BASIC ALUMINA FLASH CHROMATOGRAPHIC SEPARATION OF BULK *ORTHO*-PCBS FROM NON-*ORTHO*-PCBs, PBDEs, PCDFs, PCDDs, PCDTs, OCPs, and PCTs

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Introduction

Comprising nearly 100 congeners in environmental samples, PCBs are often still prevalent in concentrations exceeding 1 µg/g. To effectively measure PCBs, they are isolated as a group from other persistent organic pollutants using silica gel, Florisil, or alumina column chromatography for analysis by GC/MS or dual capillary column GC/ECD. When organochlorine pesticides (OCPs) and polybrominated diphenyl ethers (PBDEs) are also targeted, PCBs are often split into two chromatographic eluates. In contrast to the major *ortho*-substituted PCB congeners, much lower concentrations occur for congeners of polychlorinated- dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), dibenzothiophenes (PCDTs), naphthalenes (PCNs), and dioxin-like non-*ortho*-PCBs[1]. Such co-planar compounds are usually separated from the bulk PCBs using a carbon LC[2] or reusable porous graphitic carbon HPLC column[3] eluted forward (*o*-PCBs, mono-*o*-PCBs, then non-*o*-PCBs) before reversal with toluene (PCDFs and PCDDs) and additional separation with basic alumina to remove PCNs, polychlorinated diphenyl ethers (PCDEs), and residual lipid for PCDF/PCDD GC/HRMS analysis.

Recently, smaller particle-size normal phase adsorbents including active basic alumina have become available along with custom-made glass columns for use in low pressure flash chromatography. With low gas pressure (< 1-2 bar) and particles 32-63 µm, flash chromatography is a rapid, inexpensive technique with enhanced resolution compared to gravity column chromatography[4]. However, few environmental researchers use the technique, but basic alumina is in the automated PowerPrep LC system for PCDFs, PCDDs, PCBs and PBDEs[5]. A flash LC column is quickly dry-packed, gives improved flow performance, and has sufficient resistance to gravity flow without a shutoff valve. Contamination from lab air, dust, and sample carryover is minimized by using high purity nitrogen, much smaller eluate volumes and blown down in tubes with high purity nitrogen. The disposable adsorbent is used only once with an inert, nonleachable, reusable and cleanable glass column with glass joints and disposable glass fiber.

We evaluated basic alumina flash chromatography initially for PCBs, because Loos et al.[6] had separated 13 selected *o*-PCB congeners from three non-*o*-PCBs (77, 126, and 169) and then from PCDFs and PCDDs with eluants of 150-200 mL each from a large 25-g basic alumina (Super 1 active) column. Because the elution properties of other PCB congeners were unknown in addition to some PBDEs, PCDTs, and other compounds, we chose to evaluate basic alumina flash chromatography comprehensively. We optimized the separation of all bulk *o*-PCBs from all non-*o*-PCBs, tested other pollutants (PBDEs, PCDTs, PCDFs and PCDDs) under similar elution conditions, and finally applied the chromatographic technique to samples known or suspected to contain complex mixtures of these.

Materials and Methods

Basic (pH 9.7) alumina (active grade, 40-63 µm, surface area 150 m^2/g, Sorbent Technologies, Atlanta, Georgia, USA) was stored at 130 °C indefinitely. Alumina (4 g) was either prepacked or packed warm in a warmed glass column custom-made by Kontes (Vineland, NJ USA), topped with 1-g sodium sulfate, and quickly conditioned with 30 mL cyclopentane (1.5 mL/min at 0.5 bar) to avoid moisture. Column dimensions were 5 mm i.d. x 30 cm long topped with a 40 mL reservoir and 35/20 ground glass fitting (joint clamped with gas pressure relief valve) and a male Luer lock fitting at the end. Solvents used were hexanes, dichloromethane (DCM), and isooctane (Optima grade, Fisher), cyclopentane and nonane (B&J Grade, Burdick & Jackson), and *n*-butyl chloride (HPLC grade, Fisher). Several radiolabelled compounds used for characterization and QC were ^{14}C- PCB 52, p,p’-DDE, naphthalene, and oleic acid and ^{3}H-PCB 126. The following standards in nonane or isooctane were prepared and tested: a 20 µg-PCB mixture of equal portions of Aroclors (1242:1248:1254:1260), a mixture of 31 OCPs (Accustandard, New Haven, CT USA), a mixture of 39 mono- through hepta-PBDE congeners and ^{13}C-PBDE 209 (Cambridge Isotope Labs, Andover, MA, USA), all ^{13}C-labeled 2,3,7,8-substituted PCDFs and PCDDs and all ^{13}C-non-*o*-PCBs (Cambridge Isotope Labs), 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) totaled 10 µg from equal amounts of Aroclors 5442 and 5460.

Two GC/HRMS systems (Agilent 6890 GC/Waters Autospec Ultima and HP 5890A/VG 70S) used septumless Merlin Microseals, 2.5 m x 0.25 mm Restek Siltek-treated retention gaps, and Siltek-treated press-tight connectors to narrow bore (0.15 mm) thin film (0.1 µm) 30 m custom made capillary columns (SGE, Austin, TX USA) of BPX5 and Solgel 1 MS. A 30 m BPX5 gave excellent performance of PCDFs, PCDDs, and PCDTs within homolog groups, a short 12 m BPX5 with on-column injection worked well with all PBDEs, and a 30 m Solgel 1 MS resolved non-*o*-PCBs 81 and 77 from potentially interfering residual penta- and hexa-*o*-PCBs as shown below. Detailed GC/HRMS chromatographic information about our narrow bore columns is presented separately at Dioxin 2006[7].

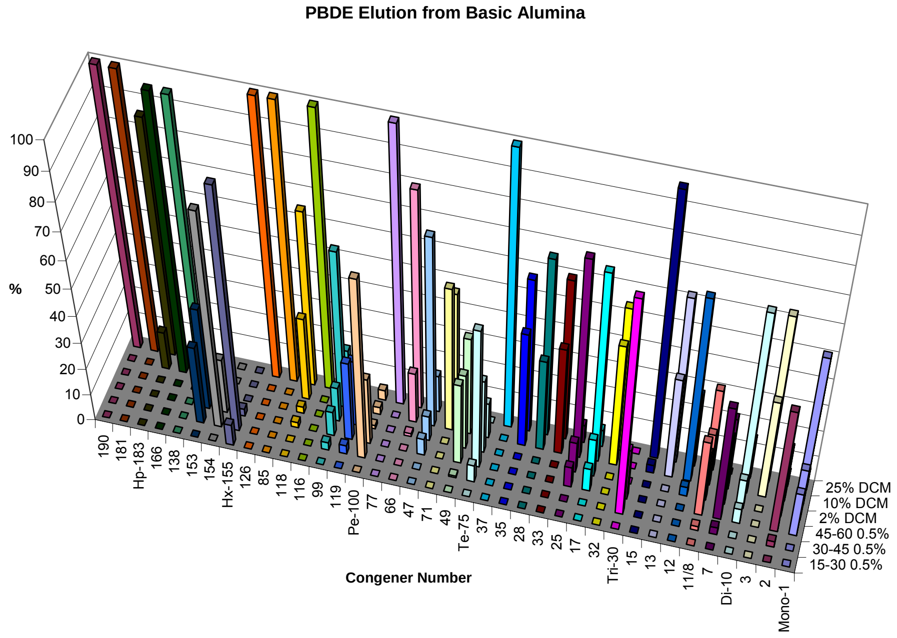
After evaluations of relative adsorbent strength and column performance, eluate test fractions were collected typically every 4-5 mL and concentrated to < 1 mL for GC/HRMS or dual column GC/ECD. Samples evaluated with basic alumina include fish (second silica gel eluate for OCPs and PBDEs, also containing PCBs and fatty acids), concentrated PCB (59 congeners) dosing solutions (propylene glycol/corn oil in isooctane) cleaned up only with reactive extraction, and sediment samples from Louisiana (USA) shortly after Hurricane Katrina.

Results and Discussion

For the first eluant, we chose cyclopentane with a minor second solvent (to be optimized) because 50 mL alone eluted most *o*-PCB congeners without any non-*o*-PCBs. Best results for the first eluant showed 32 mL of 0.5% DCM in cyclopentane (or 1.5% n-butyl chloride in cyclopentane) nearly completely separated all bulk *o*-PCBs in Aroclors from all non-*o*-PCBs. Elutions were sharp as the next 4-mL test fraction (32-36 mL) contained 40% of PCB 169 with only few residual *o*-PCBs (56/60 at 4%, 22 and 128 at 2%). In the previous 28-32 mL test fraction, last eluting *o*-PCBs were 22 (36%), 56/60 (22%), 128 (33%), 82 (9%), 40 (7%), and 105 (6%) with 1% of PCB 169. Further elution (another 28 mL) with 0.5% DCM in cyclopentane eluted non-*o*-PCB 169, 126, 81, 35 before step gradients to 10% and 25% DCM in cyclopentane were necessary to elute non-*o*-PCBs 77, 37, 15, and 3.

Under similar conditions, few OCPs (HCB, aldrin, heptachlor, o,p-DDT, and p,p'-DDE) eluted with bulk-*o*-PCBs. Most ^{13}C-2378-PCDFs and PCDDs eluted with 25% DCM except the first two hexa-PCDFs with 10% DCM. Many native PCDTs were comparably retained, but several PCDTs (OCDT, one hexa- and one hepta-PCDT) surprisingly eluted just before PCB 169. All tetra- through octa-PCNs eluted with bulk *o*-PCBs; most PCTs did not. Elutions of 39 PBDEs are shown below (Figure 1) corresponding to congeners in GC/HRMS retention time order and with bromine substitution in basic alumina elution order. Compared with PCB congeners, PBDEs show both similarities and differences. For both groups, symmetrical 2,4,6- 2',4',6'-congener 155 is least retained, while *para*-substituted congeners are strongly retained. However, PBDE 209 and PBDEs fully brominated on one ring are much more strongly retained than PCB 209 and comparable PCBs. Nearly 100% DCM was necessary to elute PBDE 209.

A Great Lakes QC sediment (DX-3, Environment Canada) was evaluated using our basic alumina with GC/HRMS analysis (Figure 2). After 95-99% bulk *o*-PCBs were eluted with 32 mL 0.5% DCM, the second eluate (15-mL DCM) is shown for non-*o*-PCBs and other compounds. Our concentrations closely match consensus values except our PCB 81 (74 pg/g) is nearly half that of 133 pg/g, which may be inflated by interference from penta-*o*-PCB 87 that our GC column resolves and alumina minimizes. PBDE 47 at 0.4 ng/g was near our blank level.



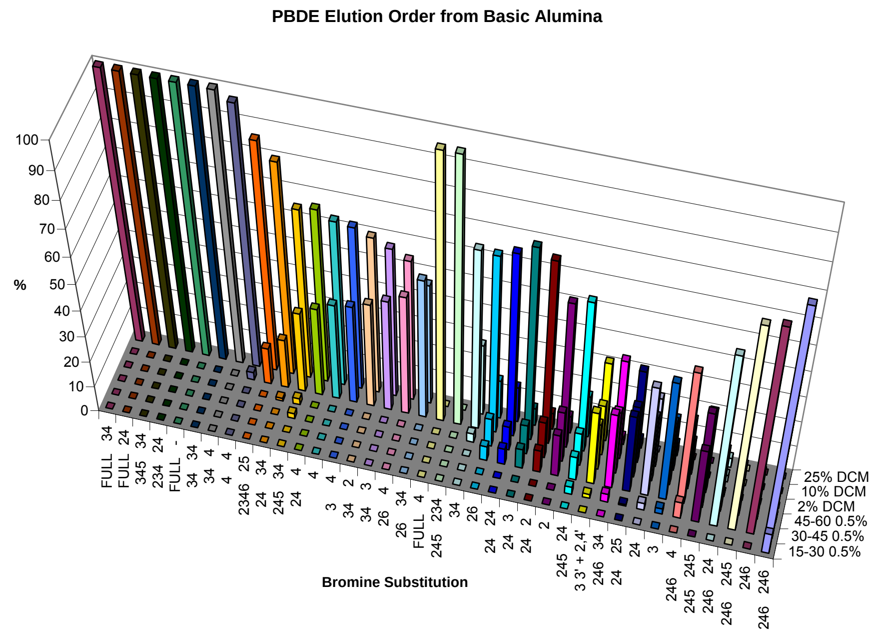


Figure 1. Elution order of 39 PBDE congeners from 4-g active basic alumina column in several 15 mL eluate fractions of 0.5% dichloromethane (DCM) in cyclopentane and subsequent 5 mL eluate fractions of 2%, 10%, or 25% DCM in cyclopentane. PBDE numbered congeners (Top chart) are tallied right to left in GC/HRMS retention time order, while PBDEs with bromine substitution (Bottom chart) are reorderd in elution order from basic alumina.

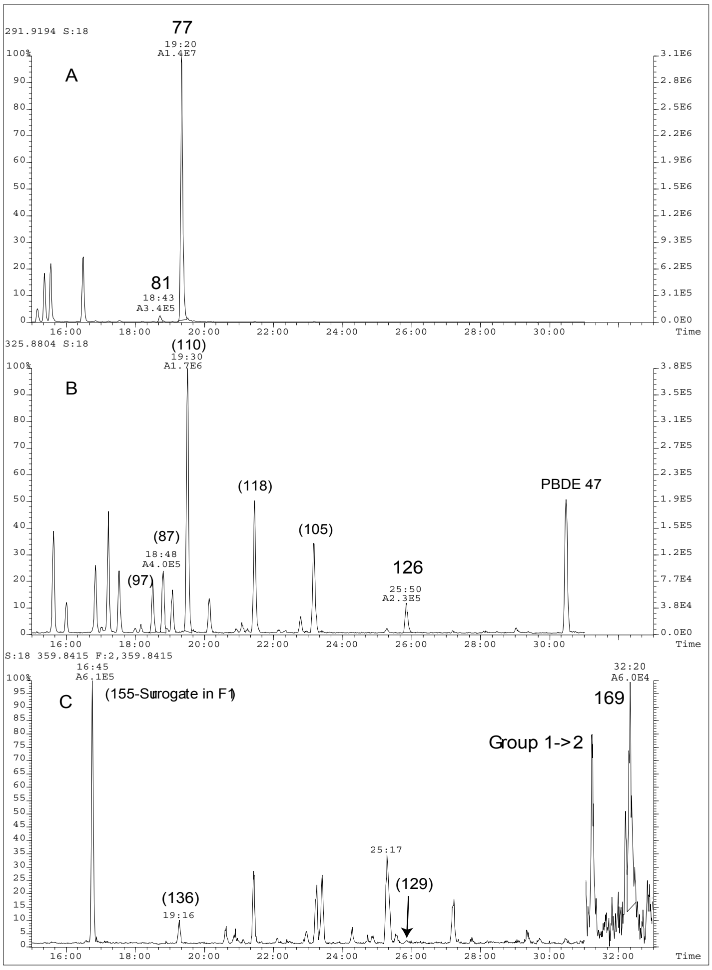


Figure 2. GC/HRMS for non-*o*-PCBs in 2nd alumina eluate (15 mL DCM) of DX-3 sediment extract (5µL/0.5 mL). Ion chromatograms (not smoothed) show tetra- (A), penta- (B), and hexa-PCBs (C) plus tetra-PBDE 47. A 30 m x 0.15 mm x 0.1µm Solgel 1 MS column began at 155 °C (1 min) to 205°C at 1.8°C/min and to 310°C at 3.6°C/min.

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