Improved separation of the 209 PCBs using GCxGC-TOFMS

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INTRODUCTION

Solving all the 209 polychlorinated biphenyl (PCB) congeners from each others in a single gas chromatographic (GC) injection has never been possible so far. Many types of column phases have been tested for that particular job, some have even been especially designed for it, but none can claim the full separation award [1,2]. The aim of the present work was to evaluate comprehensive two-dimensional GC (GCxGC) coupled to time-of-flight mass spectrometry (TOFMS) for the enhanced separation of PCBs. Several recent review articles on GCxGC are available in the litterature [3-6].

EXPERIMENTAL

Chemicals

The PCB standard solutions were obtained from AccuStandard Inc. (New Haven, CT, USA). The 9 multi-congener solutions (C-CS-01 to C-CS-09), containing all native 209 PCB congeners at concentrations of 10 µg/mL in isooctane, were used. They were mixed to produce a solution containing all 209 congeners at a concentration of 1 ng/µL.

GCxGC-TOFMS

The GCxGC-TOFMS instrument was the LN_2 quad-jet modulator Pegasus 4D (Leco Corp., St Joseph, MI, USA). Details regarding the system have been reported elsewhere [7]. The modulator had an offset temperature of 60°C compared to ^{1}D GC oven programmed as follows: 90°C (1 min), to 150°C at 30°C/min, and to 300°C at 1°C/min. The ^{2}D oven was 40°C higher than ^{1}D (iso-ramping mode). The modulator period was 3 s (0.33 Hz modulation frequency) with the hot-pulse duration set at 800 ms and the cool time between stages set at 700 ms. Helium was used as carrier gas at a constant flow of 0.8 mL/min. The inlet temperature was 280°C for splitless injections of 1 µL. The MS transfer line temperature was 250°C, as well as the MS source. The data acquisition rate was 60

scans/s for a mass range of 100-550 amu. The Leco ChromaTOF^{TM} software was used.

Different sets of columns were tested: **Column set #1:** DB-1 100% dimethylpolysiloxane (75m x 0.25mm x 0.25µm) (J&W Scientific, Folsom, CA, USA) as ^{1}D pressfitted to a high temperature HT-8 (8% phenyl)-polycarborane-siloxane (2.5m x 0.10mm x 0.10 µm) (SGE, Austin, TX, USA) as ^{2}D. **Column set #2:** DB-XLB (60m x 0.25mm x 0.25µm) (J&W Scientific) as ^{1}D and a HT-8 (2.5 m x 0.10 mm I.D. x 0.10 µm film thickness) (SGE) as ^{2}D. **Column set #3:** DB DB-XLB (60m x 0.25mm x 0.25µm) as ^{1}D and a BPX-50 (50% phenyl)-polysilphenylene-siloxane (2.5m x 0.10mm x 0.10µm) (SGE) as ^{2}D. **Column set #4:** HT-8 (50m x 0.22mm x 0.25µm) as ^{1}D and a BPX-50 (2.5m x 0.10mm x 0.10µm) as ^{2}D.

RESULTS AND DISCUSSION

Figure 1 shows the apex plots corresponding to the chromatographic distribution of the 209 PCBs into the retention plane for the different column sets. Performing the ^{2}D separation at high temperature gave ^{2}D peak widths at the base of 100-150 ms. It also reduced wrap-around ($^{2}t_R > P_M$), which was expected because of the short value of P_M (3 s) selected to comply with the GCxGC conservation rule. For all investigated column sets, one wrap-around was observed in the elution window but no coelutions between analytes issued from different modulation cycles were created and the separation profile was conserved. In Figure 1, the apex plots have been "wrap-around corrected" by correcting the $^{2}t_R$ of wrap-around compounds by the value of P_M.

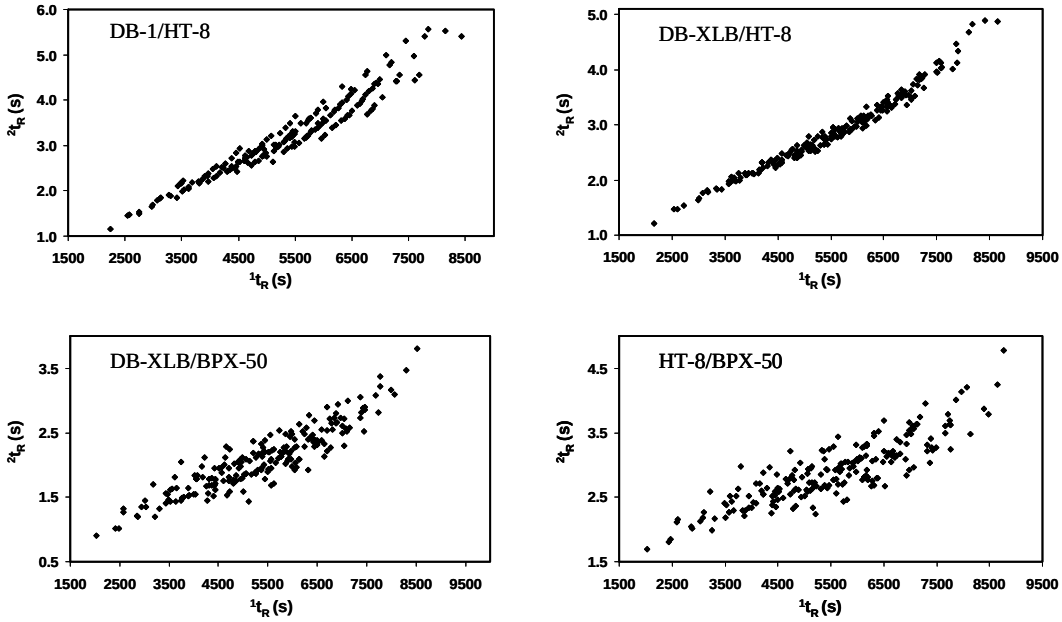

Figure 1. GCxGC-TOFMS apex plots of a standard solution containing the 209 PCB congeners at a concentration of 1 ng/µL.

The characteristic distribution of the PCBs organized by homologue series was expected for the DB-1/HT-8 column set [7,8]. The nonpolar ^{1}D stationary phase generated ^1t_R values proportional to the vapor pressure of the congeners and to the degree of chlorination. For ^{2}D, the carborane group is known to have higher affinity toward PCBs with a low degree of *ortho*-substitution. Excepted for CB-23 and CB-54, the only coelutions were for congeners within the same homologue series. Among the 209 congeners, 163 were chromatographically resolved in 140 min. Because most the coeluting compounds were characterized by similar mass spectra, the mass spectral deconvolution was not possible and the added value of the TOFMS was limited when using this particular column set, regarding the use of micro electron capture detection (µECD).

The DB-XLB and HT-8 phases are special PCB phases but they can not separate all the 209 PCB congener when used separately [9]. As illustrated in Figure 1, the congener distribution in the chromatographic space was rather limited. The degree of orthogonality of the DB-XLB/HT-8 set was not as good as for the DB-1/ HT-8

set. This resulted in a higher number of interhomologue series coelutions and a reduction of the chromatographic resolution. Nevertheless, because mass spectral deconvolution of coeluting PCBs issued from different homologue groups was possible, 21 coelutions were further resolved and only 23 coeluting PCBs remain. This approach conducted to a total separation (chromatographically and analytically) of 186 congeners in 144 min. This approach of combining the DB-XLB phase with MS detection to solve coelution issues was reported earlier as acceptable because, in most cases, the highly chlorinated compound is the minor component (low level in commercial mixtures) of the coeluting group and its dechlorination to a lower homologue group is negligible [9]. However, one should not encourage such an approach of reducing the chromatographic resolution of a system because of the potential for deconvolution of the MS signals. Such an approach might lead to identification and quantification problems in the situation where a hexa-CB congener coelutes with a tetra-CB congener. The interfering fragmentation loss of Cl_2 from the hexa-CB produces a M-70 cluster of ions that correspond to the ion cluster of the tetra-CB and makes accurate identification impossible with a low mass resolution MS instrument.

To improve the separation on the basis of a DB-XLB ^{1}D phase, we used a more polar BPX-50 ^{2}D phase. The separation was more efficient (Figure 1) than it was with the previous sets and conducted to the separation of 194 congeners after deconvolution of 3 coeluting congeners. Unfortunately, among the coeluting compounds, an unacceptable pair occurred between the tetra-chlorinated CB-77 and the hexa-chlorinated CB-144, reducing the appeal of the remaining separation. A recent study [10] however reported the successful use of the DB-XLB/LC-50 (poly-50% liquid crystalline/50% dimethyl-siloxane phase) combination and separated 181 congeners (including the 7 marker and WHO PCBs) using μECD detection.

The HT-8/BPX-50 column set resulted in a very good dispersion of the peaks into the separation space because the mechanisms involved in ^{1}D and ^{2}D clearly differed (Figure 1). Although not obvious to see in a first look, the resulting chromatogram was highly structured (Figure 2).

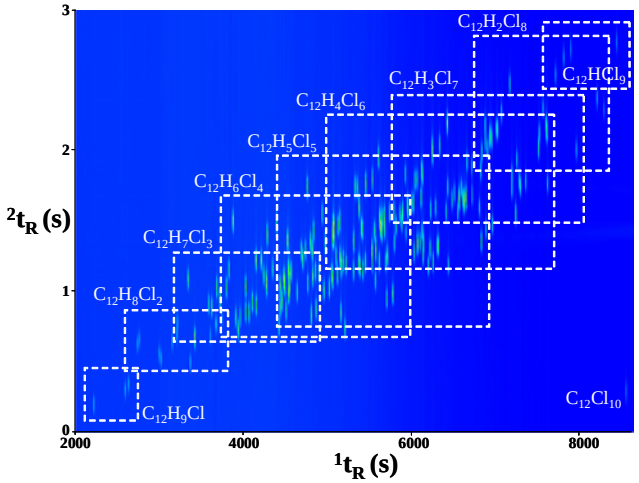

Figure 2.
GCxGC-TOFMS chromatogram of the 209 PCB congeners using the HT-8/BPX-50 set. The signal was reconstructed using the characteristic ions of each chlorination group (DIC traces) For clarity, the ^{2}D scale was shifted by 1.5 s.

Depending on the number of chlorines on the rings, up to 5 subseries were separated within each homologue group. The carborane group of the HT-8 phase showed high affinity for PCBs with a low degree of *ortho*-substitution, which splitted every homologue group across the ^{1}D retention line (Figure 3).

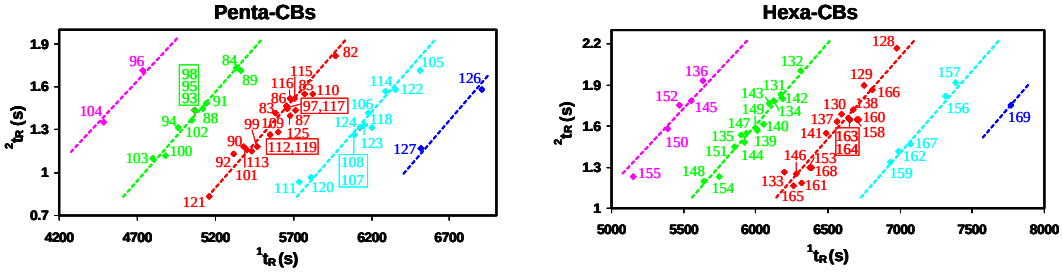

Figure 3. GCxGC-TOFMS apex plots of the penta and hexa-CB homologue groups using the HT-8/BPX-50 column set. The *ortho*-substitution level is described as follow: Tetra-*ortho*-CBs are in pink, tri-*ortho*-CBs are in green, di-*ortho*-CBs are in red, mono-*ortho*-CBs are in light blue, and non-*ortho*-CBs are in dark blue. Boxes represent coeluting congeners.

The resulting ordered structure was, thus, different than the one from the DB-1/HT-8 set. The separation of homologue groups in subseries was valuable because the increased spread out of the congeners in the chromatographic space contributes to ease the peak identification and quantification, as well as the prediction of PCB retention data in complex mixtures. Chromatographic coelution problems were minimized and the need for mass deconvolution was reduced. Figure 4 illustrates that a single-dimensional GC separation would have peaked only once (the reconstructed trace in Figure 4C), a GCxGC separation using a μECD would have peaked twice (the contour plots in Figure 4B), and the GCxGC-TOFMS permitted the identification of 3 separate analytes.

Among the 209 CB congeners, 188 were chromatographically separated and 4 (CB-132/CB-179 and CB-160/CB-175) required mass spectral deconvolution, conducting to a total of 192 congeners separated in 146 min. All WHO congeners (CB-77, CB-81, CB-105, CB-114, CB-118, CB-123, CB-126, CB-156, CB-157, CB-167, CB-169, CB-189) and marker PCBs (CB-28, CB-52, CB-101, CB-138, CB-153, CB-180), were separated from any interfering congeners (Table 1).

A recent study using GCxGC-μECD and a 60m DB-XLB/2.25m BPX-70 column set separated 194 of the 209 PCBs in 240 min [10]. A comparison shows that the input from the TOFMS detection mainly consists in an analytical speed improvement and that most of the separation can be achieved chromatographically when suitable GC conditions and column sets are selected. MS detection, however, significantly improves peak identification and offers an extra level of accuracy through mass ratio check and library searching. This is ensured by the high quality of the mass spectra that are produced [11]. Additionally, if we think beyond the PCB family and consider that many other organochlorine compounds expressing similar GC behavior might be present in PCB-containing extracts, mass spectral deconvolution might be requested to solve inter-family coelutions.

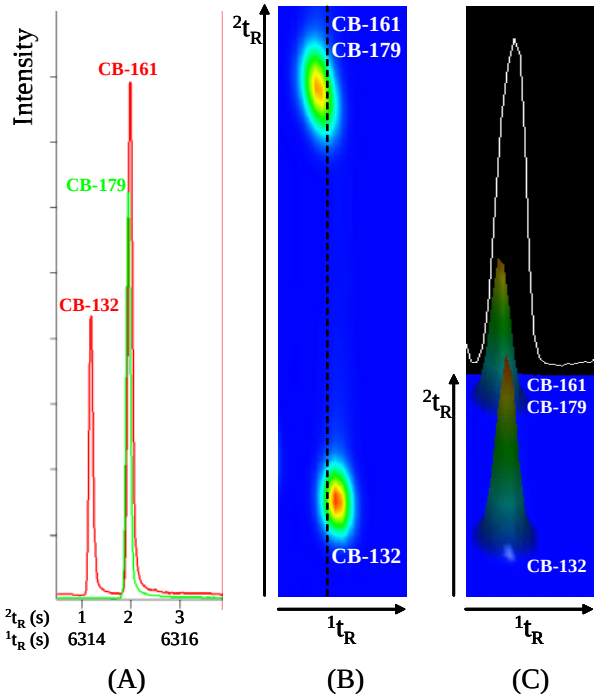

Figure 4. GCxGC-TOFMS raw data (A), contour plot (B), and 3-dimensional plot (C) for the chromatographic region where CB-132, CB-179, and CB-161 elute.

A) Only 1 cluster of peaks, corresponding to 1 modulation cycle at the time represented by the dashed line on B. The red (m/z 292x3) and the green (m/z 398) traces correspond to hexa- (CB-132, CB-161) and hepta-CBs (CB-179), respectively.

B) Contour plots corresponding to the 2 peak units chromatographically separated.

C) Reconstructed 1DGC-TOFMS trace (white) based on the sum of the signals recorded in ^{2}D.

The GCxGC-TOFMS iLOD (EI mode) ranged between 0.1 and 0.5 pg/µL injected at a scan rate of 60 scans/s with a signal to noise ratio greater than 3. Better LODs have been reported for µECD [8], but it is acceptable for measurements of PCBs in most matrices at usual background levels. Additionally, as mentioned elsewhere [10], TOFMS produce less post-column band broadening than µECD. In the present study, classical peak widths at the base in ^{2}D ranged between 100-150 ms,

which permitted easier peak identification when retention times were close. The use of either TOFMS or μECD detection should be dictated by the minimum criteria to fulfill for a particular application.

Acknowledgement
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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Table 1

Co-eluting PCBs on each of the 4 investigated column sets.

Congener	Column set			
	DB-1/HT-8	DB-XLB/HT-8	DB-XLB/BPX-50	HT-8/BPX-50
Resolved by mass				
Di-CBs/Tri-CBs	-	CB-11/CB-18	-	-
	-	CB-13/CB-27	-	-
Tri-CBs/Tetra-CBs	CB-23/CB-57	CB-23/CB-54	CB-38/CB-47/CB-62	-
	-	CB-36/CB-69	-	-
Tri-CBs/Tetra-CBs/Penta-CBs	-	CB-37/CB-68/CB-103	-	-
Tetra-CBs/Penta-CBs	-	CB-72/CB-96	-	-
	-	CB-60/CB-113	-	-
	-	CB-78/CB-116	-	-
	-	CB-47/CB-104	-	-
Penta-CBs/Hexa-CBs	-	CB-119/CB-152	-	-
Hexa-CBs/Hepta-CBs	-	-	-	CB-132/CB-179
	-	-	-	CB-160/CB-175
Unresolved by mass				
Di-CBs	-	CB-4/CB-10	-	-
Tri-CBs	CB-16/CB-32	-	-	-
	CB-20/CB-21/CB-33	CB-20/CB-21/CB-33	CB-20/CB-21/CB-33	CB-20/CB-33
Tetra-CBs	CB-52/CB-69	CB-62/CB-65	-	CB-47/CB-48
	CB-43/CB-49	CB-58/CB-67	-	-
	CB-48/CB-75	-	-	-
	CB-42/CB-59	-	-	-
	CB-41/CB-64	-	-	-
	CB-61/CB-70	-	-	-
	CB-56/CB-60	-	-	-
Tetra-CBs/Hexa-CBs[1]	-	-	CB-66/CB-155	-
	-	-	CB-77/CB-144	-
Penta-CBs	CB-98/CB-102	CB-84/CB-89	CB-84/CB-89	CB-93/CB-95/CB-98
	CB-88/CB-91	CB-90/CB-101	CB-90/CB-101	CB-112/CB-119
	CB-90/CB-101	CB-86/CB-125	CB-107/CB-123	CB-97/CB-117
	CB-83/CB-112	CB-115/CB-117	-	CB-108/CB-107
	CB-115/CB-116	CB-107/CB-123	-	-
	CB-108/CB-107	-	-	-
Hexa-CBs	CB-139/CB-149	CB-153/CB-168	CB-153/CB-168	CB-163/CB-164
	CB-134/CB-143	-	-	-
	CB-146/CB-165	-	-	-
	CB-138/CB-163/CB-164	-	-	-
Hepta-CBs	CB-182/CB-187	CB-175/CB-182	-	CB-182/CB-187
Octa-CBs	CB-196/CB-203	-	-	-
Number of separated congeners	165/209	186/209	194/209	192/209

[1]Those congeners can not be efficiently mass-separated because of the M-2Cl loss of the hexa-CB