ENHANCED GC/HRMS CHROMATOGRAPHIC ANALYSIS OF PBDES, PCDTS, AND OTHER COMPLEX MIXTURES WITH NARROW BORE THIN FILM COLUMNS

Peterman PH
US Geological Survey, Columbia Environmental Research Center,4200 New Haven Rd, Columbia, MO,USA 65201

Introduction

Many organic pollutants are comprised of various isomers and congeners, whose subtle differences cause varying degrees of biochemical responses and toxicological effects. Thus, analysts must keep striving for more specificity through enhanced chromatographic and mass spectral resolution and ideally in shorter time. For the past 20 years, long capillary columns (50-60 m) by 0.25 mm i.d. with 0.25 µm film thickness have been routinely used with high resolution mass spectrometers (GC/HRMS) to selectively detect the toxic 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, other 2,3,7,8-substituted dioxins (PCDDs) and dibenzofurans (PCDFs), dioxin-like non-*o*-PCBs from other PCBs, and many other complex mixtures. However, certain compounds such as highly brominated polybrominated diphenyl ethers (PBDEs) break down at excessively high temperatures before eluting, requiring a shorter column and thinner film to lower the elution temperature sufficiently but with some loss of chromatographic resolution inevitable[1].

Narrower diameter capillary columns have become commercially available (0.22 mm, 0.20 mm, 0.18 mm, 0.15 mm, and 0.10 mm). By using narrower (0.20 mm), higher performing, thin-film (0.11 µm) 50-m columns (Ultra-1 or 2) (Agilent Corp, Wilmington, DE USA), our GC/HRMS analyses improved for non-*o*-PCBs with the Ultra-1.[2] The Ultra-2 resolved almost all 39 mono- through hepta-PBDE congeners in a mixture[3]. Recently, we found more performance for non-*o*-PCB analysis by using a shorter (30-m) narrower (0.15 mm i.d.) Solgel 1 MS column custom made by SGE (Austin, TX USA)[4]. With that column and a similarly-sized BPX5 (5% phenyl silicone MS) column in our GC/HRMS, we comprehensively evaluated basic alumina flash chromatographic separations of many congeners constituting PBDEs, PCBs, polychlorinated dibenzothiophenes (PCDTs), and other complex mixtures[4].

In addition to optimizing analyte separations and GC/HRMS detection, certain parameters and challenges loom more importantly when using with shorter, narrower, and thin-film columns with GC/HRMS and optimizing chromatographic performance. Thus, I present significant GC/HRMS details here to complement our basic alumina research[4]. Various practical aspects about 0.15 mm capillary columns have already been thoroughly discussed in a paper by Kirchner et al.[5]. For example, they found that using an inert retention gap as a precolumn after the injector is essential for optimal performance of a 0.15 mm column because it allows significantly larger sample volumes without peak shape distortion than 0.1 µL if not used. Also, Covaci and Schepens[6] discussed various parameters as they compared using various sizes of narrow-bore columns with selected ion GC/quadrupole MS for PCBs.

Materials and Methods

Final sample extracts and standards were in *n*-nonane (B&J Brand, Burdick & Jackson). The following standards were prepared and tested: a PCB mixture of equal portions of Aroclors (1242:1248:1254:1260), a full-range mixture of 39 mono- through hepta-PBDE congeners plus added octa-PBDE 203, all three nona-PBDEs, deca- and ^{13}C-deca-PBDE 209 (Cambridge Isotope Labs, Andover, MA, USA), all ^{13}C-labeled 2,3,7,8-substituted PCDFs and PCDDs plus first and last-eluting window-defining congeners (Terrachem, Kansas City, MO USA-Wellington Labs), all ^{13}C-non-*o*-PCBs (Cambridge Isotope Labs, Andover, MA USA), tetra- through octa-PCNs in a Halowax 1014 mixture, a composite standard of mono- through octa-PCDTs from two synthetic solutions kindly supplied by Prof. Jan Andersson, and polychlorinated terphenyls (PCTs) was 10 µg from equal portions of Aroclors 5442 and 5460.

Two GC/HRMS systems (Agilent 6890 GC/Waters Autospec Ultima and HP 5890A/VG 70S) were used with Agilent 7683 or 7673 autosamplers, respectively. Helium carrier gas on both cool-on-column and split-splitless inlets in the 6890 GC were controlled electronically; the split-splitless inlet in the 5890A GC was manually set.

After anomalously low flow occurred using electronic "flow" control, constant pressure or pressure programming was used and correlated with average linear velocity based on solvent front (change in vacuum). Linear velocity was kept within 25 to 35 cm/s. Having lower flows, both 0.15 mm i.d. columns were installed in the Autospec simultaneously with reduced gas flow for the one in standby. Each split-splitless inlet will be named "direct" because each was used in a heated direct on-column mode with Siltek-treated glass liners (6890 GC with small hole and 5890A GC with Spiral Uniliner, Restek, Bellefonte, PA USA). Each inlet used a septumless Merlin Microseal (Merlin Instrument Co, Half Moon Bay, CA USA) including a prototype Microseal made for the on-column inlet. Rapid 3 µL or 5 µL injections were made into the 5890A GC direct inlet or 6890 GC direct inlet, respectively and slower 3 µL injections into 6890 GC cool-on-column inlet. Syringes required for the Merlin Microseals were straight 23 gauge or 26 gauge for direct and on-column, respectively. Restek Siltek-treated press-tight connectors were used to join the columns with Siltek-treated retention gaps. A 2.5 m x 0.25 mm i.d. retention gap was used from a direct liner to the column, but for on-column injection, a short 0.25 m x 0.53 mm i.d. retention gap was joined to a 2.0 m x 0.25 mm i.d. piece. Narrow bore (0.15 mm) thin film (0.1 µm) 30-m capillary columns of BPX5 and Solgel 1 MS were custom made by SGE (Austin, TX USA). For PBDEs, one 30-m BPX5 column was split into two 15-m BPX5 columns and subsequently one 15-m BPX5 column was shortened to 12.5 m.

The GC/HRMS systems were tuned for a mass resolution of 10,000 and calibrated with perfluoro-tetradecahydro-phenanthrene (PCR, Gainesville, FL USA) or tris(pentadecafluoroheptyl)-s-triazine for PBDEs. GC/HRMS EI selected ion monitoring (SIM) acquisition experiments typically included the two most abundant molecular ions and $(M-2Br)^+$ fragment ions for native and available ^{13}C-surrogates, a lock mass, a lock mass check ion, and ions for other compounds (chlorinated diphenyl ethers could interfere with PCDFs). Each ion delay time was 10 ms, except 20 ms for the first ion of each group. Ion dwell times varied between 20 and 40 ms, depending on the total number of ions in each group. SIM cycle scan times ranged from about 0.5 s for the fastest, earliest eluting compounds (peak width < 5 s) to about 0.8 s for later broader peaks. Trap filament current was 500 µA (70S) or 600 µA (Autospec) at 35-40 eV. Source and direct injection temperatures were within 270-290 °C. The range of GC oven program rates (1.7-4 °C/min) was chosen for optimum peak resolution with only one or two level ramps. Using nonane at several bar pressure, the initial column temperature ranged from 150 to 170 °C. The highest temperature was typically < 315 °C except for 350 °C (all PCTs), which was still below the maximum rated for each column.

Results and Discussion

Improved chromatography resulted by optimizing the injection process and rapid transfer from the liner-retention gap to the column[4,7-9]. By injecting more (3 to 5 uL) nonane to nearly fill the retention gap and optimizing the initial oven temperature, a process called "Concurrent Solvent Recondensation" significantly helped to sharpen peaks in most of the chromatogram[7-9]. With 4 bar He, the pressure-adjusted boiling point of nonane becomes nearly 40 °C higher thus providing a higher starting temperature and earlier analyte elutions. Shorter dead times with the columns allowed GC/HRMS acquisition after 3 min with the 12.5 m BPX5 (Figure 2) or 5 min with the 30-m columns. With narrower 0.15 mm columns, the phase ratio (film thickness to diameter) was reduced and provided a higher than expected dynamic range (0.1 pg to 10 ng) especially ideal for non-*o*-PCB analysis (with Solgel 1 MS)[4].

A 30-m BPX5 direct analysis for PCDFs/PCDDs and PCDTs (Figure 1) shows nearly baseline separation of 2,3,7,8-TCDD with other TCDDs, 123478- from 123678- for both hexa-PCDFs and hexa-PCDDs, and OCDD from OCDF. Nearly all PCDTs eluted within PCDF/PCDD homolog windows, but PCDTs were selectively detectable because they have one less chlorine than similarly-eluting PCDDs. Homologs were nearly discrete except the first penta-PCDF eluted a few sec before the last tetra-TCDF, as may also occur for penta and tetra-PCDTs. If present, penta-PBDEs potentially interfere with one ion of the hepta-PCDFs (m/z 408). With the 30-m BPX5, PBDE 118 eluted 30 sec earlier than the first hepta-PCDF. For PBDEs, a 12.5-m BPX5 on-column analysis (Figure 2) separated most PBDEs (including 33 from 28) within a 55-min linear ramp (3 °C/min) and enhanced the elution of deca-PBDE 209 by pressure programming faster than constant flow to lower its elution < 310 °C.

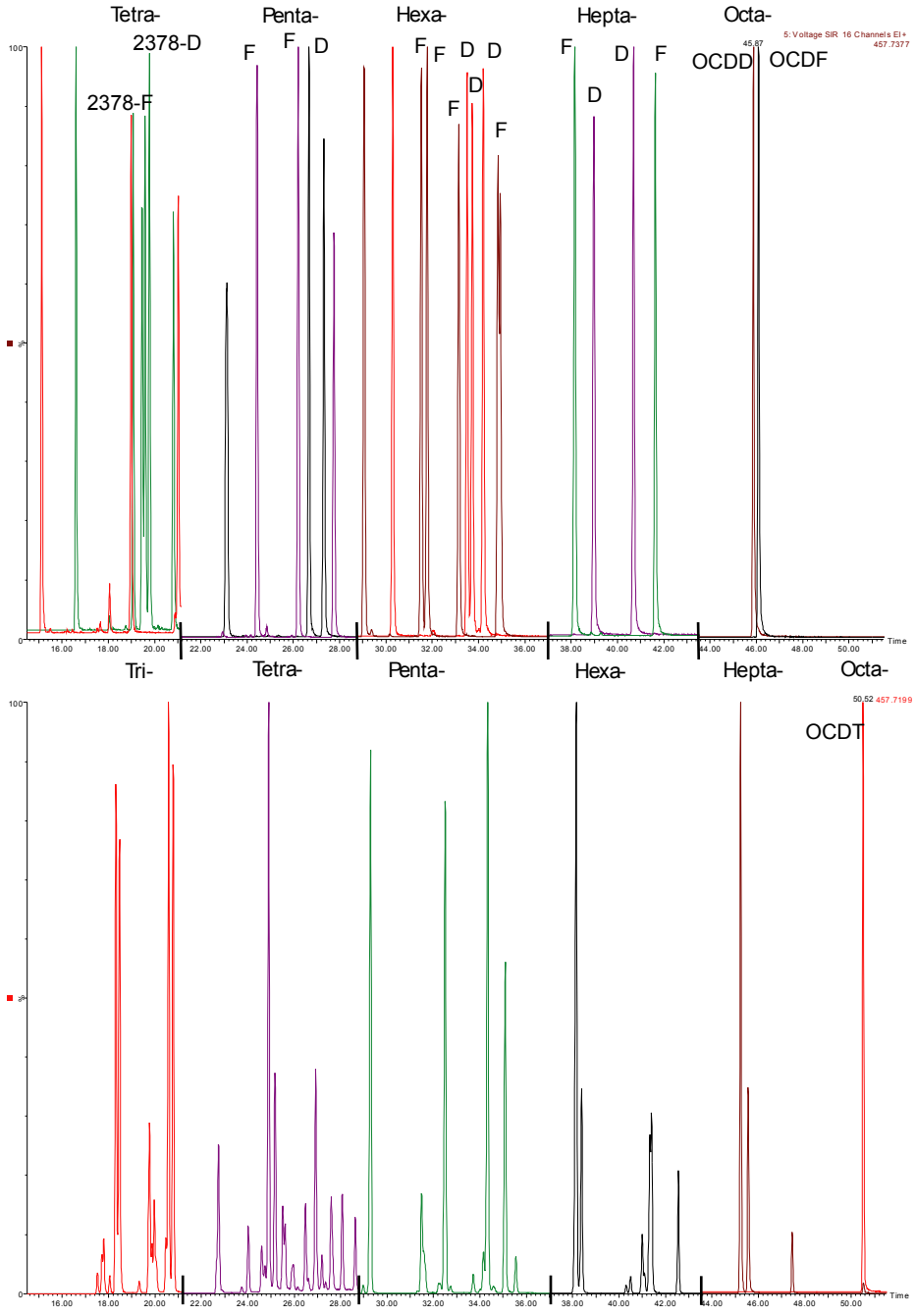

Figure 1. GC/HRMS (Autospec) SIM 5-Group Analysis of PCDF/PCDD Daily Calibration Window Standard (Top) at the start of a sample set and Tri-Octa-PCDTs in Composite PCDT Standard (Bottom) 31 direct injections later when liner-retention gap was less active. A 30 m x 0.15 mm x 0.1 µm BPX5 column with constant He pressure (440 kPa) was ramped from 170 °C (1 min) to 190 °C at 10 °C/min to 255 at 1.7 °C/min and to 315 °C at 4 °C/min.

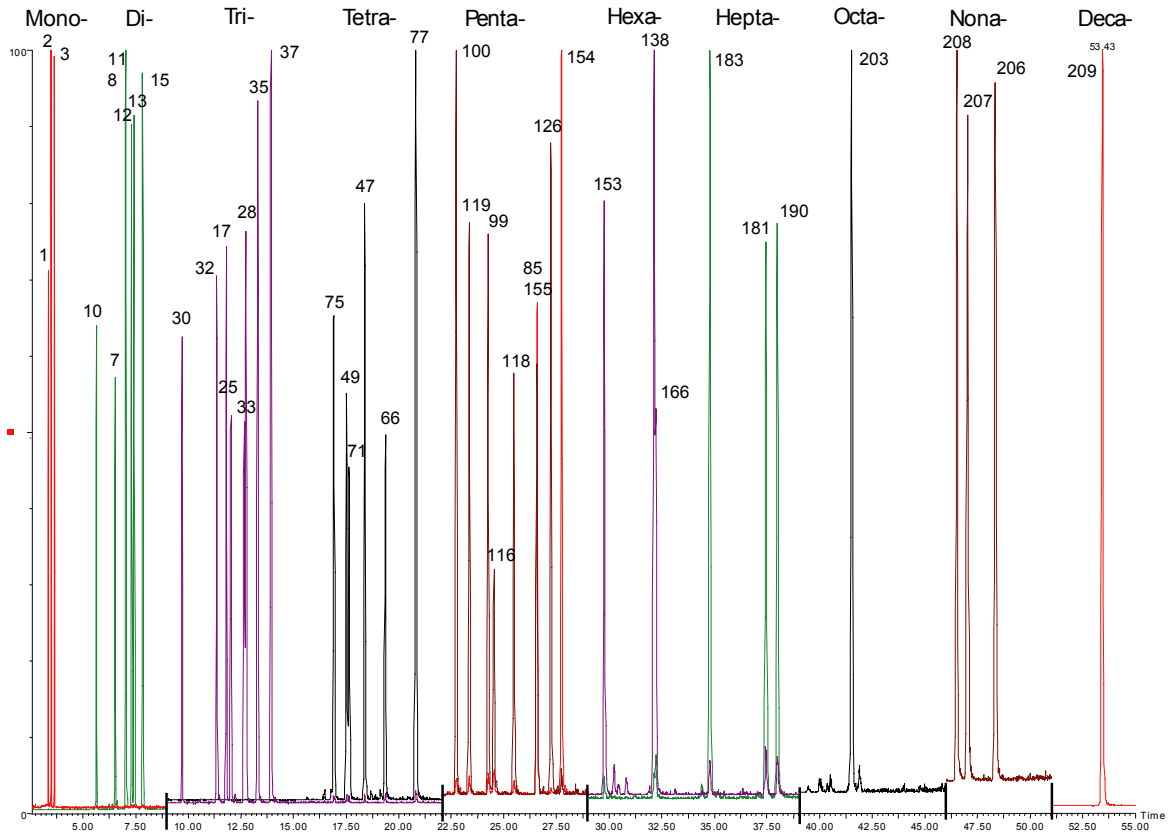

Figure 2. GC/HRMS (Autospec) SIM 7-Group Analysis of 44 PBDE congeners from mono- to deca-PBDEs via cool-on-column (3-µL). A 12.5 m x 0.15 mm x 0.1 µm BPX5 column was pressure programmed (245-415 kPa) and ramped from 150 °C to 315 °C at 3 °C/min. Deca-PBDE 209 eluted at 310 °C; PBDEs 33 and 28 partially resolved.

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