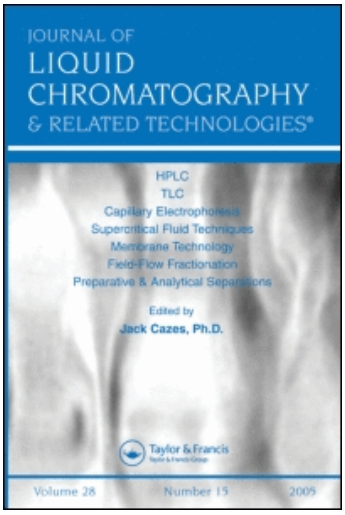

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RECENT DEVELOPMENT IN THE APPLICATIONS OF COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPH

Yuwen Wang[a]; Qian Chen[a]; Daniel L. Norwood[a]; John McCaffrey[a]
[a] Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, Connecticut, USA

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RECENT DEVELOPMENT IN THE APPLICATIONS OF COMPREHENSIVE TWO-DIMENSIONAL GAS CHROMATOGRAPH

Yuwen Wang, Qian Chen, Daniel L. Norwood, and John McCaffrey
Boehringer Ingelheim Pharmaceuticals, Inc., Ridgefield, Connecticut, USA

□ *In this review, the recent and significant advances in comprehensive two-dimensional Gas Chromatography (2D-GC or GC × GC) and their associated applications during the past three years (2007 to present) are reviewed and discussed. The discussion includes a brief overview of the recent advances in 2D-GC techniques and theory, followed by examples of new applications and instrumentation for the various 2D-GC analysis techniques. Example applications in biological/clinical, environmental, petroleum, food, fragrance, and forensic analysis are provided. This report clearly shows that the applications of 2D-GC are active and growing. The technique is penetrating to different areas with new applications being reported regularly.*

Keywords applications, comprehensive two dimensional GC, 2D-GC

INTRODUCTION

Since the introduction of fundamental definitions for multidimensional separations by Giddings[1] in 1984, this family of analytical techniques, especially multidimensional gas chromatography (MDGC) has developed very rapidly. Initially, MDGC separations involved "heart-cutting" techniques, in which a fraction (or several consecutive fractions) of eluent from a primary column was introduced into a secondary column coated with a different stationary phase.[2] Such techniques permit the further separation of sample components eluting in selected regions of the chromatogram from the primary column. Heart-cutting techniques have, however, proven inadequate for separation and analysis of many very complicated samples. A different and significantly more powerful approach is to make the second column's analysis time short enough to generate at least one complete chromatogram for each single peak eluting from the primary column. A

Address correspondence to Yuwen Wang, Boehringer Ingelheim Pharmaceuticals, Inc., 900 Ridgebury Rd., Ridgefield, CT 06877, USA. E-mail: yuwen.wang@boehringer-ingelheim.com

complete set of secondary chromatograms can then be generated in real-time as the primary chromatogram develops. Since all sample components are subjected to two-dimensional separation, the technique is called comprehensive two-dimensional gas chromatography (2D-GC or GC × GC)[3] and can provide significantly increased resolution, sensitivity, peak capacity, and selectivity in separations. The first practical implementation of 2D-GC was demonstrated by Liu and Phillips[4] in 1991. Since then, rapid development of this technique has occurred, and applications in many areas of research have been reported.

Over the years, a number of reviews on 2D-GC have been published.[5–19] Initially, these reviews focused on the fundamental principles and experimental set-ups of the technique along with basic theory. In the last three years, the majority of the published reviews are devoted to practical applications. Areas such as biological/clinical, environmental, food, and petroleum have seen significant published applications, with the numbers of citations being directly related to the complexity of samples (i.e., biological/clinical and environmental are on the top of the list) (Figure 1). Figure 2 shows the trend in publications for applications of 2D-GC (note that in order to better depict the trend in the development of 2D-GC, the time period in Figure 2 is extended back to the year 2000). The numbers of the publications related to applications of 2D-GC grew rapidly between 2000 and 2005 but have since leveled off with about 70 publications yearly.

The basic principle of 2D-GC is illustrated in Figure 3. The sample components separated in the primary column are periodically collected

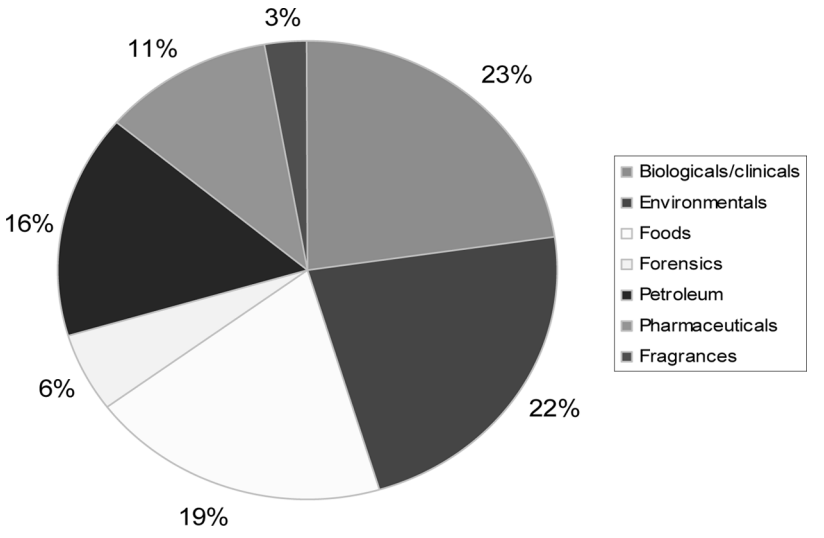

FIGURE 1 Applications of GC × GC in different areas compiled from the publications from 2005 to current (July, 2009).

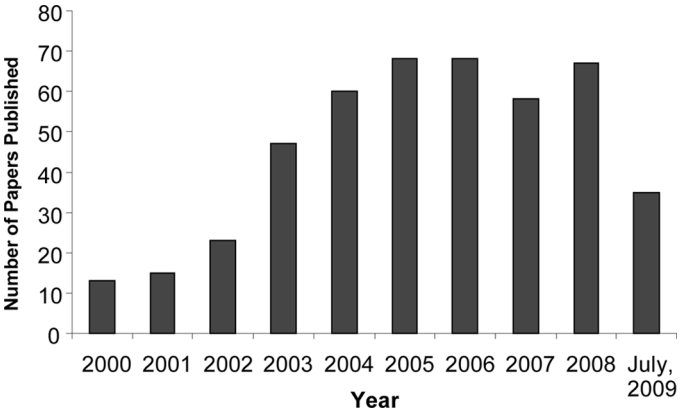

FIGURE 2 Number of publications of the applications of 2D-GC from year 2000 to present (July 2009).

in an interface called the "modulator," which collects a band of analytes for a specified period of time. Each fraction collected is then reinjected onto a secondary column as a narrow band. While the chromatographic separation of the reinjected analytes is occurring in the secondary column, the modulator collects a subsequent fraction from the primary column. After chromatographic separation in the secondary column is completed, the modulator injects the next collected fraction into the secondary column. In this process, the modulator periodically collects the eluent from the primary column and repeatedly reinjects these fractions throughout the entire 2D-GC analysis.

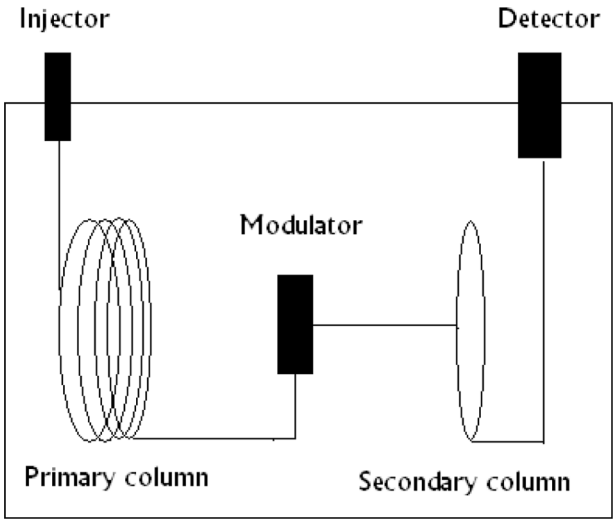

FIGURE 3 Block diagram of 2D-GC.

The modulator is the critical component in the overall analytical technique. Since the first heater-based modulator was introduced in 1991 by Liu and Phillips,[4] cryogenic modulators[20–23] and valve-based modulators[24,25] have been developed. To see the details of the evolution of modulators in 2D-GC, the interested reader is referred to Gorecki et al.[26] To quantify individual analytes in a 2D-GC analysis, the peaks in each secondary dimension chromatogram corresponding to a specific analyte are integrated and the areas of all these peaks are summed.[27]

The approximate twenty year history of 2D-GC[4,28] includes many fundamental advances in instrumentation and technology, as well as numerous applications. In this review, technological advances and applications of 2D-GC since 2007 are summarized and discussed, along with recent trends and anticipated future developments.

PETROLEUM

Crude oil is a very important natural resource whose chemical composition has been studied for more than a century. Gas chromatography has played and continues to play a central role in the characterization of petroleum derived distillates and other petroleum derived materials. Petroleum and petroleum derived materials contain hydrocarbons with a wide structural diversity as well as many "hetero-compounds," which incorporate sulfur, nitrogen, and/or oxygen. The presence of hetero-compounds in crude oil is considered undesirable for various reasons, including the fact that they are potent poisons for commonly used catalysts in chemical reactions. Due to the complexity of petroleum derived samples, one-dimensional GC analytical methods often cannot accomplish required separations, and therefore 2D-GC based methods are seeing increasing application.

Dutriez et al.[29] reported the development and optimization of a high temperature 2D-GC method for the qualitative and quantitative analysis hydrocarbons up to nC_{60} in vacuum gas oils (VGO, representing heavy cut petroleum distillates). 2D-GC is useful for this application because the number of hydrocarbon isomers increases significantly with the number of carbon atoms. The 2D-GC separation conditions (including primary and secondary column dimensions, temperature program, and cryogenic temperature of the modulation spray) were optimized using an experimental design approach and evaluating the bidimensional separation criteria of: 2D resolution, 2D asymmetry, efficiency, modulation band broadening, and orthogonality. The experimentally selected and optimized analytical conditions were used for the qualitative and quantitative analysis of a sample of vacuum gas oil and represents the first application of

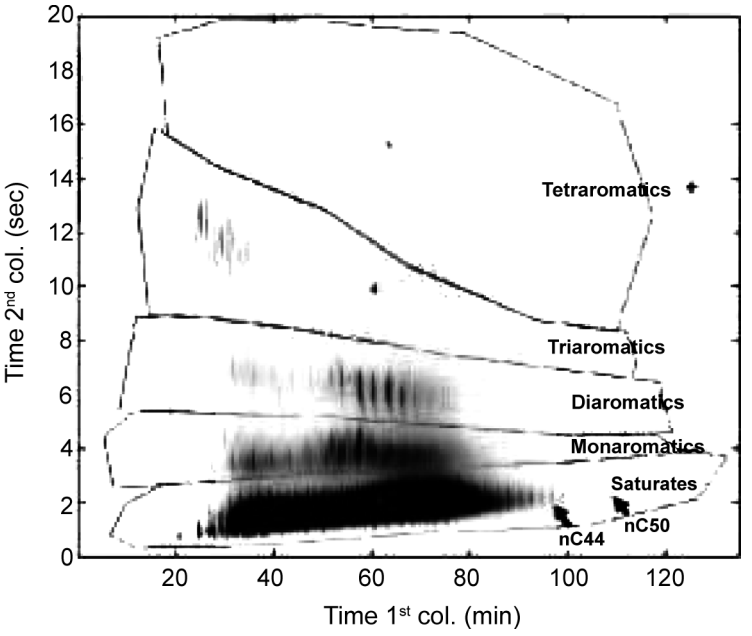

FIGURE 4 HT-2D-GC contour plot of straight run VGO. (Figure reprinted from Dutriez et al.[29] with permission from Elsevier, copyright 2009.)

high-temperature 2D-GC to the separation of all the components in a VGO. Favorable comparisons with other standardized methods illustrate the high potential of HT-2D-GC for heavy petroleum distillate fraction analysis. Figure 4 shows a high temperature 2D-GC contour plot for the VGO sample.

To understand the low temperature property of lubricant oil via the dewaxing processes, monitoring the change of key components (such as paraffins) is critical. Wang and Zhang[30] reported a method to separate the components in Group the II Lubricant Oil using 2D-GC. A detailed separation of chemical components of Group II type lubricant oil was achieved. The major advantage of these techniques is the ability to obtain the detailed fingerprint of major paraffin components by 2D-GC. This technique provides a good separation between the paraffins and naphthenes in the lubricant oil. Based on the quantitative results of the analysis, the authors are able to develop a formula to correlate the paraffin chemical components to viscosity measured by a mini-rotary viscometer (MRV). The ability of correlating the paraffin chemical component to MRV viscosity enables a better understanding of the roles of various paraffin molecules in MRV viscosity. The 2D-GC results can be used in the lubricant base oil formulation, in the additive package development, and as a model to predict MRV viscosity from the chemical components.

The 2D-GC technique has also applied in the separation of the middle distillate in the petroleum industry. Adam et al.[31] described a method to test all the hydrocarbons in middle distillates by liquid chromatography coupled with 2D-GC (LC-2D-GC). This article aims at promoting the implementation of LC-2D-GC for the quantitative determination of hydrocarbon distribution in the middle distillates, including naphthenes. In this configuration, LC allows the separation of hydrocarbons into two fractions, saturated hydrocarbons and unsaturated hydrocarbons. After the LC separation, 2D-GC was used to further separate the components in each fraction. The paper discusses the choice of 2D-GC conditions to achieve the separation and identification of hydrocarbons by chemical classes is discussed. Under these conditions, naphthenes are separated according to the number of saturated rings in the structure. This is the first time that the separation of di-, tri-, and tetra-naphthenes resulting from the hydroconversion of aromatics has been reported. The quantitation procedure for the determination of the distribution of hydrocarbons, including the distribution of naphthenes according to the number of saturated rings, was also proposed and discussed in detail. LC-2D-GC is found to provide an unequaled degree of information that will widely contribute to a better understanding of hydroconversion processes.

Concerns over the greenhouse gas emission from the increasing consumption of fossil fuels, government policies now lead to the mandatory introduction of fuels of renewable sources of energy such as biomass to fossil fuels. The mixing of fatty acid alkyl esters from vegetable oils (also known as biodiesel) with conventional diesel fuel is one of the technologies available to use (e.g., B5 blends, up to 5% esters in fossil fuel are marketed over Europe). Therefore, it is important to monitor the biodiesel origin (i.e., the fatty acid ester distribution) and its content when it is blended with petroleum diesel for quality control and for forensic reasons. 2D-GC is a preferable choice of technique due to its excellent separation power. Adam et al.[32] has demonstrated the separation of oxygenates in middle distillates. A 2D-GC method was developed for the individual quantitation of fatty acid esters in middle distillates matrixes. Several first and the second dimension columns were studied and their performances to achieve a group type separation of hydrocarbons and individual identification and quantitation of fatty acid ester blend with diesel are reported and discussed. Finally, the quantitative 2D-GC results were compared with the results from reference methods. The 2D-GC results show the benefits of fast and reliable separation of the individual fatty acid esters in one single run. With the developed separation conditions, the quantitation of group types of hydrocarbons is also possible, meaning that simultaneous quantification of hydrocarbons and fatty acid esters can be achieved in one single run.

As mentioned in the beginning of this section, hetero compounds including oxygen, nitrogen, and sulfur are very important in the petroleum industry because they are potent catalyst poisons. The monitoring of the content for these compounds is very challenging for the analytical chemists due to the complexity of the petroleum. Adam et al.[33] reported a method to analyze both the basic and neutral nitrogen compounds in middle distillates using 2D-GC. For the evaluation of the best chromatographic conditions, a nonpolar and a polar stationary phase approach was chosen. This study shows that the implementation of a polar secondary column having free electron pairs improves the separation of N-nitrogen compounds. The presence of a permanent dipole-permanent dipole interaction between neutral N-compounds and the stationary phase enhanced the separation. Nitrogen chemiluminescence detectors (NCD) showed improved selectivity for the N-compounds in the 2D-GC separation. Due to the higher resolution power and enhanced sensitivity achieved using developed chromatographic and detection conditions, it was possible to identify and to quantitate nitrogen containing compounds. Finally, the authors compared the results obtained from 2D-GC nitrogen-chemiluminescence detection (2D-GC-NCD) with the results obtained from conventional GC. The results from 2D-GC-NCD show a better correlation with the results obtained by American Society for Testing Materials (ASTM) methods for the determination of basic/neutral nitrogen ratio in the diesel samples. Dartiguelongue et al.[34] studied the distribution of basic and non-basic nitrogen compounds in diesel feeds from various origins using 2D-GC. In their study, 2D-GC-NCD was applied to the identification and quantification of basic and neutral nitrogen compounds from an extended database of diesel feeds from various origins. 2D-GC-NCD proved to be extremely sensitive and efficient to allow the separation of nitrogen compounds into well-defined families including pyridines, anilines, quinolines, acridines, indoles, and carbazoles.

The analysis of total sulfur content and for speciation of individual sulfur-containing compounds in middle distillates for an efficient catalyst selection and for a better understanding of the kinetics of the reactions involved in hydro treatment processes are required. Due to the high resolution power and enhanced sensitivity, 2D-GC coupled with sulfur chemiluminescence detection (2D-GC-SCD) has recently been used as a powerful tool for improving characterization and identification of sulfur compounds in middle distillate. Ruiz-Guerrero et al.[35] compared 2D-GC-SCD to other methods commonly used in the petroleum industry, such as X-ray fluorescence, conventional GC-SCD, and high resolution mass spectrometry, for total sulfur content detection and speciation. Different samples of the middle distillates have been analyzed to demonstrate the high potential and important advantages of 2D-GC-SCD for quantitative

analysis of sulfur-containing compounds. More accurate and detailed results for benzothiophenes and dibenzothiophenes are presented, which shows that 2D-GC-SCD would become, in the future, an essential tool for sulfur speciation analysis.

The determination of aromatics in light petroleum product is critical. In the standard methods of oil testing, the concentrations of aromatics and naphthalene hydrocarbons in light petroleum products must be detected using more than two methods. To simplify the procedures, Li[36] introduced a 2D-GC method for the determination of aromatics in light petroleum products. The method can achieve the group separations of paraffins, olefins, naphthenes, and aromatics with one to two rings and also target some components in light petroleum products. The reproducibility and precision of the method are very good. The recoveries for the standard compounds were 89.5%–106.1%, and the relative standard deviations of repeatedly analyzing the components were all lower than 5.8%. The total run times are about 30 minutes. Heavy naphtha and a fluid catalytic cracking cut were also analyzed by the 2D-GC. Olefins were also separated in an Ag-doped silica trap and followed by 2D-GC. It was demonstrated that the resolution between all chemical groups in a complex mixture of C8-14 hydrocarbons are excellent.

Very interestingly, the 2D-GC technique has been used to quantitate biomarkers in sediment samples to examine the fate of the oil in coastal seawater.[37] Fifteen sediment samples were collected down-current from the oil seeps. Petroleum content and individual biomarkers were also quantified using 2D-GC. Similarities in hopane biomarker distributions linked sediment oil with fresh seep oil ($n = 5$) and the underlying reservoirs ($n = 3$), although sediment oil was heavily weathered. The oil spatial distribution forms a plume along the continental shelf suggested to represent a chronic fallout pattern for heavy oil from persistent surface slicks; average surface currents appear to modulate this fallout distribution over a 0.4–5 day period. The extent of hydrocarbon loss is consistent for all sediment, indicating a common limit to the oil weathering with contributions from evaporation, biodegradation, and dissolution. Considering the amount of oil and quantity of impacted sediment, the authors estimated a sediment oil burden of 0.3×10^{12} to 3×10^{12} g in the study area, which is equivalent to 8–80 spills of the size of the Exxon Valdez accident of 1989. Figure 5 shows the chromatograms.

The steady rise in the price of petroleum has increased the attractiveness of adding biofuels to petroleum-based fuels. For example, E85, a gasoline substitute that contains approximately 80% ethanol and 20% gasoline, currently (3/28/08) has an average price per gallon that is 16% lower in price than gasoline. In addition, many states are mandating that renewable fuels be added to petroleum-based fuels. Thus, there is clearly a need for

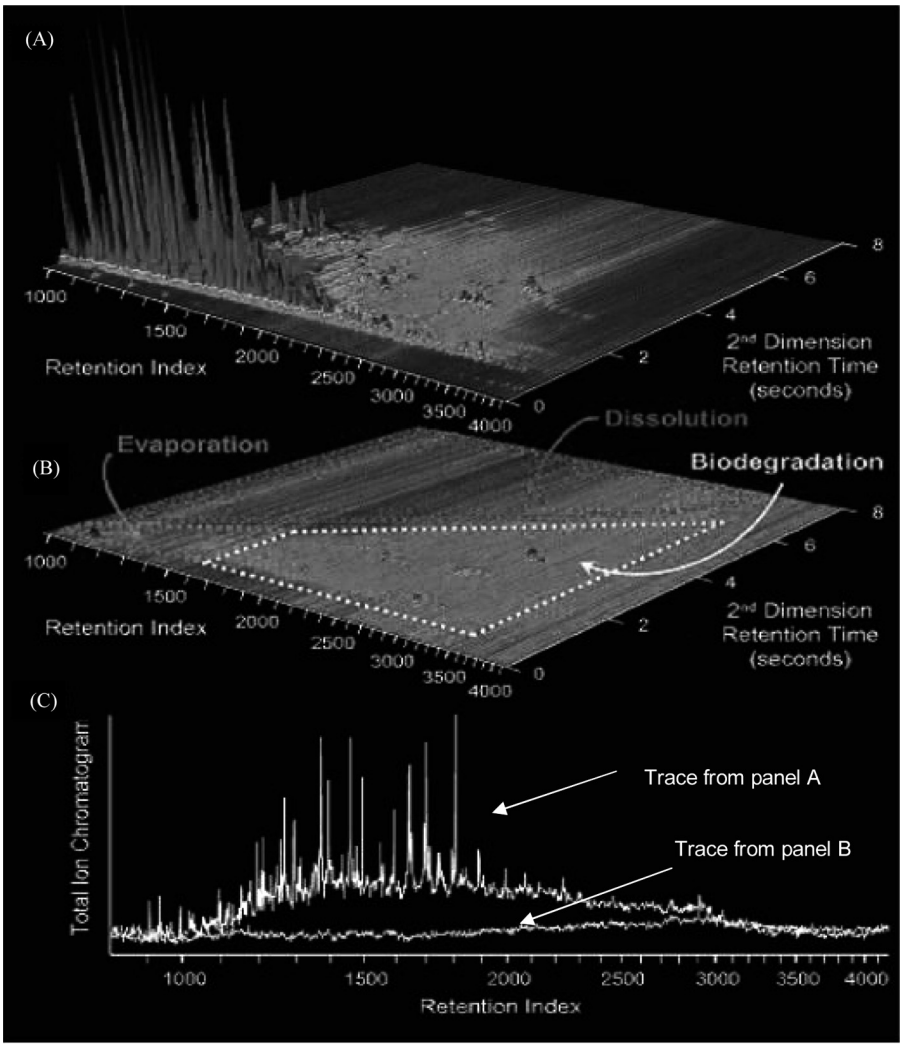

FIGURE 5 2D-GC-TOFMS chromatograms of (A) surface slick oil collected from the sea surface above Trilogy seep; (B) sediment sample oil from BC-14, with regions of the chromatogram marked corresponding to the likely associated forms of weathering; and (C) comparison of TIC traces from panels A and B collapsed into one dimension. Retention indices in all three panels are consistent with carbon number, i.e., RI = 1000 is n-C_{10}, RI = 1500 is n-C15, etc., and each chromatogram is scaled to 17α(H),21β(H)-28,30-bisnorhopane. (Figure adapted from Farwell et al.[37] with permission from the American Chemical Society, copyright 2009.)

robust analytical methods that will provide both quantitative and qualitative information on the compounds in biofuel/petroleum blends. The characterization of the blends with single-column GC is difficult due to the numerous co-elutions between petroleum hydrocarbons and biofuel oxygenated organic compounds. Seeley et al.[38] have recently developed

a 2D-GC technique to characterize biodiesel/petroleum diesel blends and ethanol/gasoline blends. The total quantity of fatty acid methyl esters was determined for biodiesel blends ranging from B1 to B20. The results were in excellent agreement with manufacturer reported values. The levels of ethanol and other trace alcohols were detected in E85 fuels. The measured levels were found to be in excellent agreement with results obtained using slower and more cumbersome analyses based on conventional heart cutting GC.

ENVIRONMENTAL

The analysis of persistent halogenated compounds in the environment is still an attractive subject. The detrimental effect of these compounds has been known for a long time. The detailed analysis of polychlorine substituted biphenyl compounds (PCBs), chlorinated boranes, and camphene is perhaps the greatest challenge for the analytical chemists. Owing to the high resolution of the technique, 2D-GC has been explored for this analysis. For example, Osemwengie et al.[39] separated 209 chlorinated biphenyl congeners by 2D-GC-TOFMS. The authors used a newly developed 40 m RTX-PCB column as the primary column and a 1 m DB-17 as the secondary column. In two GC runs, 201 PCB congeners were separated, including 43 of the 46 pentachlorobiphenyl isomers. Many of the chlorinated biphenyls that could not be resolved chromatographically were resolved with the deconvolution program containing the ChromaTOF software. Another advantage of this technique was also made of the "ortho effect," which can distinguish PCBs having 2,2′-; 2,2′6-; and 2,2′,6,6′-chlorine substituted congeners from those important compounds without these substitutions. This 2D-GC technique provides the analytical chemist with a new tool for a better front-end separations of PCB-specific congeners and, potentially, for more accurate human and environmental exposure data for risk assessments. Muscalu et al.[40] determined PCBs, OC pesticides, and chlorobenzenes in sludge and sediment samples using a 2D-GC Electron Capture Detector (2D-GC-ECD) in a single run. The enhanced selectivity of 2D-GC enables the separation of persistent environmental contaminants. Bordajandi et al.[41] reported a method to screen for the persistent organohalogenated pollutants in environmental samples by 2D-GC. Separations of eight persistent organohalogenated classes of pollutants, organochlorinated pesticides (OCPs), polychlorinated biphenyls (PCBs), polychlorinated di-Ph ethers (PBDEs), polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), polychlorinated naphthalenes (PCNs), polychlorinated terphenyls (PCTs), and toxaphene (CTT) by 2D-GC were evaluated. Columns with different polarity and

selectivity, including ZB-5, HT-8, DB-17 and BP-10, were selected as first dimension column and then combined with columns of increasing polarity in the second dimension (i.e., HT-8, BPX-50 and Carbowax) totaling nine column combinations. Because the main interest of the study was fast screening of the test xenobiotic families in complex matrixes, the author's attention was mainly focused on group-type separations. However, separation within the group was also considered, especially for those classes containing particularly toxic congeners such as PCBs and PCDD/PCDFs. Even though none of the tested column sets allowed the simultaneous and complete separations of all pollutants classes, some of the column combinations provided satisfactory separations among selected families and the rest of pollutants studied. For example, the combination of HT-8 and BPX-50 is for PBDEs, the PCDD/Fs, DB-17, and HT-8 is for PCNs, the OCPs and BP-10 and BPX-50 is for CTT, PCDD/Fs, and PBDEs. The feasibility of the proposed approach for the fast screening of the target classes of pollutants in complex samples was illustrated by the analysis of food and marine fat samples.

Due to the toxicity and carcinogenic nature of the polycyclic aromatic hydrocarbons (PAH), determination of the residual PAH in the environment is still a hot topic. Due to its complexity of the sample, 2D-GC has been used for PAH in different matrixes. Amador-Munoz et al.[42] reported

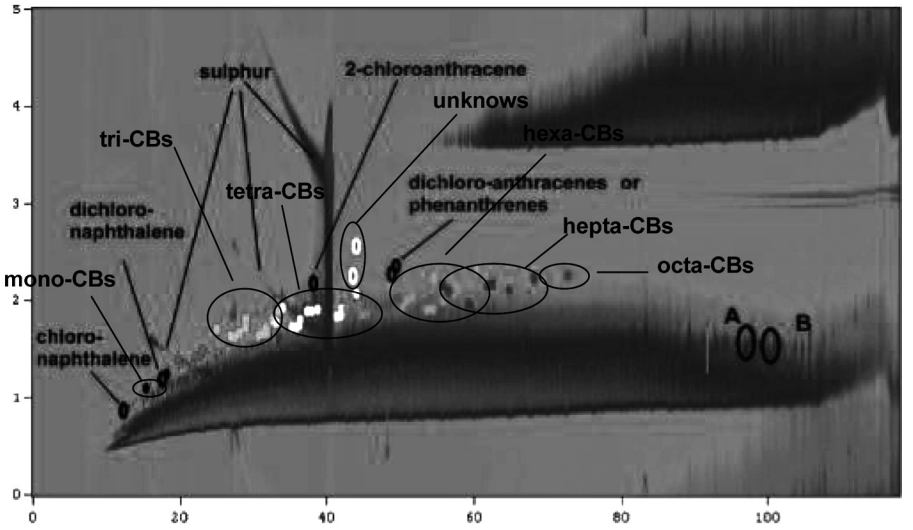

FIGURE 6 2D-GC chromatogram of the Elbe extract fraction 1 in the total ion current mode. Polychlorinated biphenols are marked as mono-CBs, tri-CBs, tetra-CBs, hexa-CBs, hepta-CBs, octa-CBs. Ovals: white: unknowns, black: chlorinated PAHs. Ovals A and B: groups of peaks with similar mass spectra. (Figure adapted from Skoczyńska et al.[43] with permission from the American Chemical Society, copyright 2008.)

a method to quantitate PAHs in air particulates based on 2D-GC isotope dilution mass spectrometry. The authors indicated that 2D-GC offers a favorable resolution and sensitivity over conventional one dimensional GC. These characteristics are of major interest when analytes in trace concentrations are present in complex mixtures, as for PAH in atmosphere particulates.

Skoczynska et al.[43] reported a 2D-GC method to identify 400 compounds in an environmental extraction sample. 2D-GC-TOFMS allows the separation of many constituents of previously unresolved complex mixtures of contaminants in sediment samples. In addition to the powerful chromatographic resolution, automated mass spectral deconvolution and identification system software enables a spectral deconvolution of closely eluting peaks. An example of highly polluted sediment sample from the River Elbe (Czech Republic) was presented in the paper. More than 400 compounds were tentatively identified from three fractions. Some of the identified analytes, although not belonging to the group of priority pollutants, are known

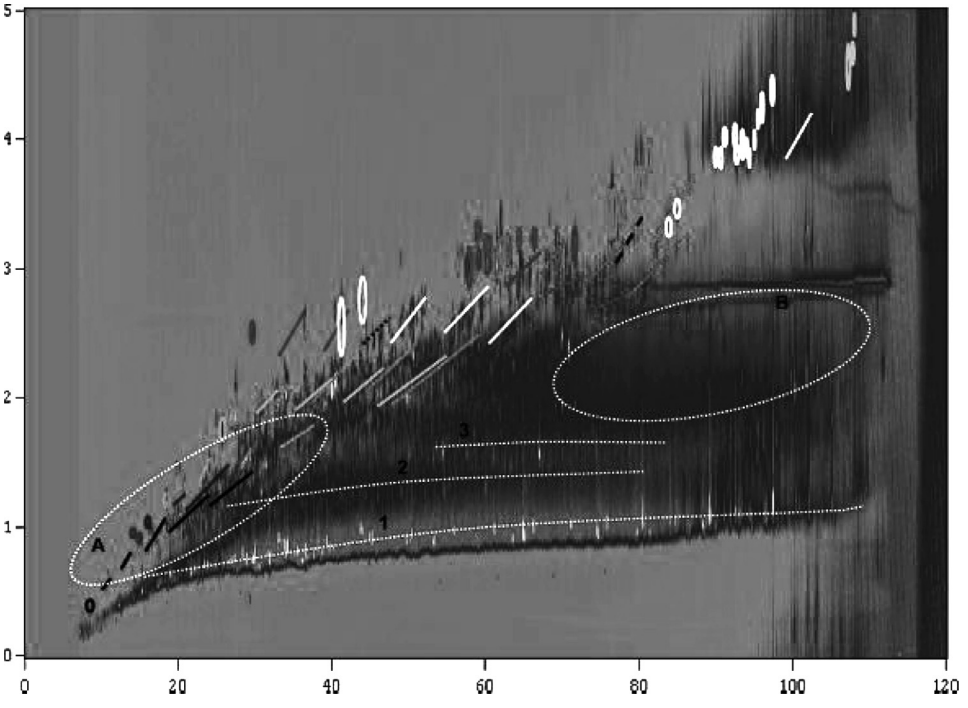

FIGURE 7 2D-GC chromatogram of the Elbe extract fraction 2 in the total ion current mode. (1) Band of alkanes, iso-alkanes, alkenes, alcohols, and ethers. (2) Band of (oxygenated) monoaromatics. (3) Band of unknowns. (A) Diaromatics and their alkylated derivatives. (B) Nonaromatics polycyclics. For descriptions of other regions, see reference.[43] (Figure reprinted from Skoczyńska et al.[43] with permission from the American Chemical Society, copyright 2008.)

to have a toxic potential. Examples are chlorinated polycyclic aromatic hydrocarbons (PAHs), alkylated PAHs, quinones, amino-quinones, dinaphthofurans, and thiaarenes. Due to the lack of a more thorough cleanup, the life-span of the GC-column is substantially reduced, whereas the ion source of the MS needs more frequent maintenance. This procedure delivers, in one run, a wealth of information that may be useful for further elucidation of the toxicological properties of the sediment samples by a tentative identification of previously unknown compounds. (See Figures 6 and 7 for the chromatograms).

The list of priority chemicals included in various government regulations such as the European Water Framework Directive, as well as the list of hazardous contaminants identified in the aquatic environment is increasing at an accelerated pace. Therefore, there is a need for broad spectrum of methods capable of simultaneously determining hundreds of contaminants. Semard et al.[44] reported a 2D-GC method to screen hazardous contaminants in urban water. A variety of drugs, personal care products, pesticides, carcinogens, and compounds toxic for reproduction were identified. Most of these compounds were removed or decreased by the wastewater treatment plant.

FRAGRANCES

In the field of fragrances, the qualitative and quantitative determination of volatile and semi-volatile components could be very challenging due to possible interferences from complex matrices. 2D-GC technique has the advantages of overcoming these separation problems in complex samples; therefore, it has been used in fragrance analysis.

For example, Cordero et al.[45] has identified and quantified the volatile suspected allergens in the *FFC02* fragrance and in the sandalwood essential oils by 2D-GC-MS. A series of OV1 and OV1701 column combinations were tested. The 0.25 mm homologous id column setup for 2D-GC offered the best separations. Figure 8 shows the resulting 2D plot and the allergen pattern in the *FFC02* fragrance. Figure 8c highlighted the increased 2D column loadability with the 0.25 mm homologous id column setup. Meanwhile, fingerprint analysis of the herbal extracts from a mixture of aromatic plants was also performed. Results demonstrated this approach as a powerful tool for the analysis of medium-complex fragrance samples containing components at variable abundances.

Another research study for the analysis of suspected allergens in fragrances conducted by Cordero et al.[46] was focused on a method development and validation using 2D-GC-qMS (2D-GC coupled with rapid scanning quadruple MS) and 2D-GC-FID. In this study, both the identification

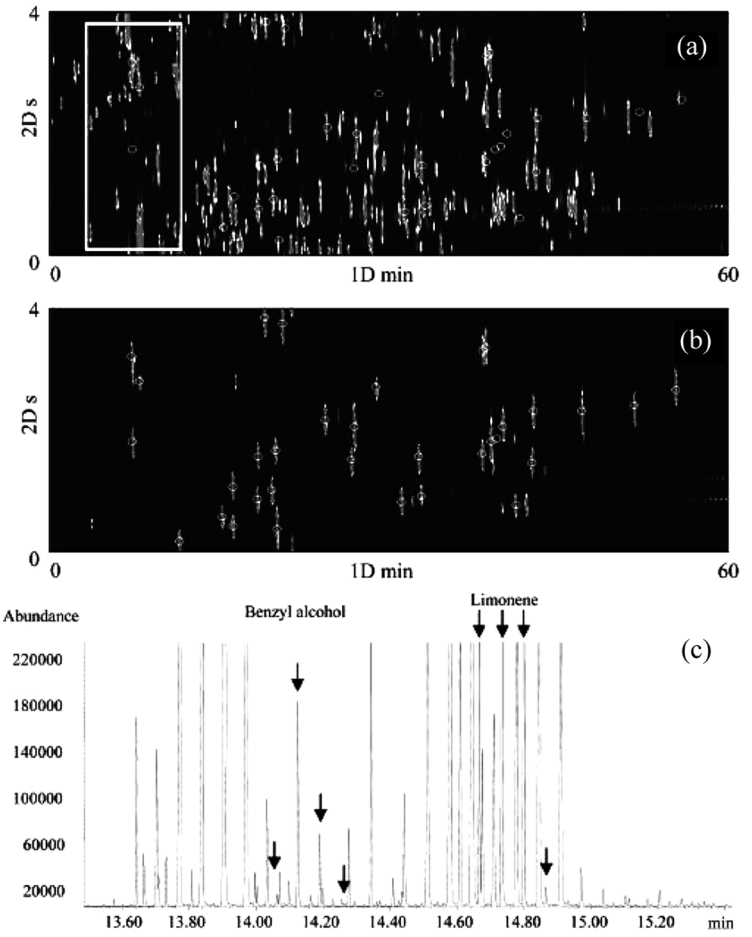

FIGURE 8 Resulting 2D plots of a TIC analysis of *FFC02* fragrance (a) and the corresponding allergens standard mixture; (b) analysed with column set 1 at a temperature rate of 3°C/min, modulation period of 4 s. The enlarged area of the 2-D plot (c) evidences the separation of benzyl alcohol and limonene from glycol isomers responsible of 2D column overloading effects. (Figure reprinted from Cordero et al.[45] with permission from Wiley-VCH Verlag GmbH & Co. KGaA, copyright 2008.)

of the target analytes by 2D-GC-qMS and the detection and quantification by 2D-GC-FID and 2D-GC-SIM–qMS were validated. Validation parameters confirmed that this approach can successfully be applied to the detection and quantitation of volatile suspected allergens in very complex fragrances.

Baier and Boehne[47] developed a method using 2D-GC-qMS for the determination of the potential allergens in cosmetic products. Direct thermal desorption from the matrix using the Optic-3 PTV injector was applied to reduce the sample preparation time. The system employed a column combination of an RTX-1 (30 m, 0.25 mm, 0.25 μm) as column one while

a Carbowax column (1 m, 0.1 mm, 0.1 μm) as column two. Results from the standard and a hand crème sample highlighted that 2D-GC-qMS is a powerful approach for both the qualitative and quantitative routine work, even with complex samples.

FOODS

With better sensitivity, greater resolution, improved peak capacity, and enhanced time saving, 2D-GC has been increasingly employed for the characterization of a variety of compounds and substances in the field of food analysis, mainly targeting on the determination of trace-level contaminants and components. Selected papers, published recently on applications and 2D-GC system set-up, are addressed in the following discussion.

A system consisting of gel permeation chromatography (GPC), an automated direct sample introduction (DSI), and 2D-GC-TOFMS was developed by Hoh et al.[48–50] The technique was applied to the analysis of organic chemicals of interest in fish oils. Figure 9 presents the diagram for the analytical approach used in one study and three additional cleanup methods including a second GPC step, silica solid-phase extraction (SPE), and acidification with H_2SO4 were compared.[48] Basically, in this approach, GPC provides initial sample cleanup which removes the bulk of the oil matrix. Sample cleanup comparison indicated that GPC-only extract provided enough cleanups for routine analysis. DSI enables the injection of larger sample size and provides for a high tolerance with dirty extracts. 2D-GC has the advantages of better separation and sensitivity. TOFMS provides full mass spectra information for the peak identification. Results of these studies demonstrated the many logistical and performance advantages using this approach, which has successfully been employed for the identification of target persistent organic pollutants and halogenated natural products, as well as un-target organic chemicals in fish oil samples. For example, seven 1,1′-dimethyl-2,2′-bipyrrole congeners were able to be tentatively identified for the first time.[48]

As a simple, fast, and easy to automate method, solid-phase micro extraction (SPME), or solid-phase micro extraction in the head-space mode (HS-SPME) has gained widespread acceptance when coupled with 2D-GC/TOFMS for the analysis of volatile and semi-volatile food components. Schurek et al.[51] used this approach for the determination of multiple pesticide residues in tea samples. In this approach, the performance of HS-SPME was optimized. Results from this research confirmed the powerful strength of this HS-SPME-2D-GC/TOFMS method as a fast and easy alternative for screening semi-volatile pesticide residues in tea samples. Purcaro et al.[52] employed the HS-SPME-2D-GC-TOFMS system for

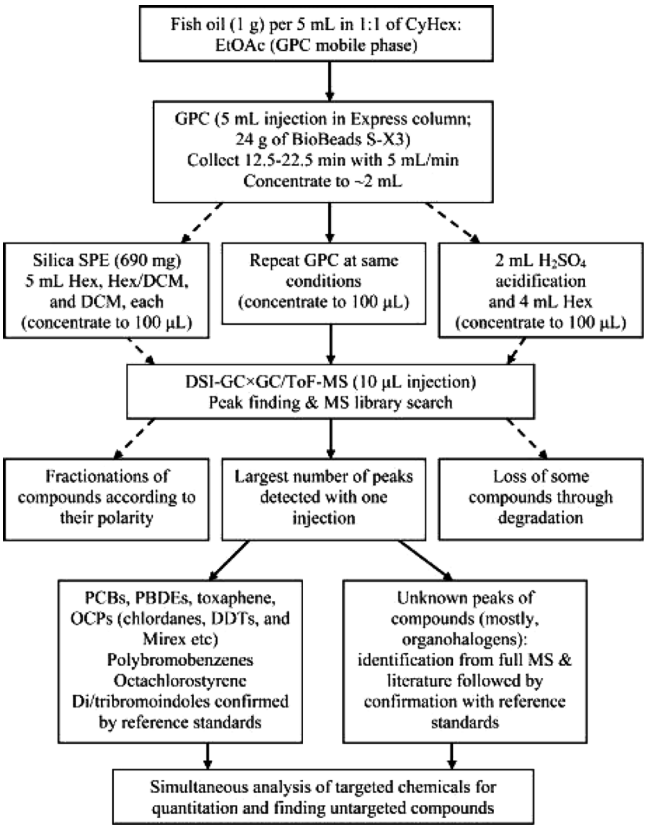

FIGURE 9 Diagram of the qualitative analytical approach used in the study. CyHex, Hex, EtOAc, and DCM signify cyclohexane, hexane, ethyl acetate, and dichloromethane, respectively. Nondashed arrows represent the approach with the widest scope. (Figure reprinted from Hoh et al.[48] with permission from the American Chemical Society, copyright 2009.)

determination of polycyclic aromatic hydrocarbons (PAHs) in vegetable oil samples. The developed HS-SPME-2D-GC-TOFMS method was validated in terms of linearity, accuracy, LODs, LOQs, and repeatability. This technique was shown to be a powerful approach for a fast analysis of PAHs. Other studies using this approach for different food products such as the analysis of honey volatiles[53] and volatile compounds in cacao beans,[54] and roasted coffee and hazelnut samples[55] were also reported.

The effect of the column combinations on 2D separations in 2D-GC was evaluated by Zhu.[56] With the analysis of Chinese liquor Moutai as an example, three "reversed-type" (polar/non-polar or medium polar) column combinations were studied in detail while a "normal" (non-polar/polar) column combination was used as a control (see Table 1). Using factor analysis, the degree of orthogonality was quantitatively

TABLE 1 2D-GC Column Sets

	1st Column	2nd Column
Set 1		
Stationary phase	HP-Innowax (Crosslinked polyethylene glycol)	DB-1701 (Crosslinked 14% cyanopropylphenylmethylpolysiloxane)
Length (m)	60	1.2
Diameter (mm)	0.25	0.1
Film thickness (μm)	0.25	0.4
Corporation	J&W Scientific, Folsom, CA	J&W Scientific, Folsom, CA
Set 2		
Stationary phase	DB-wax (Crosslinked polyethylene glycol)	DB-1701 (Crosslinked 14% cyanopropylphenylmethylpolysiloxane)
Length (m)	60	1.2
Diameter (mm)	0.25	0.1
Film thickness (μm)	0.25	0.4
Corporation	J&W Scientific, Folsom, CA	J&W Scientific, Folsom, CA
Set 3		
Stationary phase	Sol-gel-wax (Crosslinked polyethylene glycol)	DB-1701 (Crosslinked 14% cyanopropylphenylmethylpolysiloxane)
Length (m)	60	1.2
Diameter (mm)	0.25	0.1
Film thickness (μm)	0.25	0.4
Corporation	(SGE International, Ringwood, Vic., Australia)	J&W Scientific, Folsom, CA
Set 4		
Stationary phase	HP-1 (Crosslinked 100% dimethylpolysiloxane)	DB-1701 (Crosslinked 14% cyanopropylphenylmethylpolysiloxane)
Length (m)	60	1.2
Diameter (mm)	0.25	0.1
Film thickness (μm)	0.25	0.4
Corporation	J&W Scientific, Folsom, CA	J&W Scientific, Folsom, CA

(Table reprinted from Zhu[81] with permission from Elsevier, copyright 2009.)

estimated for different column combinations. The results presented the advantages of "reversed-type" column combinations in terms of correlation coefficient, spreading angle, theoretical and practical peak capacities for the analysis of Moutai liquor which mainly has some polar compounds. Moreover, the mechanism of solute-stationary phase interactions was explored through the test of Grob mixture and McReynolds constant calculation.

Time of flight mass analyzer is one of the most popular mass spectrometric detectors used in comprehensive 2D-GC systems. Other examples of 2D-GC-TOFMS applications published recently in food analysis are summarized in Table 2.

Mondello et al.[57] developed a system of 2D-GC-qMS for the analysis of trace-level pesticides contained in a complex red grapefruit extract. In this study, peak assignment was performed by a dual-filtered library search procedure. This twin-stage filter process included a minimum degree of spectral similarity as filter number one and the interactive use of linear retention indices (LRI) as filter number two. The library was constructed by analyzing commonly used pesticides by 2D-GC-qMS first, and then deriving the pure mass spectra and 2D-GC LRI values for each compound. Using this laboratory-constructed pesticide comprehensive GC-qMS library, the identification of the specific target compounds in a pesticide-contaminated red grapefruit extract was performed. Results demonstrated that this approach provided a more reliable structural elucidation of experimental MS spectra.

Other detection methods beside mass analyzers were also employed in comprehensive 2D-GC system. Khummueng et al.[58] investigated a 2D-GC system coupled to two parallel detectors: NPD and ECD. The analysis of multiclass pesticides in spinach extracts was performed. The efficiency and performance of NPD and ECD were investigated and compared. Figure 10 gave one example of the performance of NPD and ECD. Results proved that, with the dual detection system, increased information content, greater selectivity, and improved sensitivity were achieved. Haglund et al.[59] tested 2D-GC–micro electron capture detection (2D-GC-μECD) system for the analysis of polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) and dioxin-like polychlorinated biphenyls (PCBs) in food and feed. The detectability, repeatability, reproducibility, accuracy, and robustness of this method were evaluated. Although the results showed this method was a promising technique, deterioration of the chromatographic resolution caused by the postcolumn band broadening in the μECD and software for instrument control, quantification, and data visualization needed to be improved.

TABLE 2 Applications of 2D-GC-TOFMS in Food Analysis

Food Subject	Brief Description in Applications	References
Chinese Jiannanchun liquor	2D-GC/TOFMS was applied in the analysis of healthy components in Jiannanchun liquor. More than 100 kinds of functional components were found.	[82]
Potato chips	2D-GC/TOFMS was assessed among several other methods for the analysis of substituted pyrazines and related substances in potato chips. Better separation and better signal to noise ratio for target compounds were obtained with this approach. Moreover, a fingerprint screening and tentative identification of other 46 nitrogen-containing heterocyclic compounds was conducted.	[83]
Wheat grain	2D-GC/TOFMS was successfully applied for the analysis of trichothecene mycotoxins in wheat grain even without any cleanup step after extraction. It was the greater peak capacity of 2D-GC that made the separation of trichothecenes from matix interferences possible.	[84]
Coffee	2D-GC/TOFMS was used as a rapid method for the analysis of key trace-level aroma compounds in coffee products. Two different column set combinations (polar/medium polar and nonpolar/medium polar) were assessed.	[85]
Grapes	2D-GC/TOFMS was optimized for the separation and detection of pesticides in grape matrix. Compared with 1D GC-TOFMS, some co-eluted or interfering peaks of pesticides could be baseline separated and the limit of detection was significantly improved.	[86]
Animal feed sample: a cereal-based product	2D-GC/TOFMS method was developed for the target analysis of more than 100 pesticides and contaminants in a complex animal feed sample. Qualitative and quantitative performance of the 2D-GC system was characterized. This method was demonstrated to be a suitable approach for the qualitative and quantitative analysis of a wide range of pesticides and contaminants in feed samples at μg/kg levels.	[87]
Roast beef	2D-GC/TOFMS was applied for the analysis of sulfur compounds from the in-oven roast beef aroma. 50 of more than 70 sulfur compounds found were positively identified. Together with GC-SNIF analyses, six impact sulfur odorants in the roast beef top note was confirmed. The capability of direct detection of thiols in a non-derivatized form provided a more representative image of the volatiles. Moreover, some new beef sulfur compounds were identified for the first time.	[88]
Mussels	2D-GC/TOFMS technique was used for the analysis of unresolved complex mixtures of aromatic hydrocarbons bioaccumulated in mussel tissue extracts.	[89,90]

CLINICAL AND BIOLOGICAL

The publications of the applications of 2D-GC in the clinical and biological area are on the top list of all the publications of 2D-GC (23%

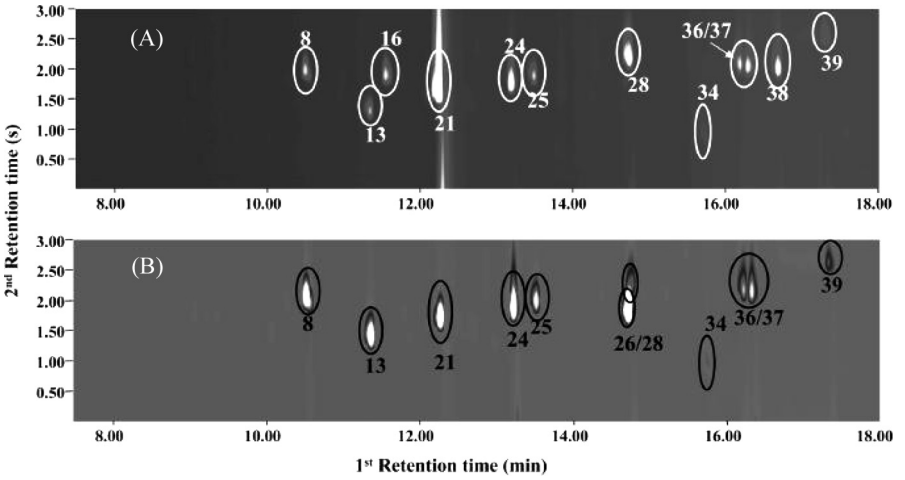

FIGURE 10 Contour plot of selected pesticides with simultaneous dual detection in 2D-GC. Chromatographic conditions used in this study were the fast temperature program. Selected pesticides comprised nine fungicides, one OP, and one OC. (A) Contour plot obtained from NPD detector. (B) Contour plot obtained from ECD detector. (The numbers on the figures represent individual pesticides listed in Khummueng et al.[58] which has been reprinted with permission from Wiley-VCH Verlag GmbH & Co. KGaA.)

of the total, see Figure 1). The 2D-GC technique has been penetrated to many different application areas in this field.

Yang et al.[60] studied targeted metabolites involved in the central carbon metabolism of the bacterium, *Methylobacterium extorquens* AM1 grown on two carbon sources, ethylamine (C2) and succinate (C4) by 2D-GC-TOFMS and LC-MS-MS. The metabolites studied are tricarboxylic acid cycle, serine cycle, ethylmalonyl-coenzyme A pathway and poly-b-hydroxybutyrate cycle. The Nucleotides, acyl-coenzyme As and a few volatile metabolites in cell extracts of M. extorquens AM1 were first separated using either a hydrophilic interaction LC or reversed-phase LC, and detected with good sensitivity by MS/MS. However, volatile intermediates within a low mass range ($<300\,m/z$), especially at low abundance (such as glyoxylic acid and others $<500\,nM$), were more effectively analyzed by 2D-GC-TOFMS which provided better sensitivity, resolution, and reproducibility. The complementary nature of the LC-based and GC-based methods allowed the comparison of 39 metabolite concentrations (the lowest level was at 139.3 nM). The authors overlapped the results obtained from both LC-based and GC-based methods of seven metabolites and provided a basis to check for consistency between the two methods and, thus, provided some validation of the quantification accuracy. The abundance change of 20 intermediates further suggested differences in pathways linked to C2 and C4 metabolites.

Kalinova et al.[61] studied the composition and the electrophysiological activity of constituents identified in male wing gland secretion of the

Bumblebee Parasite Aphomia sociella by 2D-GC. Several detection techniques including GC Fourier Transform Infrared Spectroscopy (GC-FTIR), enantioselective GC, electroantennography (EAG) GC with electroantennography detection (GC-EAD), and NMR were investigated. GC-EAD analysis of the male wing gland secretion revealed seven active areas, corresponding to 1-hexanol, 2-phenylethanol, [(R),(Z)]-nona-2,6-dien-4-olide, [(S),(Z)]-nona-6-en-4-olide, mellein, phytone, and a mixture of C18 fatty acids. SPME confirmed the presence of 2-phenylethanol, nona-2,6-dien-4-olide, nona-6-en-4-olide, mellein, and phytone in volatiles emanating from a calling male. Figure 11 shows a 2D-GC chromatogram of [(R),(Z)]-nona-2,6-dien-4-olide, [(S),(Z)]-nona-6-en-4-olide. Though

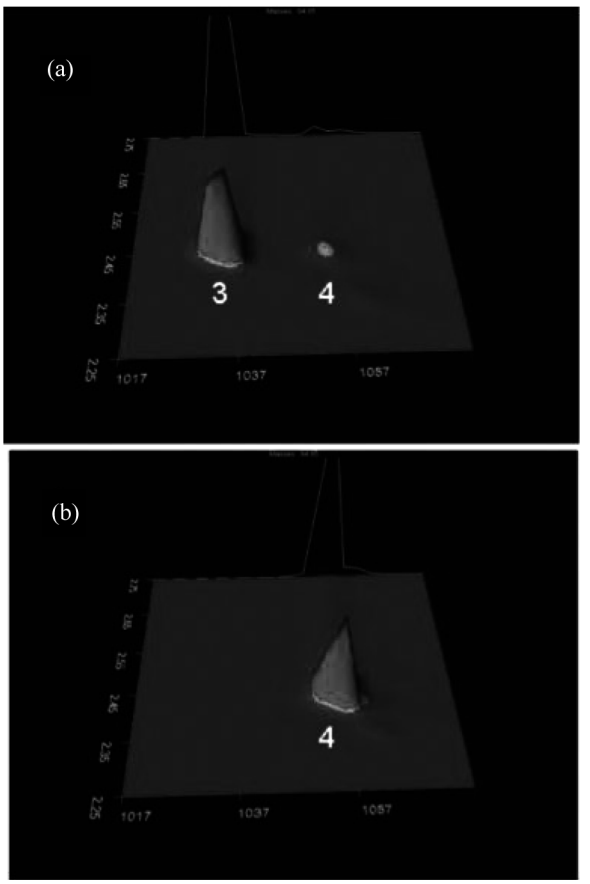

FIGURE 11 Reduction of (Z)-nona-2,6-dien-4-olide (3) and formation of (Z)-nona-6-en-4-olide (4). Two dimensional GC shows the area of lactones 3 and 4: (a) male wing gland extract; (b) product of the reduction. X-Axis shows the elution time in the first dimension; y-Axis shows the elution time in the second dimension. (Figure reprinted from Kalinová et al.[61] with permission from the American Chemical Society, copyright 2009.)

the abundance of these compounds differed slightly in SPME and gland secretion analysis, nona-2,6-dien-4-olide and mellein dominated in both samples, followed by 2-phenylethanol, nona-6-en-4-olide, and phytone. The strong antennal responses elicited by components of the secretion suggest that one or more of these compounds constitute the sex pheromone. Both sexes perceived male wing gland secretion, with females being significantly more sensitive compared to males.

Humston et al.[62] investigated Time-Dependent Profiling of Metabolites from Snf1 Mutant and Wild Type Yeast Cells by 2D-GC-TOFMS. Sixteen sample classes were investigated. The 2D-GC shows good selectivities for the sixteen sample classes and the authors demonstrated that the 2D-GC can be used in this area.

Due to the complexity of the human biology samples such as plasma, serum, or urine (usually contains about 103–107 compounds), the analysis of these samples are very challenging. Culbertson et al.[63] used 2D-GC-TOFMS for the profiling of the human plasma metabolome. The authors demonstrated that the 2D-GC-TOFMS technique is a preferable tool for the separation of the components in plasma. The authors compared the 2D-GC results from a "control" cohort (81 samples) and a cardiovascular-compromised cohort (15 samples) of individuals. The results from the 2D-GC separation provide more than an order of magnitude greater resolving power but did not affect the overall experimental run time. In addition, the 2D-GC approach provides enough information for the datasets that can be used to distinguish the control and disease sample cohorts. Such results suggest that 2D-GC-TOFMS techniques can be used for the development of personalized medicine in the disease prevention, diagnosis, and treatment due to an individual's unique molecular makeup. Challenges to full implementation of high-throughput metabolite profiling by 2D-GC-TOFMS as well as future directions are also discussed.

Ralston-Hooper et al.[64] developed 2D-GC-TOFMS methods for the separation of metabolomics used in ecotoxicological studies with invertebrates. Conventionally, 1H NMR technique is used for this kind of study. However, the major limitations of NMR-based techniques are the low sensitivity that results in an examination of a limited numbers of metabolites. An alternative approach is the use of LC or GC for separation of metabolites and mass spectrometry (MS) for their quantification and identification. In this paper, the author evaluated the 2D-GC-TOFMS technique coupled with multivariate analysis. The authors compared metabolite profiles obtained from the 2D-GC-TOFMS between Diporeia collected from Lake Michigan (declining populations) to those residing in Lake Superior (stable populations), and also between Diporeia exposed to a chemical stressor (atrazine) and controls. Overall, 76 and 302 total metabolites were detected from the lake comparison and atrazine studies, respectively.

Most of the identified metabolites are fatty acids, amino acids, and hydrocarbons. The authors also observed unique and almost non-overlapping metabolite profiles in both studies. The authors concluded that 2D-GC-TOFMS can be used to detect metabolites and to compare the differences between invertebrate groups sampled under different environmental conditions. This ability to detect unique metabolite profiles under different environmental conditions will increase our understanding on the physiology processes and whole-organism responses occurring as a result of exposure to different environmental stressors. Figure 12 shows a 2D-GC chromatogram and a mass spectrum of L-aspartic acid which was found in the investigated samples.

Ozel et al.[65] studied the chemical composition of volatile oils from leaves and flowers of Sideritis congesta using direct thermal desorption (DTD) – 2D-GC-TOFMS. Volatile oils from the leaves and flowers of Sideritis congesta, a plant endemic to Southern Anatolia, Turkey, were freed using DTD and then analyzed by 2D-GC-TOFMS. Forty one different volatile components from the plant leaves and fifty four compounds from the flowers were identified. A comparison of the flower volatiles resolved using the different thermal desorption methods indicated forty three common components. Major organic compounds found from theses samples were a-pinene (12.53–14.55%), b-pinene (17.15–25.34%), and d-cadinene (10.97–14.52%). Results from manual and automated DTD methods are similar.

Robrock et al.[66] studied the pathways of anaerobic microbial debromination of polybrominated diphenyl ethers (PBDE) by 2D-GC. Debromination pathways for 7 PBDEs by 3 different anaerobic dehalogenating

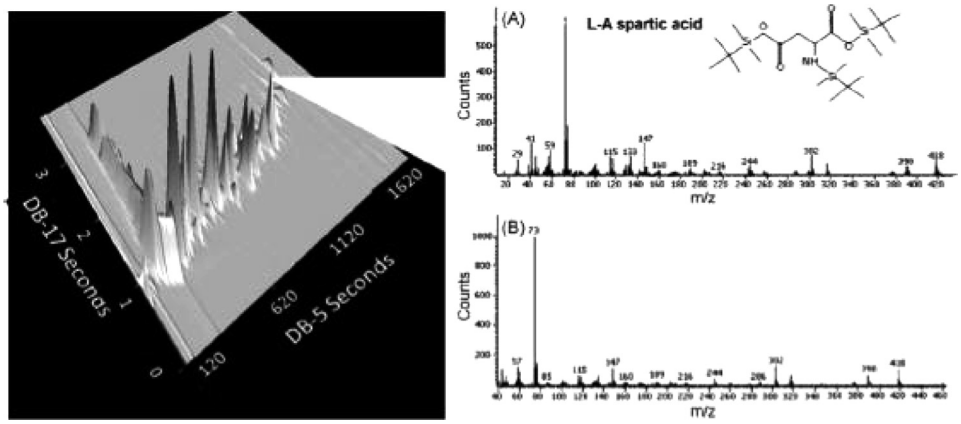

FIGURE 12 2D-GC chromatogram and mass spectra for down-regulated metabolite L-aspartic acid for the atrazine-exposed and control comparison. (A) Actual mass Spectra for L-aspartic acid and (B) matched mass spectra from NIST library. (Figure reprinted from Ralston-Hooper et al.[64] with permission from Elsevier, copyright 2008.)

bacterial cultures were examined. Five major components of the industrially used octa-BDE mixtures (PBDE 196, 203, 197 [octa-BDE], PBDE 183 [hepta-BDE], PBDE 153 [hexa-BDE]) and the two most commonly detected PBDE in the environment (PBDE 99 [penta-BDE], PBDE 47 [tetra-BDE]) were identified and quantified. The studied dehalogenating cultures included a trichloroethene-enriched consortium which contains multiple dehalococcoides species, two pure cultures, dehalobacter restrictus PER-K23, and desulfitobacterium hafniense PCP-1. PBDE were analyzed by 2D-GC with ECD to maximize separation and identify product congeners. All studied congeners were debrominated to some extent by the bacterial cultures and all exhibited similar debromination pathways with preferential removal of para- and meta-brimine. Debromination of the highly brominated congeners was extremely slow; usually $<10\%$ of nano molar PBDE concentrations were transformed after 3 months. Debromination of the lesser brominated congeners, e.g., PBDE 99 and PBDE 47, was faster; some cultures completely debrominated nanomolar concentrations of PBDE 47 within weeks.

Lu et al.[67] determined neutral chemical constituents in flue-cured tobacco by 2D-GC-TOFMS. A DB-Petro column ($50\,m \times 200\,mm \times 0.5\,mm$) was chosen as the column for the first dimension, and a DB-1701 column ($2.3\,m \times 100\,mm \times 0.1\,mm$) was chosen as the column for the second dimension. The modulation period was set at eight seconds. The initial temperatures of the two columns were set at 80°C and 85°C, respectively, and then increased with temperature programming. The 2D-GC results show that the total contents of the 24 neutral fractions in the middle leaves were the most, then in the upper leaves and the least was in the lower leaves. These contents in the flue-cured tobacco produced by Brazil were the highest, followed by Zimbabwe, Yunyan85, Zhongyan101, NC89, and K326. In four kinds of tobaccos, the total contents of the neutral fractions in Oriental tobacco were the highest, followed by Burly tobacco, Flue-cured tobacco, and Maryland tobacco.

Li et al.[68] studied the metabonomics with recognition methods by analyzing plasma from diabetic patients and healthy controls pattern with 2D-GC-TOFMS technique. With the 2D-GC-TOFMS technique, diabetic patients, and healthy control patients could be correctly distinguished based on the metabolic abnormity in plasma. Five potential biomarkers including glucose, 2-hydroxyisobutyric acid, linoleic acid, palmitic acid, and phosphate were separated and identified. It was found that elevated free fatty acids were essential pathophysiological factors in diabetes mellitus which reflected either the hyperglycemia or the deregulation of fatty acids metabolism. These potential biomarkers in plasma, e.g., palmitic acid, linoleic acid, and 2-hydroxybutyric acid might be helpful in the diagnosis or further study of diabetes mellitus. This study shows the practicability and

advantage of 2D-GC-TOFMS coupled with data analysis for metabonomics in biomarker discovery.

FORENSICS

Recently 2D-GC technique has received more and more attention in the forensics drug control area. An approach using 2D-GC-TOFMS was investigated in human doping control field.[69] The study focused on five key anabolic agents: clenbuterol, norandrosterone, epimetendiol, two methyltestosterone metabolites, and 3′-hydroxystanozolol. They were at a concentration level of two ng/ml in spiked blank urine samples. Results proved that the 2D-GC-TOFMS technique was an effective approach for the screening of the five key components at the lowest purported concentration levels. Moreover, this approach can be used as a full spectra confirmatory method for the identification of these anabolic substances. Stepan et al.[70] reported an application of 2D-GC-TOFMS for the determination of anabolic steroids and related compounds in nutritional supplements. The method was validated and the limits of quantification were calculated.

Guthery et al.[71] developed a technique using 2D-GC-TOFMS for the detection of various opiates and benzodiazepines in human serum. Solid-phase extraction (SPE) was employed for the sample cleanup. For the column combinations, the first dimension used a J&W DB-5 ms capillary column ($30\,m \times 0.25\,mm \times 0.25\,\mu m$) to separate components based on volatility and the second dimension used an SGE BPX50 column ($2\,m \times 0.1\,mm \times 0.1\,\mu m$) to separate components by polarity. In this research, the sensitivity and repeatability of the method were evaluated and the limits of detection (LODs) and limits of quantitation (LOQs) were calculated as well. The study showed that the 2D-GC-TOFMS approach provided sufficient selectivity and sensitivity for forensic drug testing. With TOFMS as detector, this approach holds a potential to be able to identify thousands of compounds in a complex biological matrix.

Heroin and cannabis are two of the most common illicit drugs in Western Europe. Groeger et al.[72] employed 2D-GC-TOFMS and 2D-GC-FID techniques for the chemical profiling of illicit drug samples. Sixteen samples of cannabis were extracted and analyzed by 2D-GC-FID, while twenty one heroin extracts were analyzed by 2D-GC-TOFMS. A pixel-based chemometric data processing method was applied. In this data processing approach, 2D data sets were first preprocessed based on single steps of background correction, alignment of chromatograms, and normalization. Then, a statistical analysis was applied to identify the chemically similar samples and to further identify promising marker compounds. The chemical profiling of illicit drug samples could be linked to the classification of

seizures. With the results achieved in this study, this pixel-based chemometric approach combined with 2D-GC has demonstrated to have the applicability for the chemical profiling of illicit drug seizures. However, more work with larger sample sets and with the optimization of sample preparation and extraction steps were still needed.

2D-GC-MS has been applied for the analysis of 3,4-methylenedioxyethylamphetamine (MDEA), 3,4-Methylenedioxymethamphetamine MDMA, and its metabolites, 3,4-methylenedioxyamphetamine (MDA), 4-hydroxy-3-methoxymethamphetamine (HMMA), and 4-hydroxy-3-methoxyamphetamine (HMA) in human plasma. Kolbrich et al.[73] reported the first 2D-GC-MS approach for the analysis of these five amine analytes in one method. In their 2D-GC-MS set-up, a microfluidic Deans switch was used to direct the outlet of the first column to either the FID detector or the inlet of the secondary column. A compressed air–cooled cryogenic focusing trap was applied at the inlet end of the second column. The outlet of the second column was connected to a HP5973 mass selective detector (MSD). This MSD was operated in electron-impact selected ion-monitoring mode. The method was validated and very low LOQs were achieved. The results proved that this approach was an effective tool for selective analyte detection and quantification in very complex matrices such as plasma, blood, and oral fluid. Earlier, Lowe et al.[74] from the same facility reported results using this approach for the simultaneous quantification of Δ^9-tetrahydrocannabinol (THC), 11-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-THC), and 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THCCOOH) in human plasma.

Meconium is a complex specimen containing materials that provide information on fetal exposure to drugs of abuse. 2D-GC is an effective technique to overcome the matrix interferences caused by the complex composition of meconium. Marin et al.[75] developed a 2D-GC/MS method for the determination of 9-carboxy-11-nor-Δ^9-THC (9-THCA) and 11-hydroxy-Δ^9-THC (11-OH-THC) in meconium. For their 2D-GC set-up, the primary column was a DB-5 MS (15 m, 0.25 mm ID, 0.25 μm), and the secondary column was a DB-17 MS (15 m, 0.25 mm ID, 0.25 μm). Electron-impact selective ion monitoring mass spectroscopy was used for 2D-GC-MS analysis. A Dean Switch assembly and a cryo trap were employed to facilitate 2D-GC separation. Conditions the sample preparation, the extraction method and for the derivatizing agent were optimized to reduce sample preparation time. With 46 positive patient specimens and 70 spiked samples, this method was validated for accuracy, precision, linearity, analytical measurement range, specificity, and carryover. Results from the comparison of GC-MS method and the Dean Switch method indicated that this Dean Switch 2D-GC-MS approach provided improved chromatography and reduced carryover. This resulted in a better, more robust method for the

analysis of cannabinoids in meconium. Meanwhile, Moore et al.[76] reported a similar approach using 2D-GC-MS for the detection of conjugated 11-nor-Δ^9-tetrahydrocannabinol-9-carboxylic acid (THCA) in oral fluid for the first time.

PHARMACEUTICALS

In the pharmaceutical area, 2D-GC has been applied on the samples with very complicated matrices such as components in herbs. Qiu et al.[77] reported a technique to determine the radix ginseng volatile compounds at different ages by 2D-GC-TOFMS. Thirty-six terpenoids were identified based on the MS library search and retention index in a ginseng sample at the age of 3 years. An obvious group-type separation was obtained in the 2D-GC-TOFMS chromatogram. The data generated by 2D-GC-FID were processed using a principal component analysis (PCA) method to classify the samples at different ages. The compounds responsible for the significant differentiation among samples were identified. It was found that the relative abundances of α-cadinol, α-bisabolol, thujopsene, and n-hexadecanoic acid significantly rise with the increase in age of the plant.

3,4-Methylenedioxymethamphetamine (MDMA, or Ecstasy) is a popular recreational drug. Analysis of MDMA and its metabolites in human plasma, particularly in pharmacokinetic studies, requires low limits of quantification. 2D-GC-MS with cry-focusing is a chromatographic technique recognized for its increased sensitivity and resolution in this area.

Kolbrich et al.[78] studied the plasma pharmacokinetics of 3,4-methylenedioxymethamphetamine (MDMA) and metabolites 4-hydroxy-3-methoxymethamphetamine (HMMA), 3,4-methylenedioxyamphetamine (MDA), and 4-hydroxy-3-methoxyamphetamine (HMA) in young adults for up to 143 hours after drug administration by 2D-GC. Seventeen female and male participants (black, white, and Hispanic) received placebo, low (1.0 mg/kg), and high (1.6 mg/kg) oral MDMA doses (comparable to recreational doses) in a double-blind, randomized, balanced, within-subject design while residing on a closed research unit. Doses were separated by one week or more. A fully validated 2D-GC-MS method simultaneously quantified MDMA, HMMA, MDA, and HMA. Calibration curves were for MDA, 1 to 100 ng/mL; for HMA, 2.5 to 100 ng/mL; and for both MDMA and HMMA, 2.5 to 400 ng/mL. The mean standard deviation at maximum plasma concentrations (Cmax) are 162.9 ± 39.8 and 171.9 ± 79.5 ng/mL for MDMA and HMMA, respectively, after low-dose MDMA. After the high dose, mean MDMA Cmax significantly increased to 291.8 ± 76.5 ng/mL; whereas, mean HMMA Cmax was unchanged at 173.5 ± 66.3 ng/mL. High inter subject variability in Cmax was observed. Mean MDA Cmax were 8.4 ± 2.1 (low)

and 13.8 ± 3.8 (high) ng/mL. HMA Cmax were 3.5 ± 0.4 and 3.9 ± 0.9 ng/mL after the low and high doses, respectively. AUC_{∞} displayed similar trends to Cmax, demonstrating a nonlinear pharmacokinetics behavior. Times of last plasma detection were generally HMA $<$MDA$<$ MDMA$<$ HMMA. Mean half-lives (t1/2) of MDMA, MDA, and HMMA were approximately 7 to 8 hours, 10.5 to 12.5 hours, and 11.5 to 13.5 hours, respectively. HMA t1/2 showed high variability. Mean MDMA volume of distribution was constant for low and high doses; clearance was significantly higher after the low dose. This study presents MDMA plasma pharmacokinetic data for the first time from blacks and females as well as measurement of HMMA and HMA concentrations after low and high MDMA doses and more frequent and extended plasma sampling than in prior studies.

Residual solvents in drug substance have to be controlled to a certain limits. Crimi and Snow[79] reported a method for the separation of International Conference on Harmonization and USP 1, 2, and 3 class pharmaceutical solvents. A 5% phenylpolydimethylsiloxane column was used as primary column and a polyethylene glycol column was used as secondary column. The total run time is 30 minutes. The significantly improved peak capacity in 2D-GC mode allows a single method for any combination of solvents and mitigates all interference. An example of separations of 1000 ppm standards of 57 of the class 1, 2, and 3 solvents were demonstrated.

Qiu et al.[80] studied the volatile oil from the traditional Chinese medicine rhizomes and radixes of Notopterygium incisum Ting ex H.T. from different regions of China by 2D-GC-TOFMS and 2D-GC-FID. A total of 769 compounds were identified and quantified in a typical sample from the Sichuan province, a producing area for the Genuine Medicinal Materials. Identification and quantitation results showed that Qianghuo from

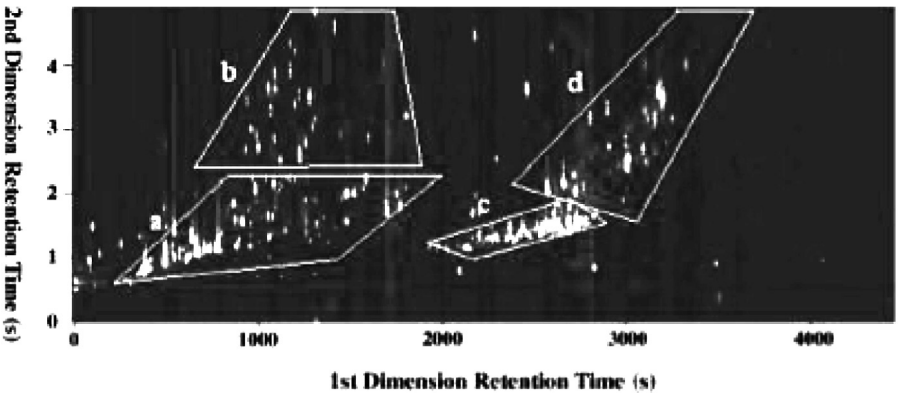

FIGURE 13 The 2D-GC-TOFMS contour plots of Qianghuo volatile oil. Zones (a–d) are mainly monoterpenes, oxygenated monoterpenes, sesquiterpenes, and oxygenated sesquiterpense, respectively. Reprinted from Qiu et al.[80] with permission from Elsevier, copyright 2007.

Sichuan province has some significant differences in the chemical content from other geographic origin for the herbs, especially in monoterpenes and oxygenated sesquiterpenes. The data for all individual peaks collected by 2D-GC-FID were processed using a principal component analysis (PCA) method to classify the samples from the different regions. The abundance of monoterpenes and oxygenated sesquiterpenes were responsible for the differentiation, which is in good agreement with the group quantitation results of 2D-GC analysis. Figure 13 shows the 2D-GC-TOFMS contour plots of the volatile oil of a Qianghuo medicine sample.

CONCLUSIONS

2D-GC combined with different detection techniques is clearly a relatively mature technique today. It has been used in variety of fields including clinical/biological, environmental, petroleum, food, fragrance, and pharmaceutical. New application areas are still being reported. The available instrumentation is capable of combining with different detection techniques such as TOFMS. From the large number of publications, we can conclude that application of the technique is still expanding, and the applications are penetrating into newer fields.

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