pesticides. The authors indicated that for such an analysis, three separate injections are required in standard routine analyses (GC–ID-TOFMS), while GC×GC–ID-TOF MS accomplished comparable results in a single run. Such approaches should prove invaluable in clinical settings, where a relatively cheap, reliable and reproducible method is needed for screening and positive rapid identification of target analytes.

Determination of pesticides in food extracts is equally important. Utilizing GC×GC–TOF MS, Dallüge et al. illustrated the separation and identification of all 58

pesticides spiked onto vegetables [52]. This was accomplished with minimal and nonselective sample preparation: a celery or carrot sample was chopped, mixed with sodium acetate and ethyl acetate, blended, centrifuged and dried. The extract was injected into the GC×GC [52]. It should be noted that although not all 58 pesticides were baseline-resolved, the high resolving power of the GC×GC method allowed proper identification of partially coeluting peaks through the use of TOF MS spectral deconvolution algorithms. The results of deconvolution are usually not as good in conventional GC–TOF MS experiments, as the method lacks the resolving power to chromatographically separate trace pesticides from the surrounding and abundant matrix components.

Recently, Korytár et al. evaluated five different GC×GC column combinations for the group separation of 12 halogenated compound classes, consisting of PCBs, PCDDs, PCDFs, polychlorinated diphenyl esters (PCDEs), polychlorinated naphthalenes (PCNs), polychlorinated dibenzothiophenes (PCDTs), polychlorinated terphenyls (PCTs), polychlorinated alkanes (PCAs), toxaphene, polybrominated biphenyls (PBBs), polybrominated diphenyl ethers (PBDEs) and organochlorine pesticides (OCPs) [53]. Although the focus of this paper was primarily group separation of different compound classes, separations within families were also illustrated. While the separation and identification of all 28 OCPs was demonstrated only for a pure pesticide mixture, most of them were fully separated even when injected alongside the other eleven compound classes [53]. Hence, it seems that with a correctly configured column set, GC×GC can be used as a primary screening step for environmental samples contaminated with pesticides alongside many other classes of pollutants, with minimal sample preparation.

Regardless of the pesticide type or the complexity of the sample, GC×GC provides a more powerful tool for rapid analysis than the presently utilized 1D methods. Increased resolution and decreased analysis time make it perfectly suitable for monitoring purposes.

Air analysis

Volatile organic compounds (VOCs) play an important role in the generation of urban photochemical smog [54]. The WHO recognizes that exposure to air particulate matter can have detrimental effects on human health [55]. Still, uncertainty exists with regard to the health effects from VOCs in urban particulate matter (PM) [56]. Thus, a rapid, reliable and informative method is required to ensure successful monitoring, identification and discovery of atmospheric pollutants.

In one of the most important applications of GC×GC, Lewis et al. illustrated the separation of more than 500 chemical species of VOCs from urban air samples (Melbourne, Australia) in one run [57]. The apparent baseline noise observed in GC–MS was determined to consist of numerous air pollutants. The presence of aliphatic, carbonyl, and aromatic bands in regions of 1D baseline noise was established. Prior to this study, many of these compounds were not known to exist in the atmosphere [57]. The authors of the study thus demonstrated the potential of GC×GC for not only monitoring applications, but also for discovery purposes. Due to the limited resolving power and sensitivity of conventional 1-D GC methods, researchers might have previously underestimated the contribution of some VOCs to urban air pollution [57].

Many PAHs and oxygenated PAHs (oxy-PAHs) are suspected carcinogens and mutagens, and so they are important target analytes in urban aerosol analysis. GC×GC–FID and GC×GC–quadrupole MS (QMS) applied to urban air samples from Finland allowed the detection of approximately 1500 peaks and identification of target PAHs [58]. The GC×GC–FID combination confirmed good reproducibility, while the MS-coupled approach was used for compound identification and quantification. Thirteen non-target PAHs were identified, and ten target PAHs were quantified. The PAH concentration range found (0.5–5.5 ng/m^3) was comparable to results obtained by standard methods in other parts of Europe [58].

Schnelle-Kreis et al. investigated the suitability of direct thermal desorption combined with GC×GC (DTD-GC×GC–TOF MS) for the analysis of organic compounds in ambient aerosol particles [59]. The use of DTD as a sample introduction method eliminated solvent use, a characteristic drawback of traditional sample preparation methods. When compared to similar analysis with GC–TOF MS, GC×GC–TOF MS exhibited a ten-fold increase in the number of peaks detected and produced highly structured chromatograms ideal for rapid screening purposes. More importantly, the comprehensive 2D-GC approach reduced the limitations of TOF MS deconvolution observed in 1D; this led to improved library matches and more confident analyte identification.

A GC×GC system developed in our laboratory was recently combined with a nondiscriminating thermal desorption/pyrolysis system [60] and applied to the characterization of urban air particulate matter [61]. Following sampling onto quartz filters, the untreated samples were thermally desorbed/pyrolyzed using the pyrolysis–GC×GC–TOF MS system. The method developed by Parsi et al. allowed the analysis of organic compounds in $PM_{2.5}$ particulate matter with no sample pretreatment. It was suitable for both low and high molecular weight semivolatiles, and generated high resolution, structured 2-D chromatograms. A chromatogram illustrating the analysis of urban air from Ottawa (Canada) is illustrated in Fig. 6. The structured nature of the chromatogram is clear, and the analytes are baseline-resolved [61].

The vast amount of information generated by GC×GC can sometimes create problems. Around 15,000 peaks were detected in GC×GC–TOF MS analysis of urban air particulate matter ($PM_{2.5}$) from Augsburg (Germany), out of which approximately 700 compounds were identified [56]. In this particular instance, the large amount of mass spectral data generated by the TOF–MS and the complexity of the GC×GC contour plot made the method unsuitable for routine air quality monitoring analysis. To overcome this

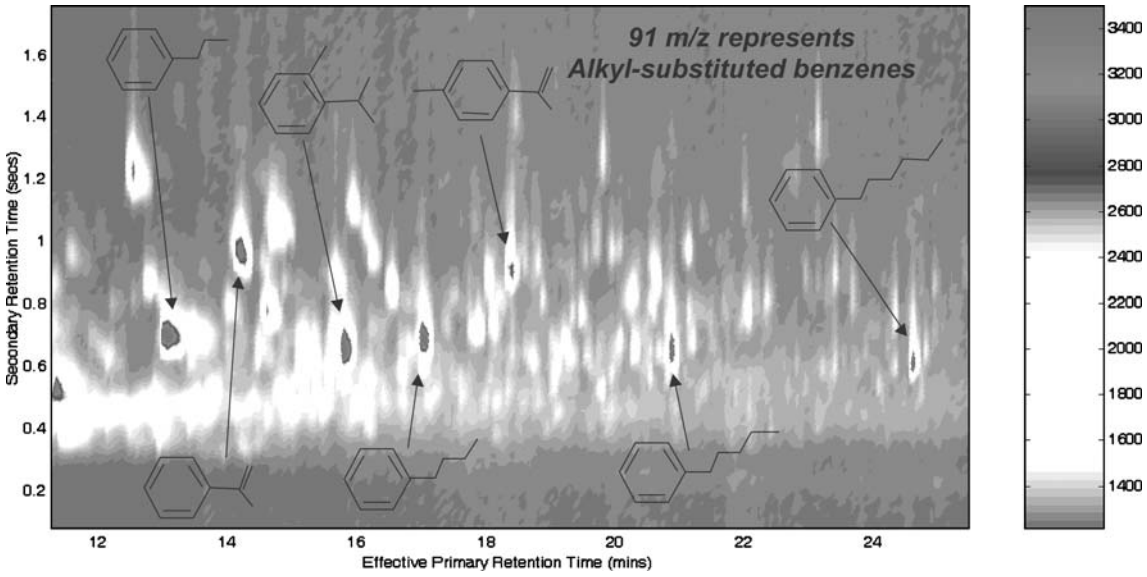

Fig. 6 Analysis of urban air particulate matter ($PM_{2.5}$) using a nondiscriminating thermal desorption/pyrolysis system coupled to GC×GC–TOF MS; extracted ion *m/z* 91 (characteristic of alkyl-substituted benzenes). Thermal desorption at 450 °C. Reprinted with permission from [61]

problem, Welthagen et al. developed a procedure for group classification of complex GC×GC–TOF MS data based on primary and secondary retention times and MS fragmentation patterns [56]. The implementation of such classification procedures simplifies the interpretation of data, making rapid peak classification, searching and identification easier.

Cigarette smoke is an extremely complex mixture estimated to consist of approximately 4,700 identified compounds and up to 100,000 unidentified components [62]. Evidently, conventional 1D-GC does not offer the separation power to effectively analyze compounds contained in cigarette smoke. Dallüge et al. utilized GC×GC–TOF MS to resolve approximately 30,000 peaks from cigarette smoke [62]. Following this, simpler samples of cigarette smoke condensate were analyzed to determine the chemical composition of the neutral fraction [63], the basic fraction [64], and the acidic fraction [65]. Xu et al. separated 200 unknown peaks and identified 115 hydrocarbons from the nonpolar neutral fraction of cigarette condensate via conventional GC–MS; GC×GC analysis of the same sample, however, achieved the separation of 4,000 compounds and the identification of 1,800 hydrocarbons [63]. In another study, GC×GC–TOF MS analysis of the basic fraction of cigarette condensates identified 377 nitrogen-containing compounds, among which 155 were pyridine derivatives, 104 quinoline/isoquinoline derivatives and 56 pyrazine derivatives [64].

Conclusions

GC×GC has quickly achieved the status of being one of the most powerful tools for the analysis of volatile organic compounds. It has established itself as a technology that is perfectly suitable for monitoring analytes in complex samples. In the area of environmental analysis, this is evidenced by the numerous examples of analysis of common environmental pollutants—including PCBs, PCDDs, PCDFs, PAHs and pesticides—in complex environmental matrices. Additionally, GC×GC has the potential to simplify the sample preparation procedures (or even eliminate them entirely), while simultaneously generating high-resolution chromatograms in a shorter overall analysis time.

For a new analytical method to be widely adopted, it must not only be reliable and reproducible, but it should also exhibit significant advantages over accepted methods. The examples reported in this review illustrate the advantages of the GC×GC method over traditional 1D-GC separations. During the first years in GC×GC history, instrumentation development was the main focus; however, since the commercialization of GC×GC systems, the number of reported applications in environmental analysis and other scientific fields have increased dramatically. Hence, we can expect a gradual transition to automated GC×GC coupled to on-line sample preparation devices for applications in routine environmental monitoring.

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