Isolation and Recovery of Selected Polybrominated Diphenyl Ethers from Human Serum and Sheep Serum: Coupling Reversed-Phase Solid-Phase Disk Extraction and Liquid–Liquid Extraction Techniques with a Capillary Gas Chromatographic Electron Capture Negative Ion Mass Spectrometric Determinative Technique

Paul R. Loconto*, David Isenga, Michael O'Keefe, and **Mark Knottnerus**

Division of Chemistry and Toxicology, Bureau of Laboratories, Michigan Department of Community Health, 3350 North Martin Luther King Jr. Blvd. P.O. 30035 Lansing, MI 48909

Abstract

Polybrominated diphenyl ethers (PBDEs) are isolated and recovered with acceptable percent recoveries from human serum via liquid–liquid extraction and column chromatographic cleanup and fractionation with quantitation using capillary gas chromatography–mass spectrometry with electron capture negative ion and selected ion monitoring. PBDEs are found in unspiked serum. An alternative sample preparation approach is developed using sheep serum that utilizes a formic acid pre-treatment followed by reversed-phase solid-phase disk extraction and normal-phase solid-phase cleanup using acidified silica gel that yields > 50% recoveries. When these percent recoveries are combined with a minimized phase ratio for human serum and very low instrument detection limits, method detection limits below 500 parts-per-trillion are realized.

Introduction

Polybrominated diphenyl ethers (PBDEs) have emerged as a class of persistent organic pollutants (POPs) that should be considered as part of a laboratory's biomonitoring program. Recently, Schecter and coworkers reported that dioxin, dibenzofuran, and polychlorinated byphenyl (PCB) levels in human blood are much lower in 2003 when compared to 1973 levels; however, the opposite is true for PBDEs. Unlike dioxin, dibenzofuran, and PCBs, they found no significant correlation between PBDE levels and age. PBDEs were manufactured and marketed as penta, octa, and deca-BDE commercial mixtures. This nomenclature is similar to the way PCBs were manufactured as various Aroclors of an earlier era. In 2001, approximately 67,440 metric tons of PBDEs were manufactured annually worldwide with a North American market that used 7100 metric tons of the Penta-BDE commercial mixture. Since 2004, the deca-BDE commercial mixture remains the only commercial mixture manufactured and sold in the United States. PBDEs are reported to be antagonists of thyroid hormones (1). Potential threats to human and animal health include neurotoxicity, endocrine disruption, peripheral nervous system damage, and cancer (2). Morland et al. (3) cite two reported adverse health outcomes in laboratory animals dosed with high levels of PBDEs to include neurological deficiencies and endocrine disruption. Schecter and coworkers (4) report on the analysis of 47 individual human breast milk samples from nursing mothers, 20–41 years of age in Austin and Dallas, Texas. They found up to 13 BDE congeners present with concentration levels that ranged from 6.2 to 419 ng/g lipid. Samples of human adipose tissue taken from 31 men and 22 women having a mean age of 53 years showed concentration levels of between 1.23 and 57.2 ng/g lipid (sum of BDEs 28, 47, 99, 100, 154, 153, 183) (5).

Robust and cost-effective analytical approaches to the isolation and recovery of selected PBDE congeners are essential for an effective biomonitoring program. Sjödin and coworkers in Sweden found elevated PBDE concentration levels in serum from workers in an electronics dismantling plant (6). Their approach used liquid–liquid extraction (LLE) techniques with acid-base partioning and silica gel/sulfuric acid cleanup. A gas chromatograph–mass spectrometer operating under dissociative resonance electron capture negative ion conditions with selective ion monitoring, abbreviated for this paper as CGC–MSD-ECNI-SIM was used in this study, whereby "bromine ions"

* Author to whom correspondence should be addressed: email locontop@michigan.gov.

become highly abundant. BDE-128 and -138 were used as the internal standards (IS). Human serum was spiked (with BDE congeners 47, 77, 85, 99, 100, 153, 154, 183, 209) at two concentration levels. A low spike with 0.03 ng/g each BDE congener yielded percent recoveries from 69% to 104%. A high spike with 0.2 ng/g each BDE congener yield percent recoveries from 77% to 104%.

Covaci, Voorspoels, and De Boer (7) reviewed the determination of brominated flame retardants with an emphasis on PBDEs through 2003 citing methods for adipose tissue, liver adipose tissue, human breast adipose tissue, and serum, while covering details of sample pretreatment, extraction, cleanup, and determinative techniques. Thomsen and coworkers reported between 56% and 111% recoveries for selected PBDEs by pretreating serum with a mixture of formic acid and 2-propanol (4:1), followed by ultra sonication and dilution with water, followed by reversed-phase-solid-phase extraction (RP-SPE) and lipid decomposition with concentrated H_2SO_4; elution with (1:1) methylene chloride (CH_2Cl_2)–methanol (MeOH), and quantitation using C-GC–MSD-ECNI (8). Sjödin and coworkers (9) reported between 69% and 85% recoveries of selected PBDEs from human serum. Following pretreatment with HCl and 2-propanol, a 1:1 mixture of hexane (Hx) and CH_2Cl_2 is used to extract PBDEs from the matrix. The extract is first washed with KCl (aq); partitioned using KOH–ethanol; treated with concentrated H_2SO_4; washed with water, acetate buffer, and a water–methanol mixture, followed by extraction with 1:1 CH_2Cl_2–MeOH and quantitation using CGC–MSD-ECNI-SIM.

Commenting that SPE remains an underused extraction technique, chemists at the Centers for Disease Control and Prevention (CDC) embarked on a series of RP-SPE/Cleanup approaches to isolating and recovering various organochlorine (OC) pesticides while subjecting various sample prep steps to automation (10). Sjödin and coworkers reported on a semi-automated high throughput RP-SPE approach to measuring PBDEs, PBBs, and PCBs in human serum while also pretreating the serum with formic acid. High throughput techniques utilized a Gilson 215 Liquid Handler (Gilson, Inc., Middleton, WI) to conduct sample pretreatment, a Rapid Trace SPE Workstation (Caliper Life Sciences, formerly Zymark Corp., Hopkinton, MA) to conduct RP-SPE, and a Rapid Vap (Labconco Corp., Kansas City, MO) to evaporate down RP-SPE eluents, with quantitation via isotope dilution gas chromatography interfaced to a high resolution MS (11).

Our laboratory has used conventional LLE techniques combined with Florisil column cleanup and silica gel column fractionation to isolate and recover over 100 OCs, PCBs, and PBBs. Our approach to sample preparation follows the methodology published previously by Najam and coworkers at the CDC (12), with minor modifications.

Recently, our laboratory was very fortunate to have acquired a new single quadrupole, low MS resolution GC–MS as part of our continued association with the Chemical Testing Laboratory Network of the CDC. Because we are designated as a Level-1 laboratory, we did not have a mandate to implement a CDC-related method on this instrument. The intense interest on the part of state epidemiologists as well as university scientists who depend on our laboratory to conduct trace analysis of human serum to determine POPs had requested that we add a selected number of BDE congeners to our existing menu. We asked our clients to review the toxicology and epidemiology literature and recommend which BDE congeners we should begin to biomonitor. We developed a CGC–MSD-ECNI-SIM separation of nine BDE congeners and two PBB congeners and quantitated each against BDE-66 that was added as the internal standard (IS). Our success with developing a highly selective and very sensitive determinative method for PBDEs will be reported elsewhere.

In this paper, we report on our findings for the isolation and recovery of BDE congeners 28, 44, 77, 100, 99, 85, 154, 153, and 183 from human serum using our exhaustive in-house LLE/Cleanup/Fractionation scheme. We also report our preliminary findings on the isolation and recovery from spiked sheep and human serum, and other aqueous matrices using the HCOOH pretreatment discussed earlier with RP-SPDE using various commercially available disks, while investigating an acidified silica gel SPE cartridge cleanup technique to remove interfering lipids. We also show how instrument detection limits (IDLs) obtained from the application of calibration statistics are used to calculate method detection limits (MDLs) for each BDE congener. As far as we know, these are the first reported percent recovery results for isolating selected BDE congeners from spiked sheep and human serum and various aqueous matrices via RP-SPDE using CGC–MSD-ECNI-SIM as the determinative technique.

Experimental

Materials and reagents

LLE and Florisil cleanup/silica gel cleanup and fractionation

Only those laboratory items unique to the method will be described. Rotary mixer (Wheaton Scientific Products, Milville, NJ); Maxi Mix II Model 37615 (Barnstead-Thermolyne, Dubuque, IA); TurboVap LV (Caliper Life Sciences); Model RC 3C Plus Centrifuge (Sorvall, Asheville, NC); SPE Cartridges, 6 mL capacity containing 3.0 g anhydrous Na_2SO_4 (Alltech Associates, Inc. Deerfield, IL); methanol (Burdick and Jackson, Muskegon, MI [B&J], #362-4); diethyl ether (B&J #106-1); hexane (B&J #216-4); concentrator tubes (Kontes, #570050-2526, Vineland, NJ); #19 glass stoppers (Kontes, #850100-0019); chromatography column, 7 mm i.d. × 200 mm (Kontes, #420100-0022); and 9 mm i.d. × 200 mm (Kontes, #420100-0023). N-EVAP Model 112 (Organomation, Northborough, MA); Florisil, 60–100 mesh (Floridin Co. Pesticide Residue Grade, Quincy, FL); anhydrous Na_2SO_4 (Fisher #S421-50, Waltham, MA); anhydrous $CaSO_4$ Drierite (VWR, #2281-040); centrifuge tubes, 13 mL (Kontes #410500-00150); chromatography column, 22 mm i.d. × 300 mm (Kontes, #420540-0233); Silica Gel Woelm, 100–200 mesh; benzene (EM Science, #BX-0212-1, Gibbstown, NJ.).

RP-SPE and RP-SPDE with and without acidified silica gel cleanup

The following hydrophobic sorbents and disks were kindly supplied and used: C8 and C18 Bond Elut (Varian, Palo Alto, CA); C18 Isoelute (International Sorbent Technology, Tucson, AZ);

C18 UCT (United Chemical Technology, Horsham, PA), all in 3-mL capacity containing 200 mg bonded silica sorbent barrel cartridge formats. Also used were C8 and C18 SD Empore disks; C2, C8 and C18 SPEC disks; MP-1, MP-3, and Focus (Varian), all in 3-mL barrel cartridge formats. The sorbent mass for the Focus Polar-Enhanced disk is 20 mg. A 24-port Visiprep SPE Vacuum Manifold (Supelco, Bellefonte, PA) was used for the RP-SPDE and a 10-port Visiprep was used for the NP-SPE cleanup work reported in this paper. A Vacuum/Pressure Station (Alltech, Deerfield, IL) provided the necessary vacuum. A 1 L safety-coated filtering flask with a side-arm served as a water trap between the vacuum pump and the vacuum manifold. Empty SPE (60 mL) reservoirs were also used and connected to the 3 mL barrel cartridge using standard Luer adapters (part #12131001, Varian). Acidified silica gel was prepared in our laboratory by first activating Silica gel 60, 70–230 mesh ASTM (EMD Chemicals, Gibbstown, NJ) overnight at 250ºC. An amount of concentrated H2SO4 was then added to yield a 2:1 w/w acid–silica gel ratio. A second amount of activated silica gel was also used without adding acid. A normal-phase SPE cartridge for removal of coextracted lipids was prepared as published previously (11). A Meyer N-EVAP Model 111 (Organomation, Northborough, MA) was used to evaporate eluents. Test tubes used as receivers were either 16 × 125-mm borosilicate disposable conical or round-bottom culture tubes (VWR, West Chester, PA).

Chemical reference standards and autoinjection considerations

PBDE stock solutions in nonane were obtained from Cambridge Isotope Laboratories Inc, Andover, MA. PBDE stock solutions in iso-octane were obtained from AccuStandard, Inc. (New Haven, CT). Stock solutions were serially diluted in 25% Hx–75% iso-octane and stored at –20°C in sealed ampoules until used as calibration reference standards, initial calibration verification reference standards (ICVs), and continuing calibration verification reference standards (CCVs) in this work. The choice of the diluent solvent composition for calibration, ICVs, and CCVs was dictated by our desire to keep the chemical composition of these standards close to that found in the sample extracts. However, the spiking solution was prepared by diluting the stock in a polar or water-miscible organic solvent to facilitate greater analyte dispersion in the sample matrix. After pre-treatment of serum, the matrix is predominantly aqueous. Chemical reference standards containing PBDEs and PBBs were placed in 300 µL conical glass inserts with spring (11-0000-100 MicroLiter Analytical Supplies, Inc., Suwanee, GA). The inserts were placed in 2 mL, 12 × 32 mm glass screw-top vials (Target DP Screw-Thread Vials, National Scientific, 66030-002, VWR). The 2-mL vials containing the inserts were placed in the GC autosampler and automatically injected. PBDEs tenaciously cling to glass surfaces. Silanized 2 mL GC vials (C4010-S2W, silanized amber target SC I-D Vial, National Scientific) are recommended for long-term storage of PBDE chemical reference standards. Compressed cylinders containing helium and methane (Airgas, Radnor, PA) supplied carrier gas and CI reagent gas to the instrument.

Instrumentation and operating conditions

A 6890 GC with a 7683 Automated Liquid Sampler interfaced to a 5973NMass Selective Detector utilizing ChemStation Software, Version D.01.02 (Agilent Technologies, Inc., Wilmington, DE) operating within Windows XP Version 5.1 (Microsoft Corporation, Redman, WA). A 30 m × 0.25 mm × 0.25 µm DB-XLB (#122-1232) or a 30 m × 0.25 mm × 0.25 µm DB-XLBMSD (#122-4432), both obtained from Agilent Technologies, were used to separate PBDEs. A CI source (G1999-60402, Agilent) was installed with methane as CI reagent gas. Reference to this instrument in this paper is abbreviated GC–MSD. Protocols were adhered to for the methane pre-tune, positive ion tune using PFDTD followed by a negative ion tune using PFDTD. A programmed temperature vaporizer (PTV) in a pulsed splitless injection mode was used throughout the study. A Graphpack-3D (007541-005-00) ferrule, a baffled deactivated glass liner (011711-010-00), and a Graphpack-2M ferrule for a 0.25 mm i.d. and 0.40 mm o.d. WCOT (all from Gerstel GmbH & Co. KG) were required to set up the PTV.

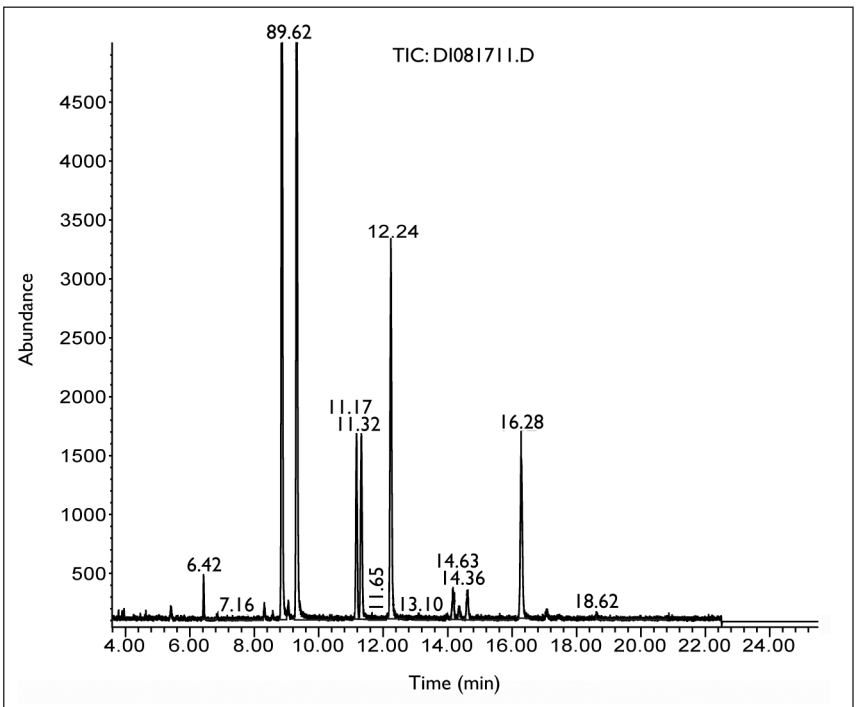

Figure 1. Total ion chromatogram from a human serum specimen from 4 mL human serum. Isolated and recovered by LLE/Cleanup. BDE-28 at 6.42 min; BDE-47 at 8.86 min; BDE-100 at 11.32 min; BDE-99 at 12.24 min; BDE-85 at 14.16 min; BDE-154 at 14.61 min; BDE-153 at 16.28 min; BDE-66 at 9.32 min added as internal standard. 30 m × 0.25 mm DB-XLB; 1 µL pulsed splitless injection PTV, T(injector) = 300°C; T(ion source) = 166°C; T(quadrupole rods) = 150°C; oven temperature program: 110°C (hold for 1 min) then to 240°C at 120 deg/min; (hold at 240°C for 1 min) then to 300°C at 3 deg/min (hold for 0.0 min) then to 110°C at 120 deg/min (hold at 110°C for 0.5 min).

Sample Preparation Methods

LLE and Florisil cleanup/silica gel cleanup and fractionation

To 4 mL of human serum that was placed in a 16 × 150 mm culture tube, 2 mL of methanol was added and vortexed at low speed for 10–20 s and then successively (3×) extracted with 2 mL of 1:1 diethyl ether–hexane. The extract was passed through anhydrous Na_2SO_4 to remove moisture and was evaporated to dryness. The weight of lipid was taken and reconstituted in 1 mL of hexane and then subjected to a micro-Florisil column cleanup, which led to two fractions: (*i*) a 6% fraction that contained PBBs, PCBs, DDT, and its two metabolites DDD, DDE, other polychlorinated organics, and PBDEs, and (*ii*) a 20% fraction that contained cyclodiene insecticides such as dieldrin, endrin, and tetradifon.

The 6% fraction was subjected to silica gel column cleanup, which yielded two fractions: (*i*) a hexane (Hx) fraction that contained aldrin, mirex, heptachlor, hexachlorobenzene, PCBs, PBBs, DDE, and PBDEs, and (*ii*) a benzene (Bz) fraction that contained DDE, DDT, DDD, technical chlordanes, *cis*/*trans* nonachlor, and PBDEs.

Extracts from the Hx and Bz fractions were injected into a dual column CGC incorporating an electron capture detector (ECD): the injected extract from the Hx fraction was split onto a DB-5 column and a DB-1701 column; the injected extract from the Bz fraction was split onto a DB-XLB column and a DB-35MS column; extracts from both fractions were combined, and then injected into our GC–MSD under CGC–MSD-ECNI-SIM conditions.

RP-SPDE with and without acidified silica gel sorbent cartridge cleanup

To 1 mL of human or sheep serum or aqueous solution was added 50 µL of a 50 ppb PBDE congener mix dissolved in 2-propanol and 1 mL of 5% acetonitrile in concentrated formic acid. The mixture was vortexed, then passed through a previously conditioned reversed-phase disk. The disk was then rinsed with distilled, deionized water, the vacuum was left on for 2 min, and water droplets clinging to the inner wall of the cartridge tube were removed with a Kim-Wipe or equivalent. A second cartridge containing 250 mg anhydrous sodium sulfate sandwiched between two polypropylene frits was placed beneath the RP-SPDE cartridge. The piggy-backed cartridges were inserted into the vacuum manifold and eluted with 1 × 500 µL of 1:1 methylene chloride–ethyl acetate and then 4 × 250 µL 1:1 methylene chloride–ethyl acetate into a round-bottom test tube as receiver. The eluent was evaporated down to near dryness. If no further cleanup was to be done, 250 µL of 25% hexane–75% iso-octane was added and the solution was vortexed. Two hundred microliters of eluent was then transferred to a 300-µL glass insert contained in a 2-mL GC vial. Twenty microliters of 90 ppb BDE-66 was added for use as the internal standard and vortexed. One microliter of eluent was injected into a CGC–MSD-ECNI-SIM. If cleanup was to be done on the eluent from RP-SPDE, the cleanup cartridge was prepared and conditioned prior to transfer of the eluent. To prepare the cleanup cartridge, to an empty 3 cc barrel cartridge was placed a polypropylene frit, 250 mg activated silica gel, a frit, 350 mg acidified silica gel (2:1 sulfuric acid, silica gel), a frit, and 200 mg anhydrous sodium sulfate. The cleanup cartridge was conditioned by first passing 2 mL of 1:1 methylene chloride–hexane, then passing 2 mL of hexane through to a receiver. The contents of the receiver were discarded. Six milliliters 6 mL hexane was eluted into a clean, dry test tube (16 × 125 mm rounded bottom), 50 µL iso-octane was added as a keeper solvent, and the solution was evaporated to near dryness under a gentle nitrogen gas purge. The test tube was removed as soon as liquid disappeared. Two hundred fifty microliters of 25% hexane–75% iso-octane was added and the solution was vortexed. Two hundred microliters of eluent was then transferred to a 300-µL glass insert contained in a 2-mL GC vial. Twenty microliters of 90 ppb BDE-66 was added for use as the internal standard and vortexed. One microliter of eluent was injected into a CGC–MSD-ECNI-SIM.

Results and Discussion

Advantages and limitations of the CGC–MSD-ECNI-SIM determinative technique

Dissociative resonance electron capture of PBDE congeners at an ion source temperature of 150°C yields highly abundant "bromine ions" at *m/z* 79 and at *m/z* 81 due to the natural isotopic abundance of the element bromine. This high abundance enables IDLs to reach down to < 1 ppb and MDLs to < 0.25 ppb. Highly reproducible GC and ECNI mass spectral detection using the rugged 6890/5973N GC–MSD with methane as CI reagent gas is advantageous. We have observed that the abundant "bromine ions" are quenched if PBDE congeners are dissolved in polar solvents. We have observed significant suppression of the *m/z* 79/81 pair when PBDE congeners are dissolved in 2-propanol versus being dissolved in either hexane or iso-octane. We consistently used a final eluent solvent mix containing 25% hexane/75% iso-octane to eliminate this matrix interference. Abundances of the 79/81 pair for BDE-66 (used as internal standard) increased if this congener was dissolved in ethyl acetate. We also found it imperative that upon adding BDE-66 to the final eluent that the contents be vortexed in order to achieve accept-

Table I. PBDEs from Human Serum analyzed on 8/17/2006 via LLE-Cleanup and CGC–MSD-ECNI-SIM*

			#ppb in extract								
Sample	ID	ChemStaFile#	28	47	77	100	99	85	154	153	183
Serum	S06-19-286	DI081711	0.07	6.12	ND	1.43	3.64	0.32	0.29	2.37	ND
Serum	S06-19-293	DI081719	D	1.10	ND	0.33	0.32	ND	ND	1.34	ND

* BDE congener results reported from the extract and not from the original serum specimen and not corrected for lipid content; BDE congeners are baseline resolved from PBB-155 (added as a surrogate to each sample) and from PBB-153 (principal congener in Firemaster); ND— not detected (i.e., below the limit of detection); D— detected but below the limit of quantitation.

able precision among replicate injections of our continuing calibration verification (CCV) standard throughout our work reported here. The CCV was injected at a frequency of once every ten samples. Quantitation, although very sensitive, lacked the selectivity afforded by electron-impact MS. It became imperative then to achieve sufficient GC resolution for any and all organobromine compounds that are likely to be present in human serum to achieve these very low IDLs. This includes non-targeted BDE congeners, PBB congeners, and hexabromobenzene. Our success in achieving adequate GC resolution for all of these organobromine compounds by optimizing the temperature program to include our measurement of IDLs for each BDE congener will be reported elsewhere.

LLE and Florisil cleanup/silica gel fractionation

Analytical results from unspiked human serum

The Hx and Bz fractions from Silica gel cleanup were combined because we found PBDEs in both fractions, contrary to our initial assumption. Figure 1 is a CGC–MSD-ECNI-SIM total ion chromatogram (TIC) of an unspiked human serum specimen. Table I lists analytical results for a study involving human serum that reveals detectable levels of BDE congeners, and includes Sample S06-19-286 (ChemStation data file DI081711). The TIC for this sample is shown in Figure 1. The peak at 11.17 min is due to PBB-155, which is added to every sample prior to LLE as a surrogate. The peak at 9.32 min is due to BDE-66, which is added to the final extract and serves as a single IS against which all targeted BDE congeners are quantitated. Figure 1 is typical of the kind of total ion chromatograms we observed with this approach in our laboratory. PBDEs can also be quantitated using CGC-ECD. A good correlation has been achieved for quantitative results for BDE congeners between the two GC determinative techniques in our laboratory. CGC–MSD-ECNI-SIM offers comparable instrument detection limits to CGC–ECD for PBDEs without any potential chromatographic interference from coeluting organochlorine compounds such as OCs and PCBs.

Percent recoveries from spiked human serum

Factors that reduce percent recoveries from an acceptable range between 70% and 120% include (7): (*i*) adsorption to glass surfaces with higher brominated BDEs more likely to adsorb; (*ii*) UV degradation such as occurs for BDE-209; (*iii*) evaporative losses such as for the lower brominated BDE-28.

Table II shows mean percent recoveries over four replicate LLEs for each targeted BDE congener. The LLE step is part of a three-step, time- and labor-intensive sample preparation scheme which is performed routinely in our laboratory. However, via exhaustive LLE, percent recoveries fell well within the acceptable range discussed earlier, while comparing favorably with those reported previously (6). The low-level % recovery study incorporated a spike concentration level that approximated the levels found in the human serum specimens whose results are reported in Table I.

RP-SPDE with and without acidified silica gel cleanup

Method development for a RP-SPDE approach with CGC–MSD-ECNI-SIM as the determinative technique

An RP-SPDE method developed and published by Covaci and Schepens for selected POPs in human serum (13) was adapted to isolate and recover PBDEs with low percent recoveries when using C18 SPDE cartridges with a suggestion to try polymeric disks (14). Figure 2 shows CGC–MSD-ECNI-SIM extracted ion chromatograms (EICs) for both bromine isotopes for the isolation and recovery of selected BDE congeners from spiked sheep serum using RP-SPDE techniques. This EIC is typical of the kind of chromatographic separation and detection for the spiked serum study. An unidentified peak occurred at a retention time

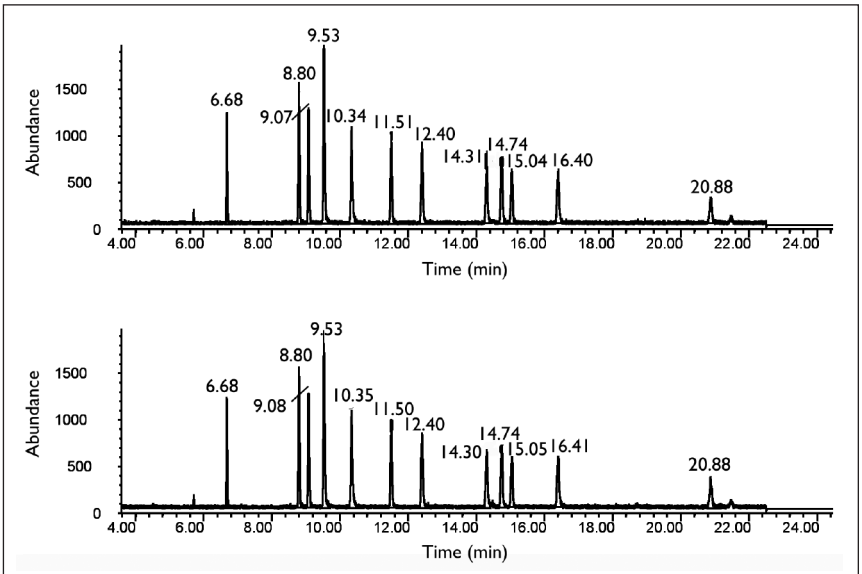

Figure 2 . Extracted ion chromatogram from 1 mL sheep serum spiked with 2.5 ng each PBDE congener. Isolated and recovered using RP-SPDE–NP-SPE techniques.

BDE#	t[R] min
28	6.68
Unknown	8.80
47	9.08
66 (IS)	9.53
77	10.35
100	11.50
99	12.40
85	14.31
154	14.74
Unknown	15.05
153	16.40
183	20.88

30 m × 0.25 mm DB-XLB; 1 µL pulsed splitless injection PTV (CIS-4, Gerstel), T(injector) = 300°C; T(ion source) = 166°C; T(quadrupole rods) = 150°C; oven temperature program: 110°C (hold for 1 min) then to 240°C at 120 deg/min; (hold at 240°C for 1 min) then to 300°C at 3 deg/min (hold for 0.0 min) then to 110°C at 120 deg/min (hold at 110°C for 0.5 min).

of 8.8 min each time sheep serum was extracted and was not present in the human serum TICs discussed earlier. C-GC–MSD-ECNI-SIM is highly selective for organobromine compounds, so it is reasonable to assume that this unknown peak is due to the presence of a brominated species. We did not pursue the identification of this unknown brominated peak in sheep serum at this time.

Percent recoveries from spiked human and sheep serum and other matrices

Tables III–VIII cite calculated percent recoveries for the nine selected PBDEs from either various spiked aqueous matrices and spiked human or sheep serum with and without subsequent eluent cleanup. Thurman and Mills have discussed the advantages of using a 1:1 mixture of CH_2Cl_2 and ethyl acetate (EtOAc) as an elution solvent for RP-SPE (15). We adopted this mixed elution solvent and observed an increase in BDE congener percent recoveries. The 1:1 CH_2Cl_2–EtOAc appears to slow the evaporation rate compared to using only CH_2Cl_2 during the N_2 purge step. We observed a significant increase in percent recoveries by replacing a borosilicate glass conical test tube with a borosilicate glass round-bottom test tube. We also observed increases in PBDE percent recoveries if we did not let the eluent from RP-SPDE evaporate to dryness. Adding a keeper solvent as a means to increase percent recoveries needs to be further explored. Although we did not conduct a time study, we noticed that percent recoveries dropped if we allowed the dried residue to stand in the test tube after N_2 purge for 30 min or more. Equation 1 shown below was used to calculate percent recoveries for the ith BDE congener, %Ri, for all results reported in Tables III–VIII (16):

$$\%R_i = \frac{A^{79}_{i,S} \Big/ A^{79}_{IS,S}}{\left(\frac{1}{N}\right)\sum_{j}^{N} A^{79}_{i,j,C} \Big/ A^{79}_{IS,j,C}} \times 100 \qquad \text{Eq. 1}$$

where $A^{79}_{i,S}$ represents the *m/z* 79 abundance for the ith BDE congener recovered from the spiked sample matrix S; $A^{79}_{IS,S}$ represents the *m/z* 79 abundance for the added IS, BDE-66, in the spiked sample matrix S; *N* represents the number of replicate injections of the control reference standard; $A^{79}_{i,j,C}$ represents the *m/z* 79 abundance for the ith BDE congener for the jth replicate injection, in a control 100% recovered chemical reference standard dissolved in solvent; $A^{79}_{IS,j,C}$ represents the *m/z* 79 abundance for the IS, BDE-66, for the jth replicate injection, in a control 100% recovered chemical reference standard, dissolved in solvent.

Percent recoveries calculated in this manner are more accurate and oftentimes avoid the > 100% results frequently reported in the literature.

Table III shows a comparison of percent recoveries for three chemically different RP-SPDE disks for an identical sample matrix without cleanup. PBDE congeners were recovered from a highly acidic and high ionic strength aqueous matrix. With a few exceptions as noted, percent recoveries were relatively independent of the chemical nature of the sorbent disk. Percent recoveries from the C2 SPEC that were comparable to the more hydrophobic disks showed a surprising outcome. Table IV shows a comparison of the same three chemically different RP-SPDE disks while replacing the acidic and highly salted aqueous matrix with sheep serum and also without cleanup. Percent recoveries were significantly higher when using a more hydrophobic surface for all nine PBDE congeners.

The Focus Polar Enhanced SPE disk is a

Table II. Mean % Recoveries from Spiked Human Serum via LLE–Florisil Cleanup/Silica Gel Cleanup and Fractionation*

BDE congener	Low level	High level
28	74.6	73.2
47	129.3	95.4
77	80.5	93.6
100	89.5	90.0
99	101.1	93.7
85	81.1	96.9
154	83.8	88.3
153	78.0	94.2
183	76.5	89.9

* *N* = 4 where N is the number of replicate LLEs performed; low level spike—15 µL of 200 ppb each BDE congener added to 4 mL human serum and recovered in a final extract volume of 1 mL; high level spike—150 L of 200 ppb each BDE congener added to 4 mL human serum and recovered in a final extract volume of 1 mL.

Table III. Influence of the Chemical Nature of RP-SPDE on BDE Congener % Recoveries*

Sorbent	28	47	77	100	99	85	154	153	183
C2SPEC	55.3	119	60.4	38.5	63.0	70.0	67.6	76.9	74.1
Focus Disk	58.0	71.2	54.3	53.5	61.7	52.5	50.0	54.8	54.7
C18SPEC	54.8	57.4	56.6	58.7	60.1	55.3	57.2	61.0	57.8

* Without cleanup; matrix—distilled, deionized water, saturated with phosphoric acid and sodium sulfate; equation 1 used to calculate % recoveries.

Table IV. Influence of Chemical Nature of RP-SPDE on PBDE % Recoveries from a Spiked Sheep Matrix*

Sorbent	28	47	77	100	99	85	154	153	183
C2SPEC	50.0	56.7	57.7	49.4	52.0	51.5	43.7	40.8	30.4
Focus	83.3	80.2	97.3	71.9	77.7	73.7	59.1	59.3	42.1
C18	83.6	80.0	88.7	70.7	76.3	79.1	59.0	60.7	54.1

*Without cleanup; matrix—sheep serum; equation 1 used to calculate % recoveries.

polymeric sorbent purported to exhibit outstanding recovery for polar as well as nonpolar analytes from serum or plasma (Varian Inc. Consumables and Supplies Catalog, 2005-2006, pp. 15–16). We used this disk and compared percent recoveries without and with acidic silica gel cleanup. Triplicate RP-SPDE experiments were conducted with the same aqueous matrix, distilled deionized water, without cleanup shown in Table V, and with cleanup shown in Table VI. Comparing percent recoveries from Tables V and VI reveal overall recovery losses when the acidic silica gel cleanup step is introduced, and this outcome is to be expected. Recovery losses were more significant, however, for the lower molecular weight congeners when compared with the higher molecular weight congeners. These recovery losses may reflect volatility differences between PBDEs that have fewer bromines per molecule versus those congeners that have more bromines per molecule. The influence of adding a cleanup step needs to be studied further. Finally, we compared percent recoveries for triplicate SPDEs using Focus between sheep and human serum without cleanup. These results, shown in Tables VII and VIII, reveal, with a few exceptions, comparable percent recoveries for the lower molecular weight congeners and differences in percent recoveries for the higher molecular congeners. All 27 percent recoveries cited in Table VII gave a calculated mean of 61.6% with an RSD in the mean of 18.5%. All 27 percent recoveries cited in Table VIII gave a calculated mean of 49.2% with an RSD in the mean of 40.7%. Percent recoveries over all PBDE congeners were more reproducible from spiked sheep serum versus those from spiked human serum. There might be homogeneity differences between sheep and human serum that could account for this difference.

MDLs for the isolation and recovery of BDE congeners via RP-SPDE

Accurately measured percent recoveries coupled to the ratio of final eluent volume to serum volume (i.e., the phase ratio) lead to MDLs for each BDE congener. Equation 2 shown below was used to calculate an MDL, for the ith BDE congener, xMDLi (16):

$$x_i^{MDL} = x_i^{IDL}\left(\frac{V_e}{V_S}\right)\left(\frac{100}{\%R_1}\right) \qquad \text{Eq. 2}$$

where x^{IDL}_i is the IDL for the ith BDE con-

Table V. % Recoveries for Triplicate RP-SPDEs Using a Focus Disk*

Sorbent	28	47	77	100	99	85	154	153	183
Focus Disk	51.9	60.8	63.8	57.4	58.7	57.2	53.6	50.9	42.6
Focus Disk	65.4	62.6	68.3	54.2	55.5	62.1	51.7	54.4	55.3
Focus Disk	82.2	83.8	85.1	69.7	75.6	76.1	69.4	71.8	56.7

* Without cleanup; matrix—distilled, deionized water, equation 1 used to calculate % recoveries.

Table VI. % Recoveries for Triplicate RP-SPDEs Using a Focus Disk*

Sorbent	28	47	77	100	99	85	154	153	183
Focus Disk	44.2	49.7	46.6	58.7	58.1	46.6	59.3	56.5	55.2
Focus Disk	41.5	46.0	47.5	58.0	57.5	41.3	61.3	59.3	61.8
Focus Disk	26.2	36.9	35.3	48.7	46.2	25.7	51.8	46.7	43.7

* With acidic silica gel cleanup; matrix—distilled, deionized water; equation 1 used to calculate % recoveries.

Table VII. % Recoveries for Triplicate RP-SPDEs Using a Focus Disk*

Sorbent	28	47	77	100	99	85	154	153	183
Focus Disk	63.0	56.5	64.5	47.7	52.2	53.3	44.4	43.8	41.4
Focus Disk	74.6	69.6	83.6	61.5	66.9	66.1	55.2	60.0	53.2
Focus Disk	77.0	73.1	83.9	61.8	66.7	72.4	58.5	57.7	54.1

*Without cleanup; matrix—sheep serum; equation 1 used to calculate % recoveries.

Table VIII. % Recoveries for Triplicate RP-SPDEs Using a Focus Disk*

Sorbent	28	47	77	100	99	85	154	153	183
Focus Disk	76.4	62.2	72.0	39.5	46.9	51.8	30.4	34.0	22.7
Focus Disk	73.1	60.9	69.1	35.9	41.9	45.2	27.1	26.7	18.1
Focus Disk	88.3	72.7	81.9	47.7	52.4	57.7	34.2	36.0	23.5

* Without cleanup, matrix—human serum; equation 1 used to calculate % recoveries.

Table IX. Calculated MDLs from RP-SPDE and CGC–MSD-ECNI-SIM*

BDE# →		28	47	77	100	99	85	154	153	183
A	IDL (ppb)	0.92	1.6	1.3	1.5	1.3	1.5	1.7	2.6	3.1
	%R	58	71.2	54.3	53.5	61.7	52.5	50.0	54.8	54.7
	MDL (ppt)	79	110	120	140	110	140	170	240	280
B	IDL (ppb)	0.92	1.6	1.3	1.5	1.3	1.5	1.7	2.6	3.1
	%R	83.3	80.2	97.3	71.9	77.7	73.7	59.1	59.3	42.1
	MDL (ppt)	55	100	67	100	84	100	140	220	370
C	IDL (ppb)	0.92	1.6	1.3	1.5	1.3	1.5	1.7	2.6	3.1
	%R	26.9	33.6	30.7	30.8	34.8	37.7	33.1	36	35.1
	MDL (ppt)	170	240	210	240	190	200	260	360	440

* A–Focus disk without cleanup for a saturated salt aqueous matrix $V_e / V_S = 0.05$; B–Focus disk without cleanup for a sheep serum matrix $V_e / V_S = 0.05$; C–Focus disk with cleanup for a sheep serum matrix $V_e / V_S = 0.05$; MDLs were calculated using equation 2. IDLs were calculated by applying calibration statistics to the ordinary least squares regression over six calibration data points including 0.25, 0.50, 2.5, 5.0, and 10.0 ppb each BDE congener.

gener; V_e is the final volume of RP-SPE eluent in the 2 mL GC vial glass insert (200 µL); VS is the accurately measured serum volume (assuming we could take up to 4 mL); % R_i is the accurately calculated percent recovery for the ith BDE congener for the specific sample matrix obtained from a study conducted independently.

Table IX considers three scenarios labeled A, B, and C, where MDLs are estimated from the IDLs using equation 2. It is assumed that the IDLs are established from a previous application of calibration statistics following an ordinary least squares regression. Starting with 4 mL serum and ending with 200 µL of eluent yields a phase ratio of 0.05. The last scenario is noteworthy in that relatively low percent recoveries still yield MDLs that are all below 500 ppt.

Conclusion

We report percent recoveries for LLE that compare favorably with previously published results. We estimated percent recoveries relative to a control reference standard for a novel sample preparation approach incorporating RP-SPDE. We have established that the isolation and recovery of PBDEs can yield MDLs that are < 500 ppt from spiked serum despite percent recoveries below 75%. RP-SPDE is ideally suited for 96 well-plate automation technology.

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