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Journal of Chromatography A, 1019 (2003) 143–156
JOURNAL OF CHROMATOGRAPHY A
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Qualitative evaluation of thermal desorption-programmable temperature vaporization-comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry for the analysis of selected halogenated contaminants

Jean-François Focant*, Andreas Sjödin, Donald G. Patterson Jr.

Centers for Disease Control and Prevention (CDC), National Center for Environmental Health (NCEH), Division of Laboratory Sciences (DLS), Organic Analytical Toxicology (OAT) Branch, 4770 Buford Hwy NE, Mail Stop F-17, Atlanta, GA 30341, USA

Abstract

The separation of 38 toxic and predominant polychlorinated biphenyl (PCB) congeners, 11 persistent halogenated pesticides, 1 brominated biphenyl (BB), and 8 polybrominated diphenyl ethers (PBDEs) has been optimized using comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC × GC–TOFMS). A thermal desorption-programmable temperature vaporization (TD-PTV) step was used for the injection. Different column sets were investigated, and a 100% dimethylpolysiloxane (15 m × 0.25 mm i.d. × 0.25 μm film thickness) narrowbore capillary column coupled to a high temperature (8% phenyl)-polycarborane-siloxane (2 m × 0.10 mm i.d. × 0.10 μm film thickness) microbore column set was selected. Of the 58 compounds investigated, only one pair of PCBs was not resolved. All other analytes were either baseline separated into the chromatographic plane or were virtually separated using the deconvolution capability of the TOFMS.

Keywords: Thermal desorption; Gas chromatography, comprehensive two-dimensional; Polychlorinated biphenyls; Polybrominated diphenyl ethers; Pesticides

1. Introduction

Many compounds are grouped under the general "halogenated environmental pollutants" appellation. Among them, chlorinated compounds such as polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs), and chlorinated persistent pesticides are at or near the top of the list in terms of toxicity. These compound classes are some of the most extensively studied within the persistent organic pollutants (POPs) group because they can be found in human matrices at significant levels. Development of high throughput specific and sensitive methods is needed to ensure rapid and reliable monitoring of

* Corresponding author. Tel.: +1-770-488-0184; fax: +1-770-488-0142.
E-mail addresses: jfocant@cdc.gov, jvf8@cdc.gov (J.-F. Focant).

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doi:10.1016/j.chroma.2003.07.007

POPs. Ideally, all these compounds should be analyzed simultaneously for the various investigated matrices. However, because of the many congeners and isomers that need to be analyzed, and the large concentration range to cover, implementation of a sample preparation step that contains all of the compounds of interest in one extract is not attainable in practice. This is related, not to any limitation or drawback of the sample preparation itself, but to the analytical tools used at the final stage of the procedure. Such tools usually are based on gas chromatography (GC) coupled to sensitive detectors such as electron-capture detectors (ECDs), or various types of mass spectrometry (MS) instruments such as high-resolution (HR) magnetic sectors, quadrupole ion storage (QIST) or time-of-flight (TOF).

Even if it is a well suited approach to measure these compound classes, some drawbacks like GC peak co-elutions (for reasonable time scales), limited acquisition rates, lack of sensitivity, and limited separation power (selectivity) can be observed. To overcome these limitations, methodologies used to measure these compound classes need to incorporate a fractionation approach to efficiently separate analytes into sub-classes depending, for example, on their structures and concentrations [1–3]. After this fractionation process, several parallel injections, analyses and data processing steps have to be performed separately before the recombination of results to produce a final report. The increase in the number of analytes (polybrominated diphenyl ethers [PBDEs], polybrominated dibenzo-*p*-dioxins [PBDDs], polybrominated dibenzofurans [PBDFs], toxaphenes, and more) being requested for bio-monitoring studies will result in more method fractions for analysis. To eliminate the multiple fractionation steps, a more versatile analytical tool is needed to accommodate a multi-group analytical procedure.

Comprehensive two-dimensional GC (GC × GC) has emerged last 15 years as a technique with many advantages over one-dimensional GC [4,5]. The most interesting characteristics are increased peak capacity, increased sensitivity (signal enhancement after zone compression) and selectivity, independent retention processes in the two dimensions (orthogonality principle) and identification of each substance by two independent retention times [6]. GC × GC therefore can be considered as a potential tool to be integrated in a multi-group analytes procedure [7–9]. By comparison to early studies carried out with less stable GC × GC systems [10], this technique has become increasingly available because of development of different robust modulation devices [11–17]. Recent reports [18–20] presented GC × GC as a highly suitable technique for congener-specific measurement of selected PCBs.

Furthermore, time-of-flight mass spectrometry (TOFMS) appears to be suited to accommodate fast measurement of such compounds [21,22], while providing MS detection for GC × GC. This non-mass-scanning device allows collection of all ions in the same time, offering valuable comprehensive mass analysis. Additionally, because all ion fragments represent the same time point on the chromatographic peak, there is no concentration bias, compared with scanning mass spectrometers. TOF, therefore, further offers spectral continuity over the entire GC peak. This important feature allows mass spectral deconvolution of overlapping peaks if the fragmentation pattern is different. This reduces the chromatographic resolution requirements and decreases the analysis time [23]. Deconvoluted ion current (DIC) can thus be used to solve chromatographic co-elution problems, and TOFMS therefore acts as an analyte-separation tool. Coupling of the GC × GC chromatographic resolution capability with the deconvolution capabilities of TOFMS is a promising tool for analyte resolution [24–27]. This combination can offer the required separation power to accommodate many more analytical peaks than classical (one-dimensional) GC–(scanning) MS.

On the sample preparation side, we investigated the area of thermal desorption (TD) to take advantage of the emerging stir-bar-sorptive extraction (SBSE) technique, which reportedly can accommodate many types of analytes [28,29]. This technique is based on static sorption of extracted analytes in a polydimethylsiloxane (PDMS) phase, as in solid-phase microextraction (SPME), except that higher quantities of PDMS are coated on a stir-bar that is immersed directly in the liquid sample to be extracted [30]. Compared with SPME, higher capacities and thus lower detection limits (LODs) can be achieved. In addition, because the present target analytes exhibit good thermal stability as well as high PDMS–water distribution coefficients, good recoveries can be expected. Because a significantly long desorption time

is required for the PDMS-coated stir-bar after extraction, the TD step requires some venting and focusing before the actual injection onto the GC column. This is achieved using a programmable temperature vaporization (PTV) injector in which desorbed compounds are cryo-trapped before rapid heating to produce a sharp GC injection. Recently reported results demonstrate low part-per-trillion (ppt) level limit of quantification (LOQ) for the analysis of selected PCBs in human sperm using SBSE–TD–(one-dimensional) GC–(scanning) MS [31].

We aimed to set up a preliminary qualitative study of a thermal desorption-programmable temperature vaporization-comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry (TD-PTV–GC × GC–TOFMS) method for the analysis of a group of 58 selected POPs. The analyte group comprised a set of 38 environmentally threatening PCBs [32,33], 11 persistent pesticides, 1 brominated biphenyl (BB), and 8 PBDEs that are routinely measured in human samples using various independent methodologies in our laboratory.

2. Experimental

2.1. Chemicals

The PCB standard solution was CS-10 (EC-5022) (1 ng/μl) from Cambridge Isotope Laboratories (Andover, MA, USA). This calibration standard solution contained a mixture of the 38 selected PCBs.

The PBDE standard solution was made from individual congeners. The following compounds were obtained from AccuStandard (New Haven, CT, USA), 2,4′,4-triBDE (BDE-28), 2,2′,4,4′-tetraBDE (BDE-47), 2,3′,4,4′-tetraBDE (BDE-66), 2,2′,3,4,4′-pentaBDE (BDE-85), 2,2′,4,4′,5-pentaBDE (BDE-99), 2,2′,4,4′,6-pentaBDE (BDE-100), 2,2′,4,4′,5,5′-hexaBDE (BDE-153), 2,2′,4,4′,5,6′-hexaBDE (BDE-154). 2,2′,4,4′,5,5′-HexaBB (BB-153) was from Wellington Laboratories (Shawnee Mission, KS, USA). The concentrations in the mixture were set to be 1 ng/μl for all the PBDEs, excepted for BDE-28 which was 2 ng/μl. The solvent was nonane with a small percentage of isooctane.

The persistent pesticides standard solution was CS-8 (ES-5019) (1 ng/μl) from Cambridge Isotope Laboratories. This calibration standard solution contained a mixture of 11 persistent pesticides routinely analyzed in our laboratory.

Using these solutions, we prepared a multi-group analyte mixture containing the PCBs, PBDEs, and persistent pesticides (Table 1).

2.2. Thermal desorption (TD) and programmable temperature vaporization (PTV)

Standards (1 μl) were spiked into desorption tubes where the stir-bars normally would sit for desorption after sample extraction. The desorption tubes were placed in a thermodesorption system (Gerstel Inc., Baltimore, MD, USA). This system consists of two PTV injectors placed in series. The first PTV is the thermal desorption unit (TDS-2) in which desorption tubes are loaded and where standard spikes were desorbed according to an optimized program (Fig. 1). The second PTV (CIS-4) injector was used to cryo-focus the desorbed compounds before the injection of the trapped analytes onto the first GC column. Details are available elsewhere [30]. Both PTVs use liquid nitrogen as coolant. Fairly high temperatures were required to efficiently desorb the targeted high-mass compounds. They were trapped in an empty baffled glass liner. As was reported in another study [31], some time was left between the end of the desorption step and the actual GC injection in order to stabilize the gas flow after closing the split valve.

2.3. Comprehensive two-dimensional gas chromatography–time-of-flight mass spectrometry (GC × GC–TOFMS)

The GC × GC–TOFMS instrument was the Pegasus 4D (Leco Corp., St. Joseph, MI, USA). This system is based on a non-moving quad-jet modulator made of two permanent cold nitrogen jets and two pulsed hot air jets that are responsible for trapping and refocusing compounds eluting from the first dimension column. This modulator is mounted in an Agilent 6890 GC oven (Palo Alto, CA, USA), and liquid nitrogen is used to create the cold jets. Fig. 2 illustrates the process responsible for the sharp re-injections of trapped analytes into the second column. The temperature of the modulator had an offset of 60 °C compared with the temperature of the primary GC oven.

Table 1
List of the compounds included in the present study

Compound	Number	MW (g/mol)
2,2′,5-TriCB	CB-18	256
2,4′,4-TriCB	CB-28	256
2,2′,3,5′-TetraCB	CB-44	290
2,2′,4,5′-TetraCB	CB-49	290
2,2′,5,5′-TetraCB	CB-52	290
2,3′,4,4′-TetraCB	CB-66	290
2,4,4′,5-TetraCB	CB-74	290
2,2′,3,4,5′-PentaCB	CB-87	324
2,2′,4,4′,5-PentaCB	CB-99	324
2,2′,4,5,5′-PentaCB	CB-101	324
2,3,3′,4,4′-PentaCB	CB-105	324
2,2′,3,4′,6-PentaCB	CB-110	324
2,3′,4,4′,5-PentaCB	CB-118	324
2,2′,3,3′,4,4′-HexaCB	CB-128	358
2,2′,3,4,4′,5′-HexaCB	CB-138	358
2,2′,3,4′,5,5′-HexaCB	CB-146	358
2,2′,3,4′,5′,6-HexaCB	CB-149	358
2,2′,3,5,5′,6-HexaCB	CB-151	358
2,2′,4,4′,5,5′-HexaCB	CB-153	358
2,3,3′,4,4′,5-HexaCB	CB-156	358
2,3,3′,4,4′,5′-HexaCB	CB-157	358
2,3,3′,4,4′,6-HexaCB	CB-158	358
2,3′,4,4′,5,5′-HexaCB	CB-167	358
2,2′,3,3′,4,4′,5-HeptaCB	CB-170	392
2,2′,3,3′,4,5,5′-HeptaCB	CB-172	392
2,2′,3,3′,4′,5,6-HeptaCB	CB-177	392
2,2′,3,3′,5,5′,6-HeptaCB	CB-178	392
2,2′,3,4,4′,5,5′-HeptaCB	CB-180	392
2,2′,3,4,4′,5,6-HeptaCB	CB-183	392
2,2′,3,4′,5,5′,6-HeptaCB	CB-187	392
2,3,3′,4,4′,5,5′-HeptaCB	CB-189	392
2,2′,3,3′,4,4′,5,5′-OctaCB	CB-194	426
2,2′,3,3′,4,4′,5,6-OctaCB	CB-195	426
2,2′,3,3′,4,4′,5′,6-OctaCB	CB-196	426
2,2′,3,3′,4,5,5′,6-OctaCB	CB-201	426
2,2′,3,4,4′,5,5′,6-OctaCB	CB-203	426
2,2′,3,3′,4,4′,5,5′,6-NonaCB	CB-206	460
2,2′,3,3′,4,4′,5,5′,6,6′-DecaCB	CB-209	494
2,4′,4-TriBDE	BDE-28	407
2,2′,4,4′-TetraBDE	BDE-47	486
2,3′,4,4′-TetraBDE	BDE-66	486
2,2′,3,4,4′-PentaBDE	BDE-85	565
2,2′,4,4′,5-PentaBDE	BDE-99	565
2,2′,4,4′,6-PentaBDE	BDE-100	565
2,2′,4,4′,5,5′-HexaBDE	BDE-153	644
2,2′,4,4′,5,5′-HexaBB	BB-153	628
2,2′,4,4′,5,6′-HexaBDE	BDE-154	644
Hexachlorobenzene (HCB)	–	285
β-Hexachlorocyclohexane (β-HCH)	–	291
Lindane (γ-HCH)	–	291
Heptachlor epoxide	–	389
Oxychlordane	–	410
trans-Nonachlor	–	444
Dieldrin	–	381
2,4′-DDT	–	355
4,4′-DDT	–	355
Mirex	–	546
4,4′-DDE	–	318

The compounds correspond to some of the major analytes targeted in the ongoing in-house epidemiologic studies. Concentrations were 300 pg/μl, excepted for BDE-28.

The primary GC oven was programmed as follows: 90 °C for 1 min, then to 150 °C at 10 °C/min, then to 250 °C at 3 °C/min, then to 290 °C at 5 °C/min and held for 2 min. The second dimension column was coiled in the secondary oven that was 50 °C higher than the primary oven (iso-ramping mode). The modulator period was 4 s (0.25 Hz modulation frequency), and the hot-pulse duration was 600 ms. Pure GC grade helium, was used as carrier gas at a constant flow of 1 ml/min.

Different dual column sets were tested during the present work. For all sets, a conventional non-polar first dimension phase was used. This first dimension column was a DB-1MS 100% dimethylpolysiloxane ($L[L = 30, 15, 8]$ m × 0.25 mm i.d. × 0.25 μm film thickness) narrow bore capillary column (J&W Scientific, Folsom, CA, USA). For all sets, the second dimension column was shorter (column ratio higher than 1) and made of a more polar phase than the first one. Different micro bore capillary column phases (2 m × 0.10 mm i.d. × 0.10 μm film thickness) were tested for the second dimension, a DB-17 (50%-phenyl)-methylpolysiloxane (Agilent, Wilmington, DE, USA), a CPWAX-52CB polyethylene glycol (Varian, Walnut Creek, CA, USA), a DB-1701 (14%-cyanopropyl-phenyl)-methylpolysiloxane (Agilent), and a high-temperature HT8 (8% phenyl)-polycarborane-siloxane (SGE, Austin, TX, USA). Among the many types of presstight connectors evaluated, the deactivated universal presstight connectors from Restek Corp. (Bellefonte, PA, USA) gave the best results for connecting narrow bore and micro bore capillary columns. Leak-free connections were ensured by careful inspection of the column cuts and cleaning the end of the columns using acetone to remove any finger grease. The connection was cured

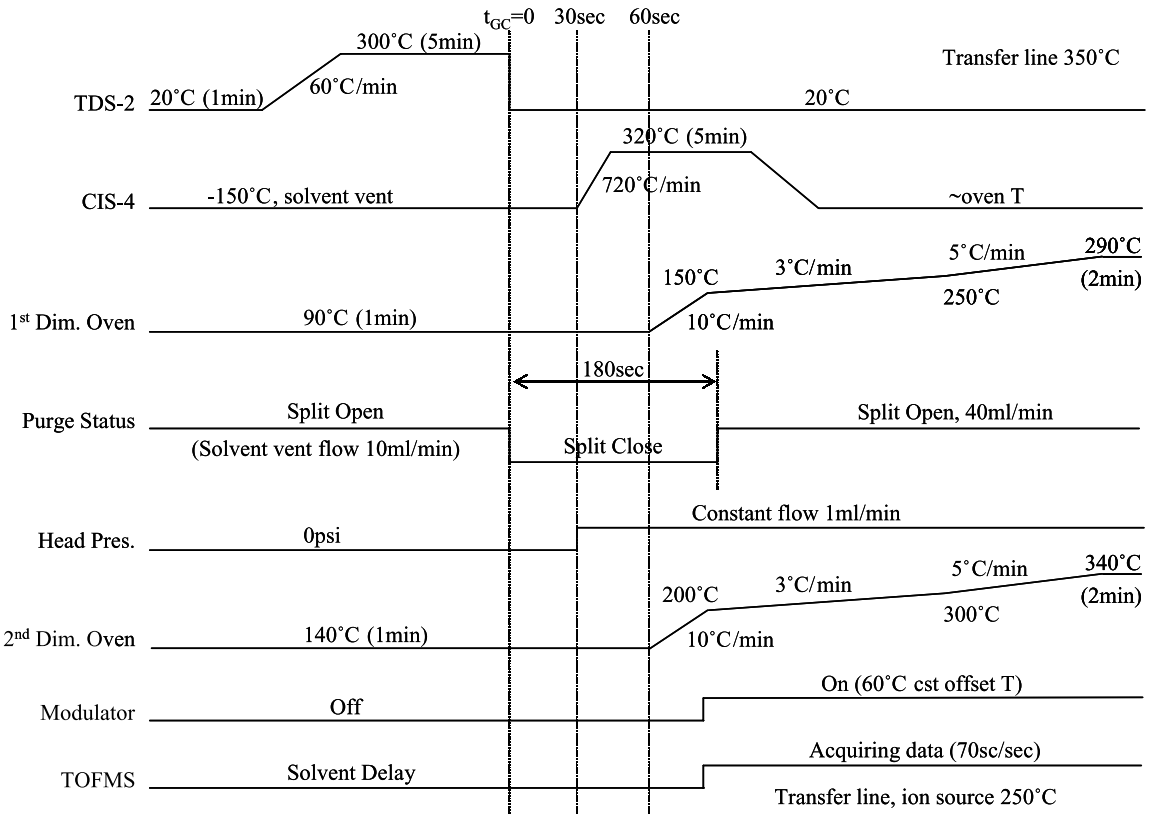


Fig. 1. Main parameters of the optimized TD-PTV–GC × GC–TOFMS procedure (not to scale).

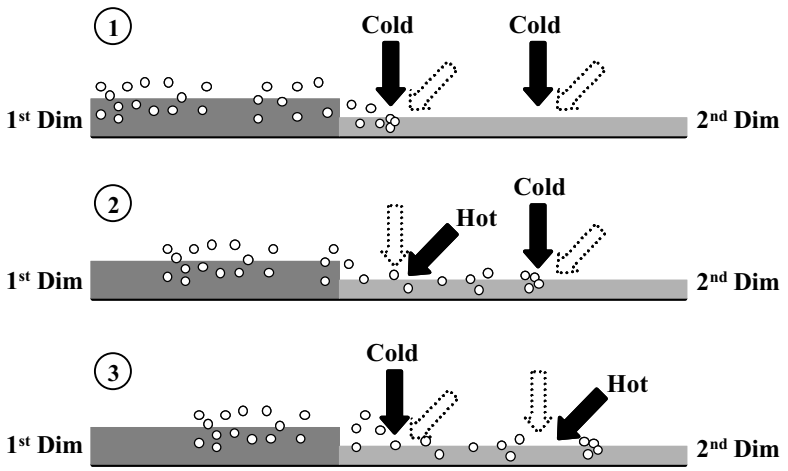


Fig. 2. Sequence of events responsible for (1) trapping, (2) releasing and re-focusing, and (3) re-injecting into the second column using a quad-jet cryo-modulator.

from 40 to 320 °C at a rate of 2 °C/min and held at the final temperature for 2 additional hours.

The transfer line connecting the secondary column and the MS source was operated at a temperature of 250 °C. The source temperature was 250 °C with a filament bias voltage of −70 V. The data acquisition rate was 70 scans/s for a mass range of 100–750 amu. The detector voltage was 1800 V. Fig. 1 summarizes the important parameters.

Data processing and display of the GC × GC chromatograms were achieved using the Leco ChromaTOF^{TM} software.

3. Results and discussion

3.1. Optimization of the TD and injection parameters

The important parameters are listed in Fig. 1. A maximum desorption temperature of 300 °C was selected because it was high enough to ensure desorption of the analytes but not excessively high for the future use of coated stir-bars that are subject to siloxane bleeding during desorption under high temperature. The 60 °C/min ramp rate was the maximum available rate. We selected the splitless flow mode for the TDS because we were looking for high sensitivity. The simultaneous use of the solvent-vent flow mode for the PTV (CIS-4) offered the possibility to use the system in a splitless–splitless mode while allowing a significant desorption flow rate. Surprisingly, a flow rate of 10 ml/min was more efficient than higher flows, especially for the lower-mass compounds. A solvent vent head pressure of 0 psi gave larger peaks than for any other higher head pressure values.

To trap the desorbed compounds using the PTV injector, we used liquid nitrogen as coolant. The selection of the best liner type was determined during the same experiments for optimization of the trapping temperature. Among the choices of carbon-packed, Tenax-packed, whole-packed, and empty baffled liners, the last one was selected with a trapping temperature of −150 °C. This combination produced the sharper GC peaks, and the use of empty liners further reduced the risk for cross-contaminations between successive injections and potential DDT decomposition. In addition, the absence of sorbent in the liner appeared to be an important parameter to ensure the release of high molecular mass compounds after trapping. Sharp injections were achieved using the maximum PTV heating rate of 720 °C/min up to a final temperature of 320 °C. The PTV temperature program started 30 s after the split valve was closed because some time is required for the flow stabilization to occur in the system, as reported in another study [31]. An additional delay of 30 s was allowed before the start of the GC oven temperature program to ensure that the release of the PTV trapped compounds was complete. A purge time of 180 s was the optimized setting over the range of values tested.

3.2. Selection of the column set

A narrow bore capillary column with a non-polar 100% dimethylpolysiloxane (DB-1) phase was selected as the first dimension to emphasize separation according to the boiling points of the compounds. A column of 0.25 mm i.d. and 0.25 μm film thickness was selected to produce sufficiently broad peaks to give the appropriate number of chromatographic slices after modulation. Three different first dimension column lengths were tested (30, 15, 8 m) with 2 m secondary columns (column ratio of 15, 7.5 and, 4, respectively). A 15 m length was selected as a compromise between chromatographic resolution and the need for short columns to elute high-boiling compounds such as PBDEs within a reasonable time frame under acceptable temperature programs. This was important because we wanted to keep a constant temperature difference between the two columns, with the secondary column operating at a significantly higher temperature. Higher temperatures on the second dimension micro bore columns were used to reduce analyte peak widths.

Different “classical” secondary dimension phases were studied. The use of the mid-polar (50%-phenyl)-methylpolysiloxane phase in the second dimension was eliminated since it was not suited to the PBDE congeners. The PBDEs remained trapped on the column and did not elute in a reasonable time. A highly polar polyethylene glycol phase (WAX) was investigated and gave a good separation of the persistent pesticides and the PCB congeners. All of the persistent pesticides were baseline separated; heptachlor epoxide and oxychlordane were separated in the second dimension. For the PCBs, only one co-elution remained

for the octachloro CB-196 and CB-203. However, the PBDEs were too strongly retained on the column. This could have been overcome if the phase would have tolerated temperatures higher than 260 °C. Decreasing the column length to as low as 0.1 m did not solve the problem. We therefore returned to a mid polar (14%-cyanopropyl-phenyl)-methylpolysiloxane phase that had previously been reported to be suitable for PBDE analyses [34]. This column, however, appeared to be temperature-sensitive when it was used at the high end of its temperature range (280 °C). The peak shapes rapidly lost their Gaussian shape and the peak width in the second dimension increased from 200 ms to values up to 800 ms, which significantly reduced the chromatographic resolution of the system. Additionally, some of the co-eluting peaks that were resolved with the WAX column were no longer resolved.

Because the temperature limitation on the second dimension column was our main problem, we tested a high temperature (8% phenyl)-polycarborane-siloxane column (HT-8) that has been commercialized for PCB applications. The combination of a 15 m DB-1 column with 2 m of this HT-8 column appeared to be a valuable column set for our application.

3.3. Optimization of the GC × GC parameters

The DB-1/HT-8 column set could be used at temperatures as high as 340 °C. We optimized a high-temperature GC program that still permitted a fairly big temperature offset (50 °C) between the two ovens. Furthermore, because we were interested in compounds with molecular weights of up to 650 amu, the ability to raise the temperature to 340 °C in the secondary oven helped the later eluting (high boiling) compounds to exit the column in about 50 min while keeping a conventional flow rate of 1 ml/min. Additionally, operating the secondary oven at high temperatures permitted us to avoid wrap-around that could be responsible for creating new co-elutions in such a complex mixture of compounds.

The modulator temperature also appeared to be important, again because of the high molecular weight of some of the target analytes. In fact, a modulator temperature offset of 60 °C appeared necessary to ensure the efficiency of the hot jets for the release of the trapped compounds during the modulation. In conjunction with that, a 600 ms hot-pulse duration was implemented to produce sharper peaks in the second dimension, especially for late-eluting PBDEs. Peak widths below 200 ms were achieved for the last eluting analytes such as BDE-153 and BDE-154. As higher brominated compounds such as 2,2′,3,4,4′,5,6-hptaBDE (BDE-183) and 2,2′,3,3′,4,4′,5,5′,6,6′-decaBDE (BDE-209) can be of interest in sample screening, we have to mention that the current analytical conditions do not currently allow to include them. Their high molecular weights and affinities for the secondary phase are challenging on the chromatographic point of view. We are currently investigating the feasibility of incorporating those analytes but, in a first approach, this would significantly increase the length of the GC run, unless some of the chromatographic resolution is jeopardized by reducing the secondary column length to a few centimeters.

3.4. Signal enhancement

The signal that can be enhanced using TOFMS as the GC × GC detector is interesting to consider. That GC × GC offers signal enhancement independent of the modulation device is quite well documented [35]. However, the selected scanning rate of the TOFMS has an important effect on the potential signal enhancement. The high scanning rate offered by TOFMS unfortunately is counter-balanced by a decrease in sensitivity as the scanning rate increases. Fig. 3 illustrates the effect of the modulation on the signal enhancement for CB-18. A comparison between the non-modulated GC peak height and the highest GC × GC peak height (the base peak) shows that the modulated peak was approximately 9.5 times more intense. The one-dimensional GC peak width at half height was around 8000 ms while the highest GC × GC peak was around 90 ms. For comparison, the peak width of modulated late-luting CB-209 peak was around 125 ms. This comparison is based on DIC traces using five characteristic ions of trichlorinated PCBs.

The net effect of using GC × GC–TOFMS is therefore a signal enhancement. However, this effect is less pronounced than what is theoretically possible [36] and results from data acquisition rate of the TOFMS. In fact, a scanning rate of 70 Hz was used for the GC × GC data acquisition to ensure sufficient sampling over the second dimension GC peaks although

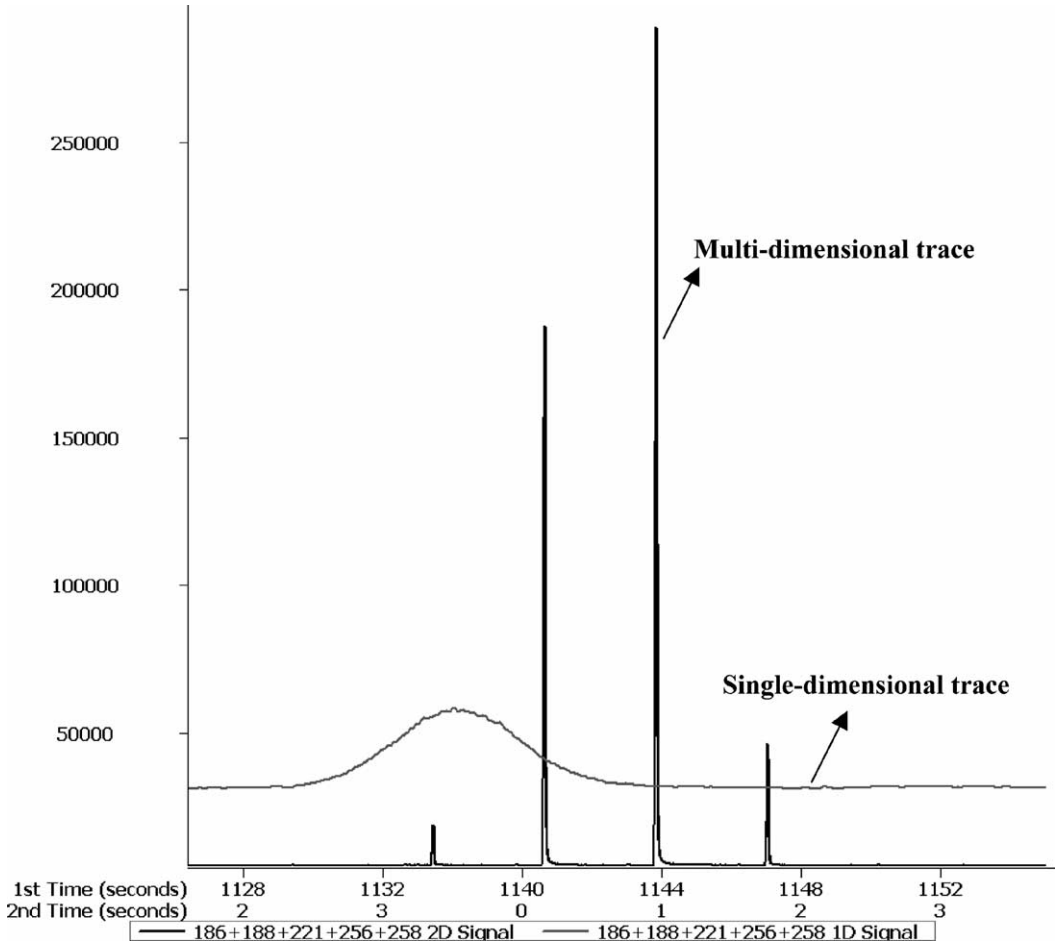
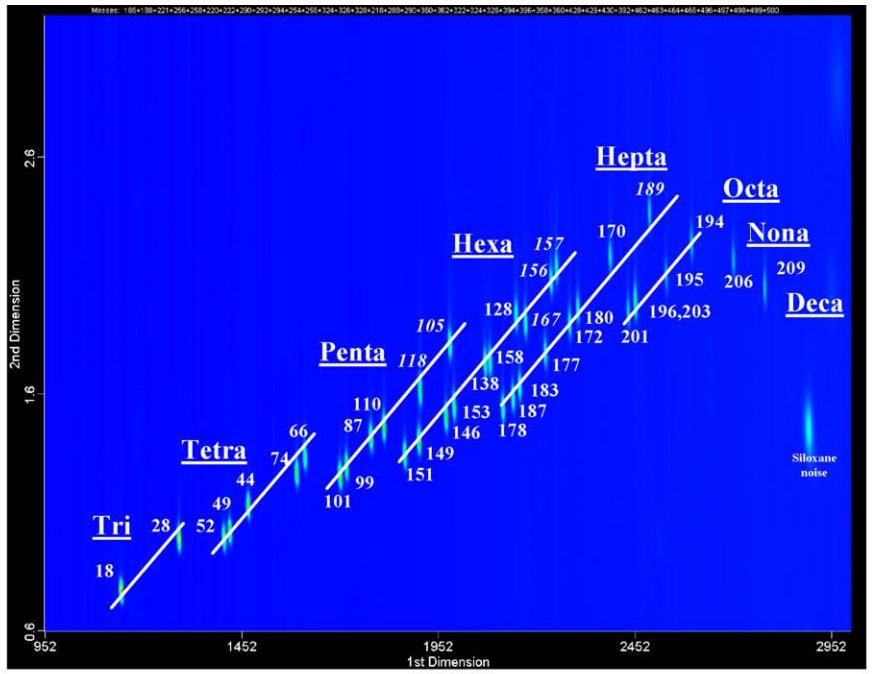


Fig. 3. Sensitivity enhancement effect after compromise between zone compression resulting from modulation and high TOFMS data acquisition rate. These DIC traces are based on the characteristic ions of the trichloro biphenyl CB-18 selected from the mass spectrum shown. Both the one-dimensional and two-dimensional chromatograms were produced using the DB-1/HT-8 dual column set. The acquired mass range was 100–750 amu. The data acquisition rates were 10 and 70 Hz for the one-dimensional and two-dimensional traces, respectively. The modulation frequency of the GC × GC run was 0.25 Hz with a hot-pulse duration of 600 ms.

Fig. 4. (A) GC × GC–TOFMS chromatogram of the 38 PCBs analyzed in human samples. The signal was reconstructed using five characteristic ions for each chlorination group (DIC traces). The DB-1/HT-8 dual column set was used. The temperature of the modulator had an offset of 60 °C, compared with the temperature of the primary GC oven. The primary GC oven was programmed as follows: 90 °C for 1 min, then to 150 °C at 10 °C/min, then to 250 °C at 3 °C/min, then to 290 °C at 5 °C/min and held for 2 min. The second dimension column was coiled in the secondary oven that was 50 °C higher than the primary oven (iso-ramping mode). The acquired mass range was 100–750 amu. The data acquisition rate was 70 Hz. The modulation frequency was 0.25 Hz with a hot-pulse duration of 600 ms. (B) GC × GC–TOFMS chromatogram of the 11 persistent pesticides analyzed in human samples. The signal was reconstructed using six to eight characteristic ions for each compound (DIC traces). The experimental conditions are identical to those in (A). (C) GC × GC–TOFMS chromatogram of the eight PBDEs and one BB analyzed in human samples. The signal was reconstructed using 6–12 characteristic ions for each compound (DIC traces). The experimental conditions are identical to those in (A). (D) GC × GC–TOFMS chromatogram of the entire set of analytes (38 PCBs + 11 persistent pesticides + 8 PBDEs + 1 BB = 58 analytes) analyzed in human samples. The signal was reconstructed using the sum of all characteristic ions to reconstruct the compound traces in the three previous chromatograms (DIC traces). The experimental conditions are identical to those in (A).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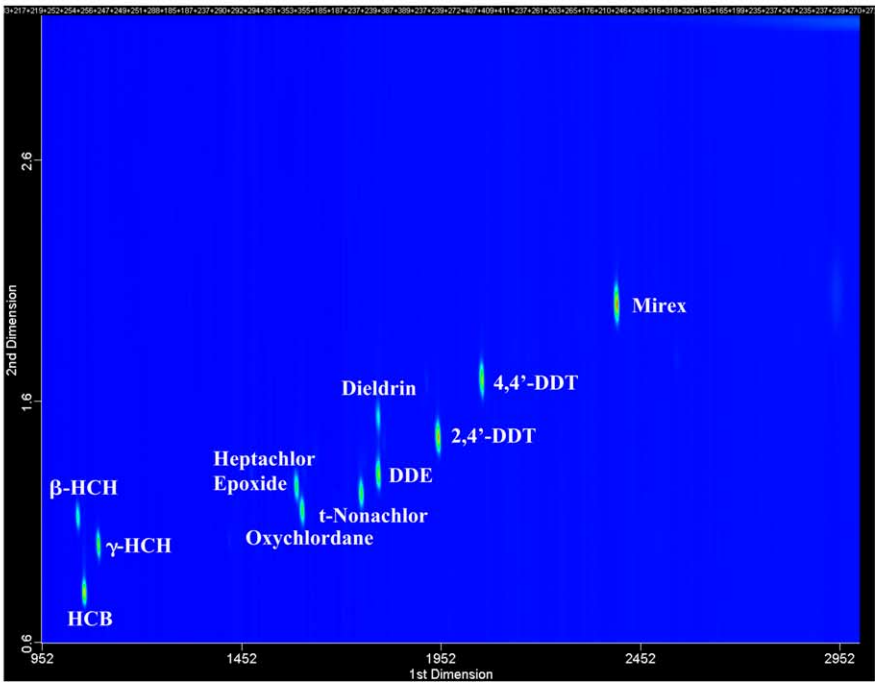
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Dieldrin
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2,4'-DDT
Heptachlor
Epoxide
DDE
β-HCH
t-Nonachlor
Oxychlordane
γ-HCH
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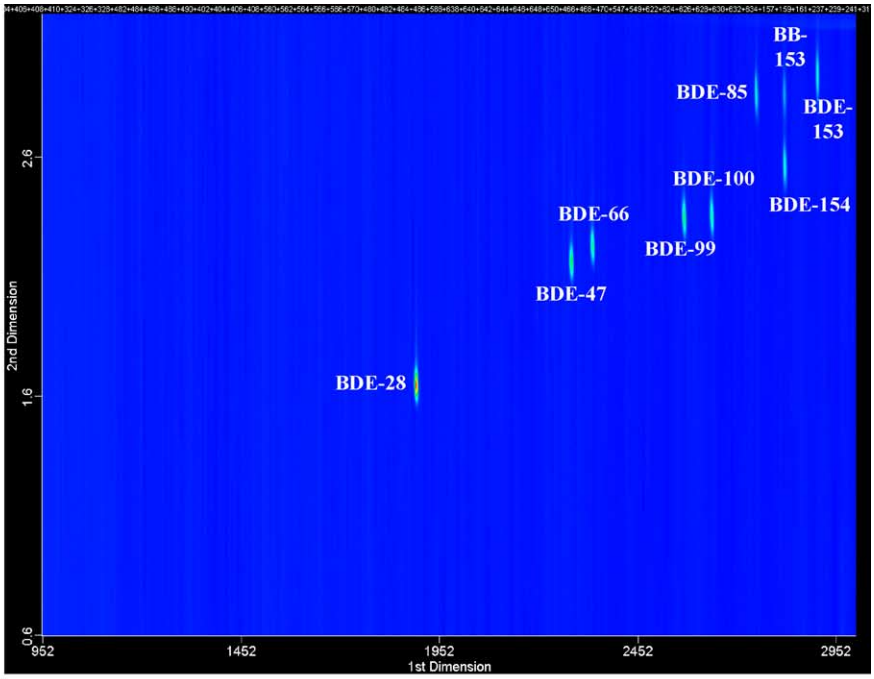
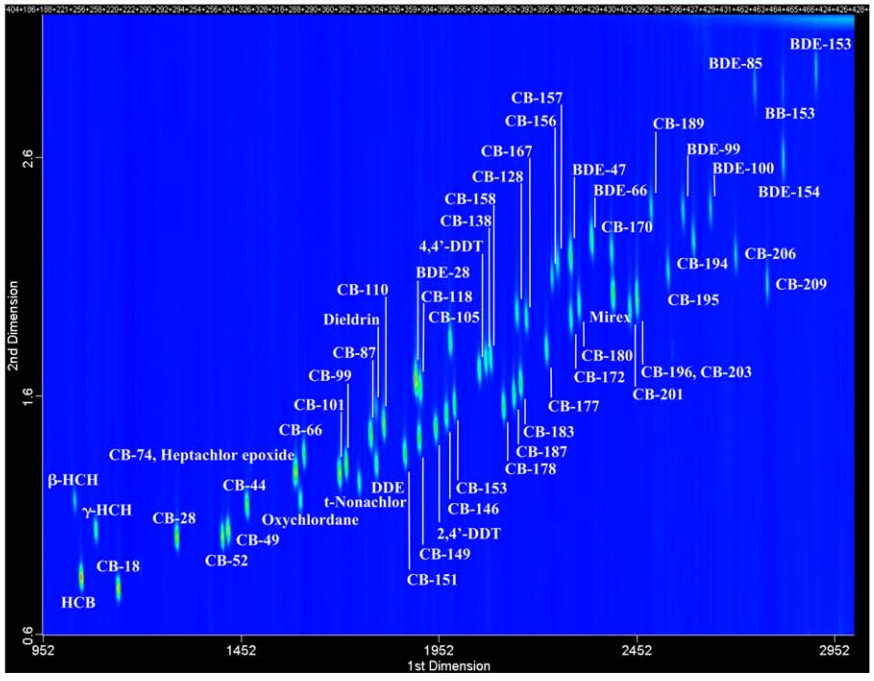
(B)



(C)



(D)

Fig. 4. (*Continued*).

10 Hz was used for the one-dimensional acquisition. The 10-fold increase thus results from the compromise between the pure GC × GC signal enhancement and the reduction of the sensitivity from the higher data acquisition rate of the TOFMS. An additional data acquisition rate effect was some loss in the TOFMS sensitivity, considering the relatively lower baseline of the modulated signal compared to the non-modulated signal (Fig. 3). The 10-fold signal enhancement was valuable because it extended the instrument LODs to the low ppt concentrations for the POPs analytes.

3.5. Chromatographic resolution versus analytical resolution

The chromatograms obtained using the DB-1/HT-8 set are presented in Fig. 4A–D. Fig. 4A shows the GC × GC chromatographic distribution of the PCB congeners, which depends on the number of chlorine atoms on the biphenyl ring. The elution of trichlorinated biphenyl (CB-18) through decachlorinated biphenyl (CB-209) took place within 45 min. A similar elution profile has been reported for another PCB mixture in about 140 min using a similar column setup [16]. Within the 38 selected PCB congeners, 7 are part of those that have been reported as compounds with a significant toxicity relative to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) [37] and requiring special attention (e.g. the need to be quantified separately). These seven mono-*ortho* PCB congeners (CB-118, CB-105, CB-156, CB-157, CB-158, CB-167, CB-189) were all baseline separated from any of the other congeners. The non-*ortho* PCBs were not included in the present work since they are isolated with the PCDD/F fraction during the sample preparation steps.

By examining the chromatogram in Fig. 4A, we found that the use of GC × GC solved the

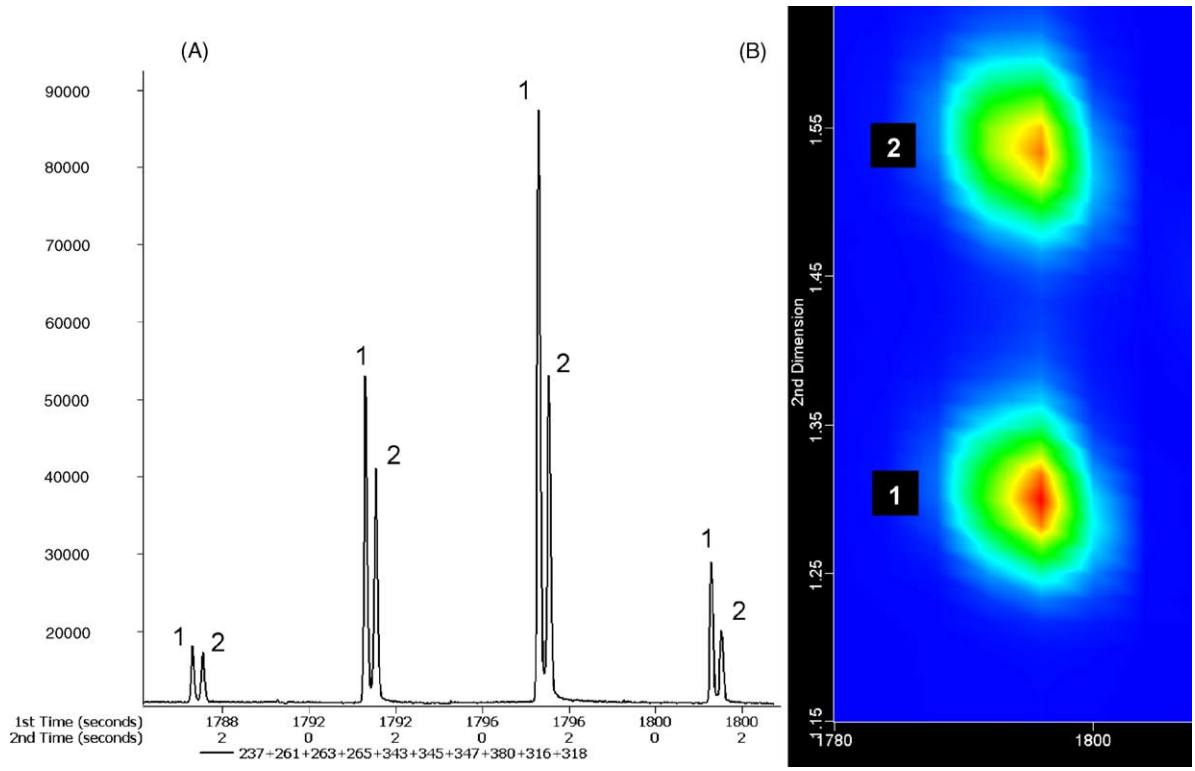


Fig. 5. A closer look at the region of the chromatogram where DDE (1) and Dieldrin (2) elute. The chromatogram has been reconstructed using the sum of the characteristic ions of the two species. (A) shows the cluster corresponding to the 'slices' that can be recombined to produce the GC × GC contour plot shown in (B). The experimental conditions are identical to those in Fig. 4A.

one-dimensional co-elutions of CB-118 and CB-149, CB-105 and CB-153, CB-128 and CB-183. Among the 38 compounds, only 2 PCBs were still co-eluting (CB-196 and CB-203). Because these two octachlorinated PCBs are characterized by the same fragmentation pattern, even the deconvolution capability of the TOFMS could not efficiently reconstruct the two separate traces. The Leco deconvolution software reportedly can deconvolute co-eluting isomers [38], and this opens some possibilities that need further investigation.

In Fig. 4B, the elution profile of the persistent pesticides uses the same conditions as those used for the PCB mixture. Each of the 11 compounds is baseline separated. The chromatographic resolution of DDE and Dieldrin in the second dimension can be seen in the figure. This is further illustrated in Fig. 5 where Fig. 5A represents the chromatographic trace as seen at the detector. The GC × GC contour plot of the two separated pesticides is shown in Fig. 5B.

The elution of the high molecular weight PBDEs (Fig. 4C) occurs at later first dimension retention times, relative to most of the PCBs and the persistent pesticides. One can see that a very useful separation is achieved between the one-dimensional co-eluters BDE-154 and BB-153. This is an important separation

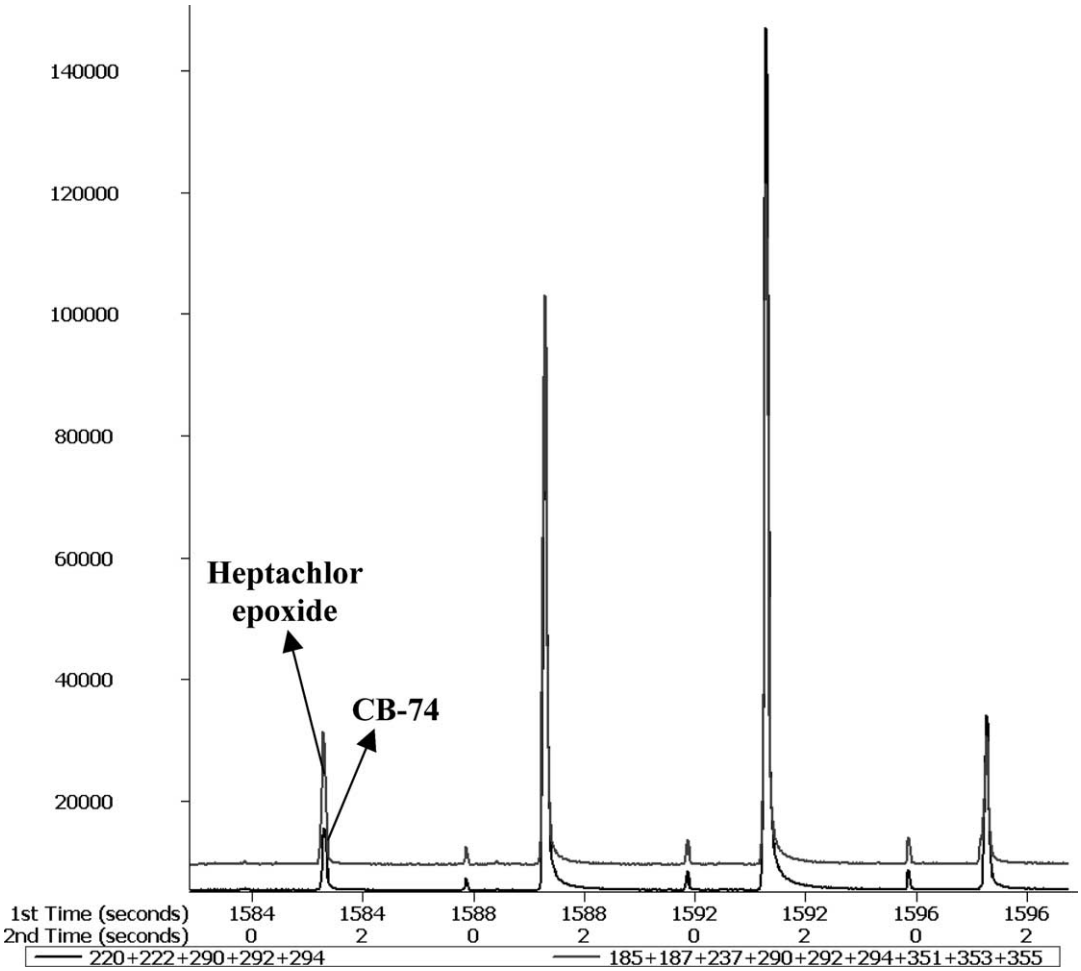


Fig. 6. Deconvoluted traces for CB-74 (black) and hepatchlor epoxyde (grey) based on their specific ions (DIC traces). These co-elutors are not resolved by GC × GC but can be separately identified using the deconvolution capability of the TOFMS. The experimental conditions are identical to the ones of Fig. 4A.

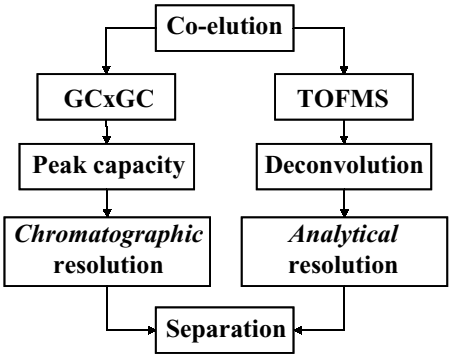


Fig. 7. The two complementary options to achieve separation of co-eluting peaks using a GC × GC–TOFMS system.

because BB-153 can be considered a marker compound to trace the impact of the hexabrominated flame retardant mixture [39]. Furthermore, in recent reports, the ratio between BB-153 and BDE-154 varied significantly, depending on the geographic origin of the samples [40,41]. In other words, neither BB-153 nor BDE-154 can be considered a negligible interference on the other, and they have to be separated for analysis of samples for brominated compounds.

The point of the present study was to include all of the analytes listed in Table 1 in a single GC injection (Fig. 4D). This chromatogram shows the separation of 56 of the 58 compounds, CB-196 and CB-203 remaining the unresolved pair. The combination of the PCBs, persistent pesticides, and PBDEs in the same chromatographic space created some extra co-elutions because some of these compounds had similar retention times (in the first or in both dimensions). For example, BDE-47 and CB-172, as well as CB-170 and Mirex, are characterized by similar first dimension retention times. Fortunately, their chemical properties permitted them to be separated on the secondary dimension column. In the case of heptachlor epoxide and CB-74, both the first and the second dimension retention times were identical. However, on the basis of differences in the fragmentation patterns of the two molecules, the traces were deconvoluted (Fig. 6).

In practice, the software requires a minimum number of data points across the peaks to deconvolute the two analytes. A peak width of 230 ms, from baseline to baseline, was produced in conjunction with a data acquisition rate of 70 Hz, and about 16 scans were obtained to describe the peaks. This is enough for the deconvolution software to separately identify the two compounds and reconstruct two co-eluting compounds. The coupling between GC × GC and TOFMS can therefore be seen as a truly efficient separation tool (Fig. 7).

4. Conclusions

We separated 38 PCB congeners, 11 persistent halogenated pesticides, 1 BB, and 8 PBDEs using comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC × GC–TOFMS). The feasibility of the coupling between GC × GC–TOFMS and the thermal desorption-programmable temperature vaporization injector has been demonstrated on a quantitative point of view. Those preliminary results show that the chromatographic resolution of GC × GC coupled with the analytical resolution of the TOFMS produces a powerful combination that solves most of the potential co-elution problems that can arise during the simultaneous analysis of several classes of compounds such as halogenated POPs. It can also ensure that there are no co-elutions during routine measurement of one class of compounds in a sample potentially containing many others.

Acknowledgements

J.-F. Focant is financially supported by the Oak Ridge Institute for Science and Education (ORISE), a Department of Energy (DOE) facility managed by Oak Ridge Associated Universities (ORAU).

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