Evaluation of Automated Stir Bar Sorptive Extraction-Thermal Desorption-Gas Chromatography Electron Capture Negative Ion Mass Spectrometry for the Analysis of PBDEs and PBBs in Sheep and Human Serum

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Abstract

Stir-bar sorptive extraction and automated thermal desorption/cryotrapping interfaced to capillary gas chromatography and electron capture negative ion mass spectrometry is shown to effectively isolate and recover polybrominated diphenyl ethers and polybrominated biphenyls from sheep and human serum. This paper describes the development of the method and demonstrates the feasibility of using Twister with spiked serum. Conditions for conducting stir-bar sorptive extraction and for automated thermal desorption that led to acceptable analyte recoveries were optimized. The approach to sample preparation introduced here significantly reduces tedious labor and solvent consumption associated with conventional liquid–liquid extraction.

Introduction

Polybrominated diphenyl ethers (PBDEs) as persistent organic pollutants are to the 21st century as DDE and related organochlorine pesticides were to the 1960s, polybrominated biphenyls (PBBs, particularly in Michigan) to the 1970s, polychlorinated biphenyls (PCBs) to the 1980s and 1990s. Unlike DDE, PBBs, and PCBs, PBDEs are currently in use. PBDEs are commercially produced as three mixtures (i.e., penta-BDE, octa-BDE, and deca-BDE, indicating the degree of bromination) (1). Protein changes have been reported in the striatum and in the hippocampus in mice given doses of egg lecithin and peanut oil containing 1.2 mg/mL BDE-99 (2). These changes have implicated the developing cholinergic and catecholaminergic systems as targets of PBDEs as a possible cause of injury. The CDC report an increase in concentration of PBDEs in human serum collected in the United States from 1985–2002 while PBB and PCB levels were decreasing during the same time period (3). The CDC report, of 2062 human serum samples from participants from the National Health and Nutrition Examination Survey (NHANES) in 2003-2004, the congener with the highest serum concentration was BDE-47 followed by BDE-153, and then BDE-99 (4). Because PBDEs are used as flame retardants, pet cats may prove to be a sentinel for humans due to their constant contact with indoor carpet, household dust, and even pet food (5).

Most analytical laboratories that use liquid-liquid extraction (LLE) coupled with various adsorptive chromatographic cleanup techniques applied to human serum to quantitate persistent organochlorine (OC) pesticides and PCBs have merely added the most environmentally significant BDE congeners to their existing menus. These techniques are tedious, labor intensive, and consume relatively large amounts of organic solvents. It has been recently demonstrated in our laboratory that nine BDE congeners and two BB congeners, 155 and 153, can be isolated and recovered with acceptable percent recoveries from sheep and human serum using reversed-phase solid-phase disk extraction (RPSPDE) coupled to capillary gas chromatography (GC) with electron capture negative ion mass spectrometry (MS) (6). The origin of the idea for a unique serum pretreatment reagent is discussed in this paper. The adaptation of a GC–MS method to separate and quantitate these nine BDE and two BB congeners with high sensitivity and selectivity has been discussed elsewhere (7).

This paper introduces the first systematic study (to our knowledge) of the isolation and recovery of BDEs 28, 47, 77, 100, 99, 85, 154, 153, 183 and BBs 155 and 153 from water, sheep, and human serum utilizing a recently developed alternative to LLE, RP-SPE and RP-SPDE: stir bar sorptive extraction (SBSE). The same GC–MS determinative technique as reported earlier is also used here (6,7). Prieto and colleagues have recently reported their findings using SBSE while extending the technique to other priority pollutants in addition to PBDEs (8). SBSE is a solventless extraction technique developed by Sandra and colleagues (9). SBSE has been shown to extract a wider range of analytes whose octanol–water partition coefficients, K_{OW}, can be as low as 100 in contrast to solid-phase microextraction due to a 50 to 250 × greater phase ratio (10). SBSE coupled to thermal

desorption (TD) and capillary GC–MS is being applied to urine, serum, plasma, and other body fluids. Organic fatty acids can be derivatized with ethyl chloroformate while urinary phenols can be acylated using acetic anhydride in an aqueous matrix so as to significantly increase analyte K_{OW}s prior to SBSE (11). SBSE can also be accomplished without automation. Analytes sorbed onto a polydimethylsiloxane coated stir bar, Twister [Gerstel GmbH & co. KG, Mülheim an der Ruhr, Germany], can be solvent eluted (desorbed) from Twister. The eluent is diluted to a precise final volume for analytical purposes (12). The eluent is injected into a GC in a manner similar to RP-SPDE. Advances in robotics, computer software, and GC inlet technology has enabled a Twister after undergoing SBSE to be thermally desorbed, cyrotrapped on the inlet liner, then rapidly thermally desorbed into a capillary GC all without analyst intervention.

BDE/BB congener data was generated utilizing automated Twister thermal desorption cryotrapping-capillary gas chromatography with mass selective detection operated in the electron capture negative ion mode using selective ion monitoring. This acronym, TD-Cryo-C-GC-MSD-ECNI-SIM, describes the instrumentation used throughout this paper.

Experimental

Materials and reagents

Silanized 2-mL GC vials [National Scientific, Suwanee, GA] are recommended for long-term storage of PBDE chemical reference standards. Concentrated formic acid (HCOOH) was obtained from EMD Chemicals (Darmstadt, Germany). Compressed cylinders containing helium and methane [Airgas, Radnor, PA] supplied carrier gas and CI reagent gas to the instrument. Liquified nitrogen, low pressure, 22 psi, is connected to the instrument from the liquid valve of the Dewar (Airgas). All organic solvents of pesticide residue grade quality obtained from various manufacturers were obtained through VWR. 5-mL headspace (HS) vials (Microliter Analytical Supplies, Suwanee, GA) were used to contain all samples for conducting SBSE. Twister, 10 mm long × 0.5-mm film thickness; desorption liners; transportation adapters for desorption liners; desorption tubes for use with the TC-2 Tube Conditioner were from Gerstel (Gerstel, GmbH & Co. KG, Mülheim an der Ruhr, Germany).

Sheep and human serum were obtained in-house (Michigan Department of Community Health, Bureau of Laboratories). Bovine calf serum was obtained from the CDC's LRN-C program that my laboratory participates in. Human serum was also obtained from the Arctic Monitoring and Assessment Programme (AMAP). All three serum sources were found to be free of background PBDE/PBB contamination because frequent blank samples were run.

To define a 100% recovered spiked sample, we placed the same aliquot of spiking solution into some glass wool that was previously inserted as a plug into a clean, dry, and empty TDU tube. The contents of the TDU tube were thermally desorbed under identical TD-Cryo-C-GC-MSD-ECNI-SIM conditions used for Twister.

Chemical reference standards

PBDE stock solutions for individual congeners 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 diluted in 2-propanol to a concentration of 1 ppm each BDE congener, then stored in amber 2-mL screw cap sealed silanized glass vials. A spiking mixture of congeners at 40 ppb each was prepared precisely by placing 200 µL of 1.0 ppm BDE congener into a 5-mL volumetric flask and adjusting to a final volume with 2-propanol to yield a 40 ppb each mixture of congeners to be used to spike various aqueous or serum matrices. A 1 ppm PBB-155 was available in-house. PBB-155 (2,2',4,4',6,6'-Hexabromobiphenyl) is the principal surrogate used in our laboratory. It is added to each and every sample prior to conducting LLE/Cleanup. A 1 ppm PBB-153 was prepared by appropriate dilution of a 35 ppm inhouse source. PBB-153 (2,2',4,4',5,5'-Hexabromobiphenyl) was the principal BB congener that was inadvertently substituted for animal feed, which led to major destruction of cattle and subsequently entered the human food chain in 1973 in Michigan (13).

Instrumentation and operating conditions

The system consisted of a MPS2 installed atop a 6890 gas chromatograph 5973N Mass 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 Gerstel MPS2/TDU bundle consisting of the following was installed: MPS2 autosampler; CIS 4 (a programmed temperature vaporizer inlet with a septumless sampling head) and C505 Controller; TDU (Thermal Desorption Unit); TDU Automation Kit for MPS2; Universal Peltier Cooling Unit for the TDU; Maestro software for MPS2/TDU/CIS-4 control.

A Gerstel TC-2 Tube Conditioner and a Gerstel 15-position Twister Stir Plate are required to reuse Twisters and to properly implement SBSE using Twisters. A Gerstel Cryo tube and connection accessories are required for the Cooled Injection System (CIS). The front-programmed temperature vaporizer (PTV) (Agilent) inlet was replaced with a TDU/CIS 4 inlet. The TDU sits atop the CIS 4 inlet. A CI source (G1999-60402, Agilent) was installed with methane as CI reagent gas. Protocols for the methane pre-tune, positive ion tune using perfluoro-5,9-dimethyl-3,6,9trioxidodecane (PFDTD) followed by a negative ion tune using PFDTD were conducted as needed. A Graphpack-3D ferrule, 7.0 cm and 7.8 cm baffled and notched quartz packed deactivated glass liners and a Graphpack-2M ferrule for a 0.25 mm (i.d.) and 0.40 mm (o.d.) (Gerstel, GmbH & Co. KG) were required to properly operate the CIS 4 and to connect a 30 m × 0.25 mm i.d. × µm (film thickness) DB-XLB (Agilent Technologies, Inc.) column. The development of an optimized temperature program to baseline resolve all BDE and BB congeners is reported elsewhere (7). The column temperature program used throughout the study was as follows: 110°C and hold for 1.0 min then to 240°C at 75 deg/min. Hold for 1 min at 240°C and then to 325°C at 4°C/min. The flow rate through the column was 1.0 mL/min using helium as carrier gas. The CI source was held at 166°C, the quad temperature was 150°C with a transfer line temperature of 280°C.

Methane was used as CI reagent gas at 40% of full control valve. *m/z* 79 (quant) and 81 (qualifier) were SIM ions monitored throughout the study.

The optimized temperature program for the TDU is as follows: 50°C for 0.2 min, then to 325°C at 420°C/min, then hold for 3.0 min. The TDU final hold time was critical to achieve 100% thermal desorption of PBDE/PBBs from Twister as will be discussed later. The relatively high temperature settings also reflect the chemical nature of PBDEs/PBBs. The optimized temperature program for the CIS-4 is as follows: –75°C and hold for 0.5 min, then to 325°C at 12.0°C/s, then hold for 3.0 min. An equilibration time of 0.5 min is set. The cryotrap temperature was initially set at –120°C. No difference in peak abundances was observed between these two cryotrap temperatures for the BDE/BB congeners. A higher cryotrap temperature aids in conserving liquefied nitrogen. The TDU transfer temperature is set at 345°C with a transfer temperature mode set at fixed. The TDU desorpton mode is set at splitless, and the TDU sample mode is set as "Sample Remove". In the ChemStation software (Agilent), it is critical to set up the front inlet as follows: mode-solvent vent, vent flow at 50 mL/min, and vent pressure at 11.0 psi. Purge flow at 20.0 mL/min with purge time of 0.75 min. For WCOT and all GC–MS related parameters, refer to the literature (8,9) for experimental details.

Sample Preparation

Various spiked and unspiked samples were prepared using distilled, deionized water (DDI), sheep or human serum in 5-mL headspace vials. Various chemical reagents were added. Twisters were placed into the 5-mL headspace vials and stirred for precise periods of time at fixed stir rates. Twisters were then removed from the vials, cleaned, dried, and then placed in their respective TDU glass tubes. Following the completion of a batch of samples, the TDU tubes were thermally desorbed automatically via TD-Cryo-C-GCMSD-ECNI-SIM.

The procedure with safety comments used to generate the data whose results are shown in Table IX is given as follows: Place from 0.1 mL to 1.0 mL of serum into a clean 5-mL six headspace vial. For serum aliquots of ~ 0.5 mL, add ~ 0.5 mL of DDI. This provides a minimum volume of liquid for adequate spiking. Spike with increasing aliquots of a 1 ppb PBDE / PBB mix. Be sure to spike directly into the liquid. Spike with 10 µL of 9 ppb BDE-66 to each and every calibration standard, QC standard, and unknown sample. Add 0.5 mL concentrated HCOOH (wear gloves and eye protection as contact with HCOOH will burn skin and eyes). Always transfer HCOOH from bottle to container in a fume hood. Add 1.0 mL DDI to yield a total sample volume of ~ 2 mL. Place a new or carefully cleaned and conditioned Twister into the mixture. Stir for 2 h at ~ 500 rpm. Continue as stated earlier. Thermally desorb the contents of the glass TDU tube via TD-Cryo-C-GC-MSD-ECNI-SIM. When finished, place HS vial and contents in bleach solution and treat as a bio-hazard.

The procedure used to generate the data whose results are shown in Table X is given as follows: 100 µL of a 1 ppb spiking reference standard dissolved in 2-propanol that contains eleven BDE/BB congeners is added to a HS vial containing 0.1 mL Hserum and 0.4 mL DDI. Then, 10 µL of a 9 ppb BDE-66 dissolved in 2-propanol is added to the serum/DDI mixture. Next, add 0.5 mL of concentrated HCOOH followed by 1.0 mL DDI. The pretreated sample is vortexed. A Twister is added and the sample stirred for 2 h at 500 rpm. Continue as stated above. The contents of the TDU tube are thermally desorbed via TD-Cryo-C-GC-MSD-ECNI-SIM.

The procedure used to generate the data whose results are shown in Table XI is given as follows: to 0.5 mL bovine calf serum (BCS) is added 100 µL of a 1 ppb spiking reference standard dissolved in 2-propanol and 10 µL of a 9 ppb BDE-66 dissolved in 2-propanol. Then add 0.5 mL HCOOH and 1.0 mL DDI, stir for 2 h at 500 rpm. Continue as described earlier. The contents of the TDU tube is thermally desorbed via TD-Cryo-C-GC37 MSD-ECNI-SIM.

Results and Discussion

TDU and CIS-4 temperature program optimization

The optimum TDU and CIS-4 temperature profiles that efficiently thermally desorbed all BDE/BB congeners was first studied. PBDE congeners span a considerable range of molecular weight (MW). For the nine BDE congeners studied in this paper, their MWs range from a low of 405.9 Da for BDE-28 (a tribromoDE) to a high of 719.6 Da for BDE-183 (a heptabromoDE). All spiking solutions used consist of BDE and BB congeners dissolved in 2-propanol so as to facilitate miscibility of extremely hydrophobic PBDEs. PBDEs possess log K_{OW} whose values range from a low of BDE-28 of 5.88 to a high of BDE-183 of 10.28 (14). It was found necessary to keep the final TDU hold time between 3 and 5 min. A final TDU hold time of only 0.5 min resulted in incomplete thermal desorption. Higher MW congeners remain retained vs lower MW congeners at a TDU final hold time of 0.5 min. If the abundance for the second thermally desorbed Twister at the 0.5 min TDU final hold time, is divided by the abundance of its corresponding BDE congener from SBSE (i.e., from the first thermal desorption) a percent car-

Table I. Percent of First TD Found in 2nd TD SBSE When Twister is Thermally Desorbed a Second Time

BDE/BB Congener #	% of 1st TD found in 2nd TD*
28	9.0
47	16.7
66	16.3
77	15.6
PBB-155	NA
100	22.8
99	23.1
85	23.4
154	29.4
PBB-153	NA
153	36.7
183	31.8

NA not applicable; analyte was not present in spiking solution

* Calculation based on:

$$\% \text{ found} = \frac{\text{Abundance (2nd TD from Twister)}}{\text{Abundance (1st TD from Twister)} \times 100}$$

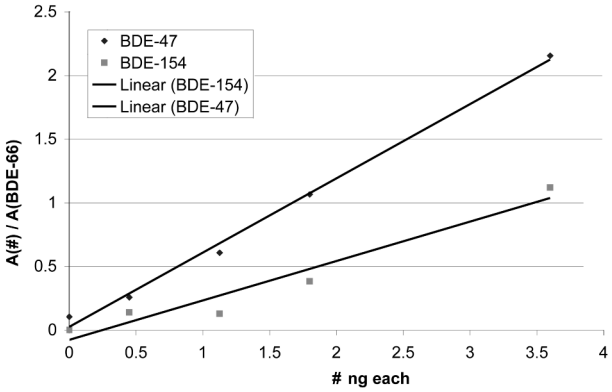

Figure 1. Calibration Plot BDE-47 vs BDE-154 using a final TDU hold time of 0.5 min; SBSE from 1.0 mL distilled, deionized water spikes with 0, 10, 20, 40, 80 µL of 45 ppb and 25 µL 90 ppb BDE-66.

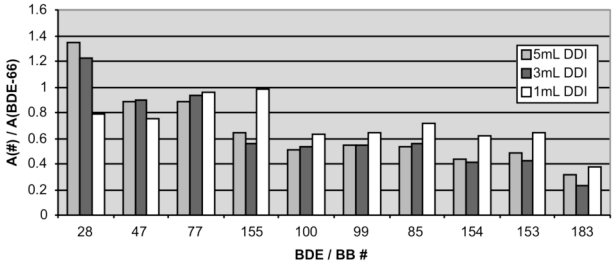

Figure 2. PBDE abundance ratio vs sample volume, 40 µL of 40 ppb each congener (including BDE-66) added to increasing volumes of DDI; samples stirred for 2 h at 180 rpm.

ryover (% carrover) can be estimated. These estimates are shown in Table I. Higher molecular weight (MW) BDE congeners require a longer hold time to be thermally desorbed efficiently into the CIS-4 inlet. We also studied the final temperature hold times for the CIS-4 and saw no difference in BDE congener abundances with increased hold times.

The first calibration attempt was conducted before the TDU and CIS-4 final hold times were optimized and is shown in Figure 1. Calibration plots showing the linear trendline for BDE-47 and for BDE-154, are compared. BDE-154 exhibits a much poorer fit to the experimental points than does BDE-47. This difference could be accounted for by the significantly higher %carryover for BDE-154 vs BDE-47 shown in Table I. CIS-4 final hold times following cryotrapping and subsequent ballistic heating of CIS-4 to drive analytes into the WCOT column, in contrast to TDU final hold times, seem to have minimal effect on BDE congener recovery loss.

Influence of sample volume on abundance ratios for SBSE

Following the successful elimination of incomplete thermal desorption of BDE congeners from DDI using SBSE, a series of increasing sample volumes of DDI were spiked with the same amount of BDE/BB congeners. Twisters were placed in each of the 5 mL HS vials. The vials were stirred for 2 h at 180 rpm. A plot of the abundance ratio of each BDE/BB congener is shown in Figure 2. Note that BB-153 was not in the mix of spiking analytes. Highest abundance ratios were found for the 1.0 mL sample volume. Note that A (BDE-66) was 2756 for the 5 mL aliquot, 2255 for the 3 mL aliquot and 11,308 for the 1 mL aliquot. An earlier abundance ratio vs sample volume experiment

Table II. SBSE From Spiked Sheep Serum: Calibration Parameters, ICVs, CCVs, IDLs

Date	Lsquares	BDE-28	BDE-47	BDE-77	PBB-155	BDE-100	BDE-99	BDE-85	BDE-154	PBB-153	BDE-153	BDE-183
1/8/08	m	1.337	1.045	1.39	0.931	0.701	0.741	0.819	0.534	0.640	0.463	0.197
	b	0.0606	0.0864	0.0707	0.136	0.0617	0.0712	0.0354	0.0623	0.0379	0.0593	0.0173
	r	0.9994	0.9987	0.9994	0.9933	0.998	0.9969	0.9979	0.9981	0.991	0.9952	0.9993
	s(m)	0.032	0.0376	0.0327	0.0764	0.0317	0.0416	0.0374	0.0236	0.0611	0.0324	0.00536
	s(b)	0.0295	0.0347	0.0302	0.0705	0.02922	0.0383	0.0345	0.0217	0.0563	0.030	0.00494
a = 0.05	t(1 – α,d_f = 4)	2.92	2.92	2.92	2.92	2.92	2.92	2.92	2.92	2.92	2.92	2.92
	y[c]	0.192	0.242	0.206	0.451	0.193	0.243	0.190	0.160	0.290	0.193	0.040
	x[c]	0.098	0.15	0.097	0.34	0.19	0.23	0.19	0.18	0.39	0.29	0.11
	x[D]	0.19	0.29	0.29	0.64	0.36	0.45	0.37	0.35	0.74	0.55	0.22
1/9/08	t[R]min	8.248	10.910	12.199	13.231	13.350	14.186	15.955	16.322	16.598	17.747	21.596
% Rel error	ICV-low	0.32	0.36	0.37	0.32	0.3	0.39	0.38	0.35	0.32	0.35	1.28
	ICV-high	1.25	1.23	1.12	0.97	1.09	1.14	1.2	1.01	1.02	1.08	1.03
	CCV	1.08	1.96	1.08	1.00	0.99	1.06	1.08	1.02	1.05	1.05	1.05

m is the slope and b is the y-intercept of the linear and non-weighted least squares regressed line.
r is the correlation coefficient, s(m) and s(b) are the standard deviations in m and in b respectively.
t(1-a,d_f = 4) is Student's t for a given level of significance (a).
The number of degrees of freedom d_f, where $d_f = N - 2$ and N = # calibration points.
y[c] corresponds to the upper confidence limit when x=0 in which a 1-a probability exists that the normal distribution of instrument responses falls to within the mean y at x = 0.
x[c], the decision limit,in ppb, is a specific concentration level for each PBDE above which one may decide whether the result of an analysis indicate detection.
"x[D], the instrument detection limit, IDL,in ppb, is a specific concentration level for each PBDE above which one may rely upon to lead to detection.
t[R] is the analyte chromatographic retention time.
ICV-low is an initial calibration verification reference standard, expect 0.3 ppb each congener.
ICV-high is an initial calibration verification reference standard, expect 1.2 ppb each congener.
CCV is a continuing calibration reference standard, expect 1.0 ppb each congener.

using 1, 2, and 4 mL sample volumes yielded similar results to that shown in Figure 2. It is evident that the amount of BDE/BB congeners partitioned into the Twister (whose dimensions are 1 cm in length with a coating thickness d_f = 0.5 mm exhibiting a 24 µL volume) and subsequently thermally desorbed, cyrotrapped, and ballistically heated to drive analytes into the XLB column, is found to be directly proportional to analyte concentration. The suppression of A(BDE-66) for V_S = 3 mL when compared to A(BDE-66) for V_S = 5 mL is surprising. The fact that the abundance ratios are comparable for most congeners with the exception of BDE-28 for the 5 and 3 mL cases can be expected. However, the significant enhancements shown for BB-155 and for pentaDEs, hexaDEs, and heptaDE (BDE-183) are an unanticipated outcome of this study.

Influence of sample pretreatment reagents on calibration and verification; preliminary SBSE recovery

Calibration/Verification

The significance of using concentrated formic acid (HCOOH) to pre-treat serum samples, with respect to RP-SPDE, has been discussed elsewhere (6). We saw in our PBDE recovery studies using SPDE techniques that addition of HCOOH to serum eliminated protein coagulation/precipitation while contributing to an overall increase in percent recoveries. We explored whether or not HCOOH would also be a suitable serum pretreatment reagent when we utilized SBSE. Using sheep serum (Sserum) as a representative sample matrix, we added HCOOH and again noted the absence of any coagulation/precipitation. We proceeded to conduct a calibration study with our internal standard BDE-66 added prior to SBSE. Table II shows results for: the ordinary least squared regressed slope (m); y-intercept (b); correlation coefficient (r) for all nine BDE congeners and two BB congeners.

Confidence band calibration statistics was applied to the calibration data. This leads to a decision level, decision limit, and method detection limit (MDL, since determinative measurement and sample preparation are mutually inclusive) as shown and defined in the notes beneath the table (15). Correlation coefficients, r > 0.990, were found for all congeners. IDLs ranged from a low of 0.19 ppb (BDE-28) to a high of 0.64 ppb (BB155). Initial calibration verification (ICV) and continuous calibration verification (CCV) standards were run after the calibration standards and results are also shown in Table II. For example, consider BDE-47, one of the most ubiquitous PBDEs found in humans. Percent relative errors range from a high of 20% (ICV-L) to a low of 2.5% (QC-H). These % relative errors are reasonable when one considers that SBSE couples both sample preparative and determinative measurements. Figure 3 is the chromatogram from QC-H and this chromatogram is typical of the high GC resolution, high sensitivity and high selectivity associated with SBSE coupled to TD-Cryo-C-GC-MSD-ECNI-SIM when applied to organobromine compounds such as PBDEs and PBBs that are isolated and recovered from Sserum.

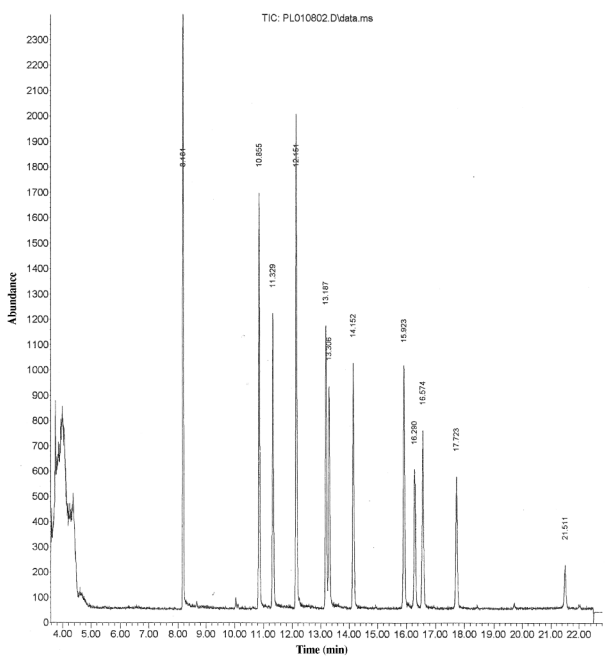

Figure 3. TD-Cryo-C-GC-MSD-ECNI-SIM chromatogram of QC-H, an ICV reference standard from spiked Sserum using SBSE. Peaks at indicated chromatographic retention times (min) are identified as follows:
BDE-28 at 8.164, BDE-47 at 10.855, BDE-66 (internal standard) at 11.329, BDE-66 at 12.151, BB-155 at 13.187, BDE-100 at 13.308, BDE-99 at 14.152, BDE-85 at 15.923, BDE-154 at 16.290, BB-153 at 16.574, BDE-153 at 17.723, BDE-183 at 21.511.

Preliminary recovery study

Since we demonstrated feasibility in the use of HCOOH for a Sserum matrix spiked with BDE/BB congeners, we explored the influence of HCOOH on analyte recovery. Figure 4 compares duplicate SBSEs of BDE/BB congeners for each pretreatment reagent used recovered from spiked Sserum. Duplicate results are shown to demonstrate method precision. Two replicate aliquots of Sserum were prepared with HCOOH and two identical Sserums were prepared with DDI. The use of acetonitrile and triethylamine as additional sample pretreatment reagents when using HCOOH originated with Pauwles and co-workers (16). Based on further experiments conducted, the addition of these reagents to a Sserum matrix did not affect recovery of analytes. As Figure 4 demonstrates, the lower MW congener BDE-28

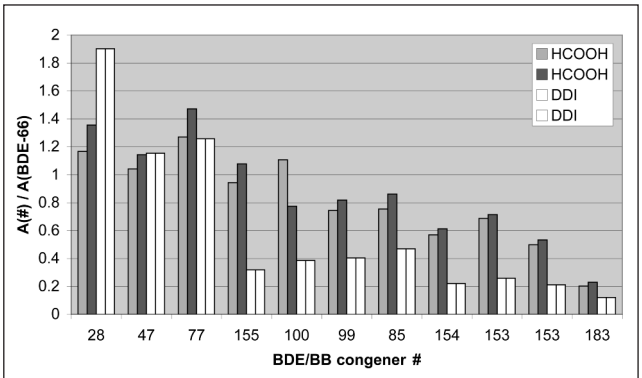

Figure 4. Comparison of two pairs of SBSEs of BDE/BB congeners recovered from spiked Sserum HCOOH vs DDI. A(#) / A(BDE-66) vs pre-treatment reagent: 1.0 mL sheep serum, 40 µL 40 ppb PBDEs/40 µL 40 ppb BDE-66, 1.0 mL HCOOH or DDI; 50 µL acetonitrile, 10 µL triethylamine; stir for 2 h, 155 refers to BB-155, first 153 refers to BB-153; all others are BDEs.

partitions into Twister to a greater extent in DDI in contrast to HCOOH. However, from BDE-77 (a tetrabromoDE) all the way to BDE-183 including both BB congeners, the presence of HCOOH significantly increases partitioning from a Sserum matrix. From this particular set of experiments, the ratio of BDE-66 abundance (from SBSE) to BDE-66 abundance from placing an identical aliquot of a 40 ppb spiking solution containing PBDEs in 2-propanol onto glass wool placed in a TDU tube (100% recovered control) yielded a percent recovery of ~16%. This low percent recovery to a first approximation is surprising. We next varied the % HCOOH in the spiked sheep serum matrix and found a further dependence of HCOOH on analyte recovery. As Figure 5 shows, with the exception of BDEs 28, 47, and 77 (all lower MW congeners), all higher MW congeners (from BB-155 through to BDE-183) show a significant increase in abundance ratio for serum treated with HCOOH vs serum treated with DDI. These results also represent a significant outcome of this study.

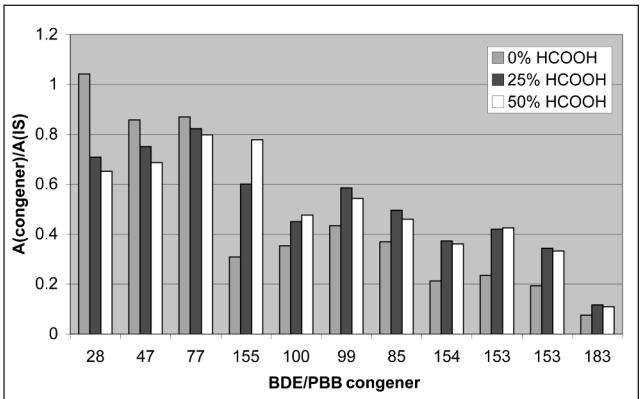

Figure 5. Abundance Ratio vs %HCOOH for SBSE of BDE/BB congeners recovered from spiked Sserum. Ratio of analyte abundance to IS abundance vs % Formic Acid in Spiked Sheep Serum [stir 2 h at 1000 rpm] 1.0 mL sheep serum + 1.0 mL (DDI and/or HCOOH) + 50 µL acetonitrile + 10 µL triethylamine; 155 refers to BB-155, first 153 refers to BB-153.

Table III. Statistical Relationships and Definitions Used in Text

$\bar{X} = \frac{\sum_i X_i}{N}$	Mean of N replicate measurements xi
$S = \sqrt{\frac{\sum_i (X_i - \bar{X})^2}{N-1}}$	Standard deviation in the mean of N replicate measurements xi
$RSD(\%) = CoeffVar = \left(\frac{S}{\bar{X}}\right) \times 100$	Relative standard deviation or coefficient of variation in the mean
$\%RR_j = \frac{\bar{A}_j^{SBSE}/\bar{A}_j^{SBSE,BDE-66}}{\bar{A}_j^{Control}/\bar{A}_j^{Control,BDE-66}} \times 100$	Percent relative recovery from SBSE for the ratio of the mean abundance of the jth BDE/BB congener to the mean abundance of the internal standard divided by their respective ratios for the control
$\%R_j = \frac{\bar{A}_j^{SBSE}}{\bar{A}_j^{Control}} \times 100$	Percent recovery from SBSE for the jth BDE/BB congener
$\%RR^j(s_R^j) = \frac{x^j(s^j)}{x_c^j(s_c^j)} \times 100$	Gereralized percent relative recovery from SBSE for the jth BDE/BB congener showing respective standard deviations.
$\frac{s^j{}_R}{RR^j} = \sqrt{\left(\frac{s^j}{x^j}\right)^2 + \left(\frac{s^j{}_c}{x^j{}_c}\right)^2}$	Propagation of variances between sample and control for the jth BDE/BB congener
$RSD_j = \left(\frac{s^j{}_R}{RR^j}\right) \times 100$	Relative standard deviation in the relative recovery expressed as a percent

Relative percent recoveries from Sserum/DDI and Sserum/HCOOH compared to their corresponding absolute percent recoveries.

Definition of and rationale for using relative percent recoveries

In consideration of the success with HCOOH in isolating and recovering PBDEs and PBBs from Sserum without coagulation/precipitation, we next carried out a percent recovery study. As mentioned earlier, we found that absolute abundance of BDE-66 was ~16% when compared to the absolute abundance of BDE-66 that had by-passed SBSE altogether. We spiked triplicate glass wool plugged TDU tubes and thermally desorbed each so as to generate a set of triplicate data. We proceeded to spike Sserum, add DDI, and conduct SBSE in triplicate. We also proceeded to spike serum, add HCOOH, and conduct SBSE in triplicate. We calculated a mean abundance ratio for each BDE/BB congener, a standard deviation in the mean and a relative standard deviation using equations found in Table III. Table IV lists all abundance ratios, means, and RSDs for 9 BDE congeners and 2 BB congeners isolated and recovered from spiked Sserum. Triplicate thermal desorptions (TDs) from spiking a clean and empty TDU tube with analytes into glass wool show a mean abundance ratios that range from a low of 0.302 (BDE-183) to a high of 1.04 (BDE-77). Both BB congeners show abundance ratios of ~ 0.9. Triplicate SBSEs using DDI as pretreatment reagent show mean abundance ratios that range from a low of 0.124 (BDE-183) to a high of 0.925 (BDE-28). Triplicate SBSEs using HCOOH as pretreatment reagent show mean abundance ratios that range from a low of 0.117 (BDE-183) to a high of 0.824 (BDE-77). Table IV is the first quantitative results reported in this paper that demonstrates measured precision in the isolation and recovery of PBDEs/PBBs from Sserum using SBSE.

Percent relative recovery vs percent recovery

Results in this paper are reported in terms of a % relative recovery (%RR). $\%RR_j$ for the jth congener is calculated using the equation shown in Table III. %RRs show to what extent a specific congener prefers to partition from the spiked sample to Twister or off of the spiked TDU tube

Table IV. Summary of Abundance Ratios for the Isolation and Recovery of PBDEs and PBBs via SBSE*

Date	A(#)/A(IS)	BDE-28	BDE-47	BDE-77	PBB-155	BDE-100	BDE-99	BDE-85	BDE-154	PBB-153	BDE-153	BDE-183
1/14/08												
TD	control	0.886	0.939	1.24	1.11	0.726	0.894	0.897	0.852	1.02	0.887	0.298
	control	0.67	0.757	1.02	0.9	0.616	0.757	0.771	0.742	0.89	0.776	0.294
	control	0.619	0.662	0.86	0.757	0.51	0.593	0.63	0.563	0.69	0.591	0.313
	mean	0.725	0.786	1.04	0.922	0.617	0.748	0.766	0.719	0.867	0.751	0.302
	RSD(%)	19.6	17.9	18.3	19.3	17.5	20.1	17.4	20.3	19.2	20	3.3
SBSE	DDI	0.778	0.749	0.831	0.437	0.43	0.596	0.56	0.449	0.52	0.48	0.232
	DDI	1.06	0.985	0.783	0.265	0.595	0.531	0.303	0.177	0.193	0.164	0.064
	DDI	0.936	0.749	0.737	0.249	0.297	0.391	0.301	0.176	0.2	0.167	0.075
	mean	0.925	0.828	0.784	0.317	0.441	0.506	0.388	0.267	0.304	0.27	0.124
	RSD(%)	15.3	16.5	6	32.9	33.9	20.7	38.4	58.9	61.4	67.2	76
SBSE	HCOOH	0.647	0.704	0.823	0.706	0.392	0.487	0.401	0.328	0.4	0.285	0.124
	HCOOH	0.656	0.651	0.777	0.619	0.439	0.48	0.409	0.305	0.352	0.25	0.088
	HCOOH	0.683	0.764	0.872	0.829	0.501	0.621	0.504	0.414	0.489	0.364	0.14
	mean	0.662	0.706	0.824	0.718	0.444	0.529	0.438	0.349	0.413	0.3	0.117
	RSD(%)	2.8	8	5.8	14.7	12.3	15	13.1	16.5	16.8	19.5	22.7

* SIM at m/z 79; qualifier ion at *m/z* 81

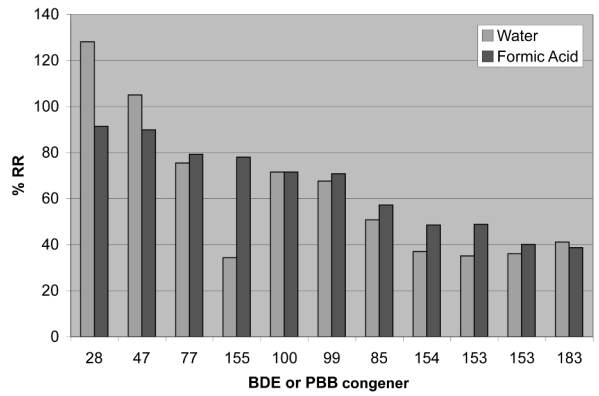

Figure 6. %RR vs sample pretreatment reagent for SBSE of BDE/BB congeners recovered from spiked Sserum. Stir Bar Sorptive Extraction: Matrix Effect on PBDE/PBB % Relative Recoveries: 1.0 mL sheep serum + 1.0 mL water vs 1.0 mL conc HCOOH; acetonitrile and triethylamine added [2 h stir at 500 rpm];155 refers to BB-155, first 153 refers to BB-153.

Table V. Comparison of %Relative Recoveries and %Recoveries for BDEs 47, 99, and 154 from Spiked Serum using HCOOH*

BDE#	%R	%RR
47	44.5	89.8
99	35.3	70.7
154	24.1	48.5

* Refer to Table III for equations.

relative to BDE-66. %RRs are used here for comparative purposes in the development of a new approach to sample preparation using SBSE. A more scientifically correct recovery result is to find the absolute congener abundances between a SBSE and a control. The imprecision due to the inherent instability of mass spectrometric detection requires that BDE congener absolute abundances be ratioed against the absolute abundance of a selected internal standard. Otherwise unacceptable %Rs would result. The $\%R_j$ for the jth congener is calculated using the equation shown in Table III. %RRs for all congeners studied comparing the significant effect of sample matrix is given in Figure 6. A significant outcome from the comparison of DDI to HCOOH % RRs is the increase in % RRs for all BDE congeners starting with BDE-77 and moving to higher MWs whereas the two lowest MW BDE congeners favor DDI.

This result is consistent with that shown in Figure 5 in which abundance ratios were used. Significantly lower RSDs were found for all congeners when HCOOH is added to spiked Sserum in contrast to when DDI is added as shown in Table IV. This is a significant outcome in this study. Even though BDE-28 shows a higher % recovery in DDI vs HCOOH, the RSD among triplicate SBSEs in HCOOH is only 2.8% vs 15.3% in DDI.

Table V compares %RRs and %R for three representative BDE congeners. Not only are %R values about half of the corresponding %RR values, a RSD for the absolute abundance of BDE-47 in a HCOOH matrix is 29.7% while the RSD in the abundance ratio for BDE-47 is only 8.0%. The topic of relatively low %Rs for the isolation and recovery of BDE/BB congeners at the level of concentration in spiked serum shown in Table II is due to the finite capacity of a Twister to extract these congeners. This topic will be addressed in a subsequent section in this paper.

Propagation of error between sample and control

Table VI summarizes %RRs for each BDE/BB congener isolated from serum modified with DDI and HCOOH. Also included are the propagated RSDs in the abundance ratios between SBSE and controls. The calculation of RR^j involves dividing x^j by x^j_C. Mathematically, the only way to calculate $s_R{}^j$ in the %RR is to propagate error between x^j by x^j_C. A RSD can be calculated by

taking into account the summation of variances using the equation shown in Table III. These mathematical concepts were applied to the results shown in Table VI. %RRs as shown Table VI, with the exception of BDEs 28, 47, and 183, are significantly greater (%RR) when HCOOH is used as a pretreatment reagent for serum. For all BDE/BB congeners, propagated RSDs, incorporating uncertainty in both the controls and SBSE samples are significantly lowered by use of HCOOH. The study was repeated, propagated RSDs in %RRs were again calculated. The results are shown in Table VII. Again, use of HCOOH as an important sample pretreatment reagent yielded enhanced %RRs. The most significant enhancements are seen from both PBBs, BB-155 and BB-153. Again, the lower MW BDE congeners show little preference for either DDI or HCOOH while the higher MW BDE congener %RRs are significantly enhanced with use of HCOOH as pretreatment reagent for a Sserum matrix.

Table VI. Comparison of BDE/BB Congener %RRs and RSDs Between DDI and HCOOH Isolated from Spiked Sheep Serum*

	DDI		HCOOH	
BDE#	%RR	RSD in%RR	%RR	RSD in %RR
28	128	24.9	91.3	19.8
47	105	24.3	89.8	19.6
77	75.4	24.3	79.2	19.2
BB-155	34.3	38.1	77.9	24.3
100	71.5	38.2	71.5	21.4
99	67.6	28.6	70.7	25.1
85	50.7	42.2	57.2	21.8
154	37.0	62.3	48.5	26.2
BB-153	35.1	64.3	48.8	25.5
153	36.0	70.1	40.0	27.9
183	41.1	76.1	38.7	22.9

* Refer to Table III for equations.

Table VII. Comparison of BDE/BB Congener %RRs and RSDs Between DDI and HCOOH Isolated from Spiked Sheep Serum*

	DDI		HCOOH	
BDE#	%RR	RSD in%RR	%RR	RSD in %RR
28	155	11.8	123	6.9
47	95.8	8.8	102	15.5
77	83.2	8.7	94.4	5.3
BB-155	22.9	3.7	89.2	1.3
100	47.4	7.5	82.1	10.6
99	50.1	6.5	80.4	10.9
85	43.7	10.6	67.8	5.1
154	21.2	7.1	70.2	1.2
BB-153	17.6	9.0	70.3	4.4
153	18.5	5.6	59.2	0.48
183	12.0	2.9	54.9	2.3

* Refer to Table III for equations.

Sample matrix effects on BDE/BB congener recoveries

Influence of sample matrix on propagated RSDs

At this point, it appeared that the direct deposition of aliquots of spiking standard onto a glass wool plug inserted into a TDU tube could serve as a valid control. This enabled consistent and reproducible %RRs from replicate SBSEs to be achieved. The propagated relative standard deviation, RSD, in the %RR was obtained from triplicate SBSEs. Four matrices were identified: 1, serum, DDI only; 2, serum, DDI, acetonitrile, triethylamine; 3, serum, HCOOH, only; 4, serum, HCOOH, acetonitrile, triethylamine.

RSDs were plotted vs each BDE/BB congener and compared for each of the four matrices as is shown in Figure 7. The lower the magnitude of RSD, the more precise is the %RR. For all congeners, with the exception of BDE-100, the effect of adding HCOOH to Sserum is to reduce the RSD. This reduction in RSD is more pronounced for the two BB congeners.

Effect of additional reagents/dilution on abundance ratios and on %RRs

Methanol (MeOH), ethyl acetate (EtOAc), and 2-propanol were also considered as matrix modifiers. Absolute abundances for all congeners recovered using EtOAc and 2-propanol were ~ 10x less than absolute abundances for all congeners when DDI, MeOH, or HCOOH was used. Figure 8 compares abundance ratios for BDE/BB congeners isolated from Sserum via SBSE using DDI, MeOH, and HCOOH. Lower MW congener recovery favors DDI while higher MW congener recovery favors either MeOH or HCOOH. A dilution of the reagent/serum mix adding DDI resulted in better recovery of lower MW congeners. Results are shown in Figure 9. Lower MW congener recovery favors a diluted MeOH or HCOOH serum matrix while higher MW congener recovery 26 favors the undiluted reagents. Both PBB congeners show a significant preference for an undiluted reagent. Recovery of higher MW BDE congeners as well as both PBB congeners favor a diluted HCOOH vs a diluted MeOH modification (Figure 9).

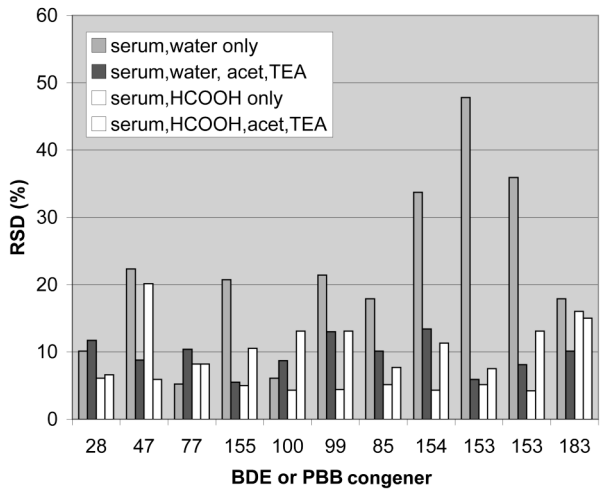

Figure 7. %RR vs sample matrix for SBSE of BDE/BB congeners recovered from spiked Sserum. Stir Bar Sorptive Extraction: Matrix effect on RSDs in the Percent Relative Recovery for each BDE and PBB congener' 155 refers tp BB-155, first 153 refers to BB-153.

Assessing applicability to human serum (Hserum)

The total ion chromatogram shown in Figure 10 demonstrates the feasibility of using Twister to extract PBDEs from only 1 mL of Hserum from AMAP. Figure 11 compares %RRs from spiked DDI vs duplicate spiked blank W03 (AMAP). Triplicate controls are first prepared. A blank DDI sample spiked only with BDE-66 shows an absence of laboratory contamination. A spiked DDI and two aliquots of W03 Hserum are spiked and taken through SBSE. Again, lower MW BDE congeners show little if any matrix effects (water vs Hserum) while the effect of a Hserum matrix suppresses %RRs for higher MW BDE congeners. PBB-155 shows a preference for the Hserum matrix vs DDI. A repeat of this experiment incorporating a packed liner while comparing %RRs vs a DDI or a Hserum matrix show nearly identical differences to that shown in Figure 11. In fact, PBB-155 recovery again favored Hserum vs DDI. These studies demonstrate no additional matrix effects when SBSE is accomplished using Hserum.

SBSE precision vs different CIS-4 inlet glass liners

We conducted triplicate SBSEs from Sserum after installing a 7.8 cm × 2 mm i.d. baffled liner into the CIS-4 inlet. We then replaced the liner with a 7.8 cm × 2 mm i.d. notched quartz packed liner. We conducted triplicate SBSEs using this liner. We calculated abundance ratios for all BDE and BB congeners. What we found when we compared results from using the two different liners is listed below: (*i*) Mean abundance ratios over triplicate

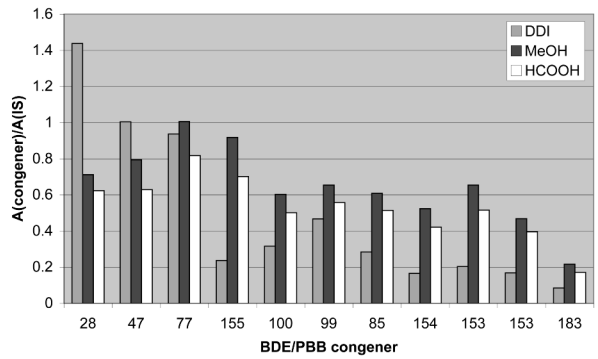

Figure 8. Abundance Ratio vs chemical nature of sample pretreatment reagent of BDE/BB congeners recovered from spiked Sserum. Ratio of analyte abundance to IS abundance vs serum matrix (stir 2 h at 500 rpm) 20 µL 40 ppb PBDEs + 20 µL 45 ppb IS; 155 refers to BB-155, first 153 refers to BB-153.

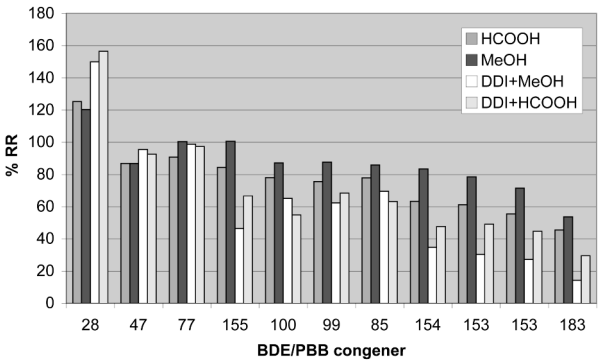

Figure 9. %RR vs degree of reagent dilution of BDE/BB congeners recovered from spiked Sserum. SBSE: %Relative Recoveries from sheep serum: effect of diluted vs undiluted HCOOH and MeOH matrix modifiers (stir for 2 h at 500 rpm); 155 refers to BB-159, first 153 refers to BB-153.

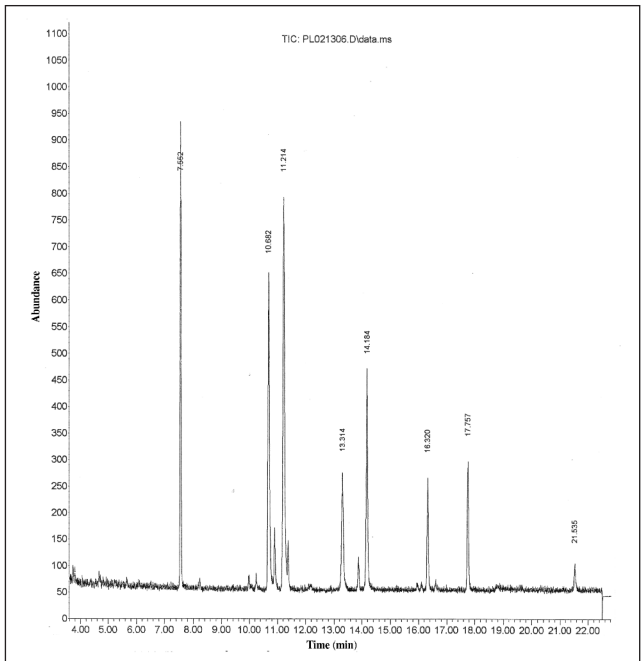

Figure 10. TD-Cryo-C-GC-MSD-ECNI-SIM chromatogram of unspiked AMAP Hserum using SBSE. BDE congeners identified in the TD-Cryo-C-GC-MSD-ECNISIM chromatogram include: BDE-28 at 7.552 min, BDE-47 at 10.682 min, BDE-66 at 11.241 min (added as internal standard), BDE-100 at 13.314 min, BDE-99 at 14.184 min, BDE-154 at 16.320 min, BDE-153 at 17.757 min. To summarize how this sample was prepared: in a 5 mL headspace vial is placed 1.0 mL of AMAP serum. The Hserum is spiked with 20 µL of 45 ppb BDE-66 in 2-propanol. An aliquot of 1.0 mL HCOOH is added. An unused Twister is then added to the modified sample. The sample is stirred for 2 h at 500 rpm. The Twister is removed from the sample, placed in DDI to remove biological material and then dried with a Kim-Wipe. Twister is then placed in a clean TDU glass tube and subsequently thermally desorbed, cryogenically trapped and desorbed into a 30 m x 0.25 mm DB-XLB column for separation and detection of recovered BDE congeners.

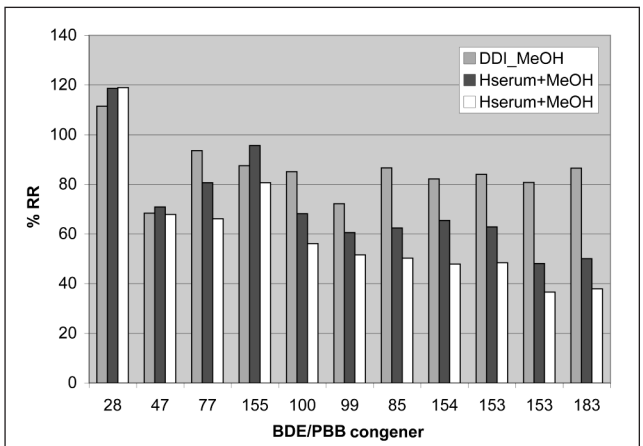

Figure 11. %RR vs sample matrix for SBSE of BDE/BB congeners from spiked Hserum. SBSE: % Relative Recoveries DDI vs AMAP Human Serum (W03-02 and W03-05) (stir for 2 h at 500 rpm) 2-15-08; 155 refers to BB-155, first 153 refers to BB-153.

SBSEs for each BDE/BB congener were significantly higher with the baffled liner installed than with the notched liner installed. (*ii*) Relative standard deviations (RSDs) in the mean abundance ratio for all congeners were comparable between liners. RSDs ranged from a low of 3% (BDE-77 with a baffled) to a high of 40% (BDE-183 with a notched). (*iii*) We experienced difficulty in overshooting the setpoint CIS-4 pressure after a TDU tube containing a Twister was inserted into the TDU and locked by the pneumatics when the notched liner was installed.

Octanol–water partition coefficients (K_{OW}) for PBDEs and prediction of theoretical Twister capacity

BDE congener K_{OW} values and Twister capacity

When Twister is placed into an aqueous matrix, PBDE molecules, being hydrophobic, get distributed between the nonpolar PDMS and the more polar sample matrix. SBSE should follow the model previously developed for SPME when the fiber is immersed into a liquid (15). Rapid stirring in SBSE creates a steady state in which molecules that arrive at the PDMS surface are quickly partitioned into the PDMS. This creates a depleted region that surrounds the stir bar. This depletion zone sets up a concentration gradient throughout the bulk sample. A concentration gradient is also established within the PDMS coating thickness. In contrast to LLE whereby both extractant and liquid sample share the same surface area interface, mass transfer via diffusion is the principal kinetic means for SBSE to occur. Diffusion coefficients become important and perhaps this is where the sample matrix exerts its most influence. The extent to which BDE congeners are partitioned into Twister after equilibrium is established and determined for the ith analyte according to:

$$K^i_{SBSE} = \frac{C^i_{PDMS}}{C^i_S} \qquad \text{Eq. 1}$$

where CiPDMS is the equilibrium concentration of the ith analyte in Twister while CS is the equilibrium concentration of ith analyte remaining in the sample.

K^i_{SBSE} for a given organic compound can be estimated from its K_{OW} (17). Log K_{OW} values for BDE congeners of interest in this paper are given in Table VIII. Those BDE congeners asterisked were not originally found in the EPA Suite database. However, we estimated K_{OW}s for those asterisked using the SMILES algorithm (18). All BDE congeners exhibit log K_{OW}s that reflect extreme hydrophobicity. A theoretical percent recovery for the ith chemical analyte initially dissolved in an aqueous sample matrix is given by:

$$\%R^i_{SBSE} = \frac{K^i_{SBSE}\beta}{1 + K^i_{SBSE}\beta} \times 100 \qquad \text{Eq. 2}$$

where β is the phase ratio and $\beta = V_{PDMS}/V_S$. V_{PDMS} represents the volume of the polydimethylsiloxane (PDMS) coated Twister and V_S represents the volume of sample. If the K_{OW} for a specific BDE congener is substituted for its KSBSE value in Equation 2, a calculation of a theoretical percent recovery can be made. Since we know the amount of spike added to a final volume of sample, Equation 1 can be utilized to estimate the capacity of one Twister to partition one BDE congener. We develop these calculations for BDE-47 below. Log K_{OW} values are so large such that all other BDE congeners would be expected to behave similarly.

Theoretical % recovery and capacity for BDE-47

From the EPI Suite database, for BDE-47, log K(ow)= 6.77 so that K(ow) = 5.9 × 10. Assume a sample volume that consists of 1 mL serum and 1 mL reagent so that V_S = 2 mL = 2000 µL; V_{PDMS} = 24 µL based on a 10 mm long and 0.5 mm thick Twister (16). Substituting into equation 2 gives:

$$\%R^{BDE-47}_{\text{SBSE}} = \frac{(5.9 \times 10^6)(24/2000)}{1 + (5.9 \times 10^6)(24/2000)} = \frac{(5.9 \times 10^6)(0.012)}{1 + (5.9 \times 10^6)(0.012)} \times 100 \approx 100$$

The calculation predicts PBDEs should demonstrate 100 % recovery. Does this suggest that Twister can remove all BDE congeners irrespective of the total amount of BDE congeners in a 2 mL volume of sample? Stated differently, what is the capacity of Twister to extract PBDEs such as BDE-47? How many pg of BDE-47 can be extracted?

Consider an initial concentration of BDE-47 in serum as C0 (expressed in terms of a #pmoles / µL). A rewriting of equation 1 for BDE-47 is shown below:

$$K^{BDE-47}_{SBSE} \approx K^{BDE-47}_{ow} = \frac{C^{BDE-47}_{PDMS}}{C^{BDE-47}_S} = \frac{xpmoles/V_{PDMS}}{C_0 - (xpmoles/V_s)} \qquad \text{Eq. 3}$$

where the amount extracted is expressed in picomoles (pmoles). Upon substituting experimental values for V_{PDMS} and for V_S in equation 3 gives:

$$5.9 \times 10^6 = \frac{xpmoles/24\ \mu L}{C_0 - xpmoles/2000\ \mu L}$$

Solving the above equation x gives:

$$x = [(2.0 \times 10^3) \cdot C_0] \text{ pmoles BDE-47} \qquad \text{Eq. 4}$$

Equation 4 is the mathematical basis for quantitative analysis utilizing SBSE since the amount of analyte "extracted" is directly proportional to the original sample concentration. One Twister ought to be able to extract up to a maximum of [2000] *[C_0] pmoles BDE-47, given a molecular weight of 485.80 Da for BDE-

Table VIII. Octanol–Water Partition Coefficients for BDE Congeners Studied in this Paper

Congener #	log K_{OW}
28	5.88
47	6.77
66	7.61*
100	8.50*
99	7.66
85	7.66
154	9.39*
153	9.39*
183	10.28*

* calculated using SMILES

47 and assuming that we spike 2 mL of sample (serum + reagents) with 10 µL of a 4.5 ppb BDE-47 solution in 2-propanol, this gives an estimate for the original concentration of BDE-47 in the sample:

$$C_0 = \frac{4.63 \times 10^{-5} \text{ pmoles}}{\mu\text{L BDE-47}}$$

Therefore,

$$x = [2.0 \times 10^3\ \mu\text{L}] \cdot [4.63 \times 10^{-5}\ \text{pmoles}/\mu\text{L}] = 9.26 \times 10^{-2}\ \text{pmoles BDE-47}$$

and

$$9.26 \times 10^{-2}\ \text{pmoles} \times 485.8\ \text{pg/pmole} = \textit{45 pg BDE-47}$$

Equation 4 predicts a capacity of one Twister to extract 45 pg BDE-47. This amount corresponds to a theoretical 100% recovery of BDE-47 from a given sample. All other BDE congeners would exhibit this capacity as well. Equation 3 can be viewed in terms of only variables. If $K_{OW} >> V_S$ (the case for BDE congeners), it can be shown that (15):

$$x = [V_S] \times C_0$$

Estimating an experimental Twister capacity

Figure 12 shows the results of conducting three successive SBSEs on the same spiked serum sample. Keeping in mind the calculated capacity of BDE-47 above, 800 pg each BDE/BB congener was spiked and the final volume adjusted to 2 mL of sample. If one SBSE recovered > 50% of each congener, subsequent SBSEs on the same sample would be expected to give significantly less peak abundances. The significant absolute abundances for BDE-66 from the second successive SBSE are even greater than that from the first. Despite a 100% theoretical percent recovery for BDE / BB congeners, our data indicates that the 800 pg spike amount significantly exceeds the capacity of Twister.

Successive SBSEs keep extracting out a certain percent of total congener originally present in the spiked sample. Consider the following scenario, if 45 pg of BDE-47 can be extracted out each time SBSE is performed, than if would take ~18 successive SBSEs to extract out all of the 800 pg present in the spiked sample.

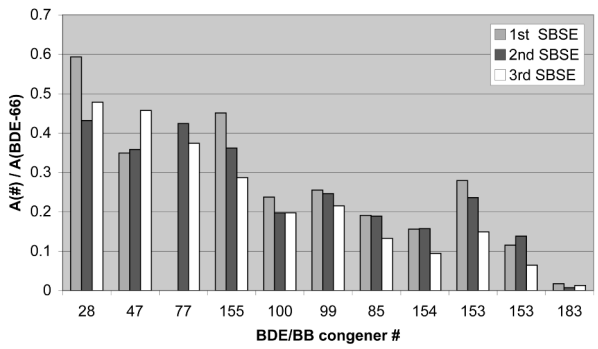

Figure 12. Abundance Ratio vs successive SBSE. A(#) / A(BDE-66) vs successive SBSE: 1.0 mL human serum + 1.0 mL HCOOH + 200 µL acetonitrile: 7.8 cm × 2 mm i.d. quartz packed notched liner: stir for 2 h at 500 rpm; 155 refers to BB-155, first 153 refers to BB-153.

To test this hypothesis further, two different spiked amounts were placed into identical sample volumes of DDI. DDI was chosen instead of serum to eliminate any matrix effect. We will focus on BDE-66 only. All other BDE and BB congeners behave similarly. Absolute peak abundances from sample #1 from a second successive SBSE (241 vs 11,666 for BDE-66) were found when 20 pg each congener was added to 2 mL of sample. Absolute peak abundances from sample #2 were treated the same as for sample #1 showed (3717 vs 14,260 for BDE-66) were found when 80 pg each congener was added. These results are consistent with an estimated Twister capacity of 45 pg each congener derived from theoretical principles discussed earlier. We repeated this experiment a second time, this time adding 45 pg BDE / BB congeners to serum and undergoing SBSE. After removal of the first Twister, a second Twister was placed into the sample and stirred for exactly the same amount of time and at an identical stir rate. We again used DDI in this set of experiments instead of serum to rule out any matrix interferences. In this experiment 22.5 pg each BDE-66 spiked into 2 mL sample were more efficiently recovered from the first SBSE. However, 45 pg BDE-66 spiked into 2 mL sample were not as efficiently recovered after one SBSE. All other BDE/BB congeners behaved in a similar manner to BDE-66. Results from these studies experimentally verify the finite and very low capacity of Twister for PBDEs and PBBs.

Calibration/verification considerations at concentration levels below Twister capacity

After recognizing that 45 pg each BDE / BB congener might represent the maximum amount of analyte that can be extracted by Twister from spiked serum, experimental calibration data points were generated whereby abundance ratios for each BDE / BB congener can be plotted against concentration in parts-per-trillion (ppt). Six representative plots are shown in Figure 13. BDE-28, a tribromo DE yielded a good ordinary least squares (OLS) regression fit. A plateau is seen above 25 ppt. BDE-47, a tetrabromoDE, shows significant deviation from an OLS fit due to blank twister contamination from reused Twisters. This contamination is reflected in a y-intercept of 4.28. BB-155, a hexabromo biphenyl, is added to all serum samples in our laboratory, yielded a good OLS fit. BDE-85, a pentabromoDE, yielded a good OLS fit. BDE-153, a hexabromoDE, deviated somewhat from the OLS fit. BDE-183, a heptabromoDE, yielded a good OLS fit. These plots demonstrate the high sensitivity of SBSE / TD-Cryo C-GC-MSD-ECNI-SIM resulting in a linear dynamic range from 2.5 ppt to 20 ppt.

Three calibration plots were prepared for 2 mL (total samples containing 0.5 mL DDI, 0.5 mL Sserum and 0.1 mL Hserum over nearly the same range of analyte concentrations. OLS parameters are shown in Table IX. Refer to the experimental section for procedural details. Comparing OLS slopes for the three matrices (DDI, sheep, and human serum) shows a greater sensitivity for PBDE/PBB analytes from DDI vs serum. However, a significant increase in OLS y-intercept is seen for the DDI matrix.

Coefficient of determinations are for the most part > 0.990 with the following exceptions. BDEs 47 and 99 show in some Twisters, evidence of blank Twister contamination which dis-

torts the OLS fit and BBs 155 and 153 along with some other BDE congeners in the DDI matrix. However, coefficients for these significantly improved in both sheep and serum matrices. Triplicate 2 mL samples that contained 0.1 mL spiked Hserum and run as ICVs were quantitated against the 5/19/2008 OLS calibration parameters. Results are shown in Table X. The majority of congeners fall to within ± 10 ppb of the expected value of 50 ppb. BDEs 47 and 99 again show a significant deviation from the ICV-2 Twister and acceptable deviations from the ICV-1 and ICV-3 Twisters. This difference in deviations among triplicate SBSEs is attributed to blank Twister contamination with respect to ICV-2. All of this calibration work reported here points to the feasibility of using SBSE as a quantitative sample preparation technique within the limitations mentioned. Table XI shows a second precision study using spiked calf bovine serum. Refer to the Experimental section for procedural details. A blank serum sample underwent SBSE and this resulted in no detectable BDE/BB congener peaks. In light of the high selectivity and sensitivity of our determinative technique, this absence of congeners in the blank serum demonstrates our success in effectively removing BDEs 47 and 99 from used Twisters. The technique we developed for efficiently removing traces of congeners from used Twisters followed by reconditioning them will be discussed below. Quadruplicate spiked bovine calf serum samples were taken through SBSE and then TD-Cryo-C-GCMSD-ECNI-SIM as shown in Table XI. Except for blank Twister contamination for BDEs 47 and 99, so noted earlier, RSDs varied from a high of 17.1 (BDE-183) to a low of 3.2 (BDE-154). These values demonstrate excellent precision given that uncertainty exists in both sample preparation and in the determinative technique used. These RSD values are quite comparable to LLE / Cleanup and RP-SPDE techniques (6).

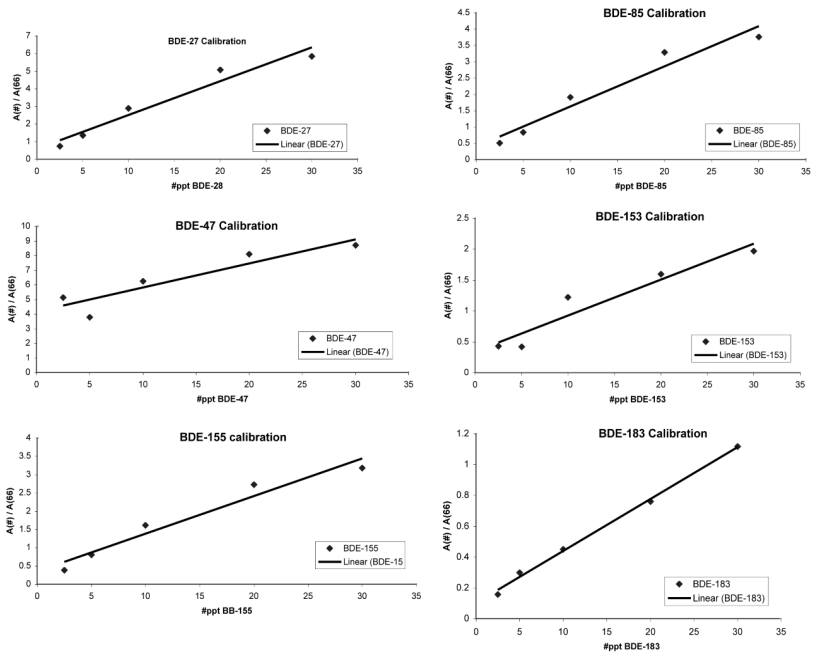

Figure 13. Six representative BDE/BB congener calibration plots.

Re-use of Twisters to isolate and recover PBDEs

We have found that Twisters can be used and then reused two or three times before a noticeable memory effect occurs. PBDEs tenaciously adsorb to glass surfaces. Used Twisters were initially soaked in 80:20 acetonitrile–methanol and then reconditioned as suggested by Gerstel, Inc. We found that these techniques insufficiently removed PBDEs. This problem is best described by viewing a series of C-GC-MSD-ECNI-SIM

Table IX. Summary of OLS Parameters for DDL Sheep and Human Serum Matrices: PBDEs, PBB-155, PBB-153

Date	OLS(sample matrix)	N	BDE-28	BDE-47	BDE-77	PBB-155	BDE-100	BDE-99	BDE-85	BDE-154	PBB-153	BDE-153	BDE-183
5/21/08													
5/12/08	m(0.5mL DDI)	4	0.01	0.008	0.012	0.009	0.0073	0.007	0.0085	0.0065	0.012	0.0055	0.0034
5/14/08	m(0.5mL Sheep Serum)	4	0.0068	0.0054	0.0077	0.0067	0.0051	0.0056	0.0057	0.0044	0.0053	0.0038	0.0021
5/16/08	m(0.1mL human serum)	6	0.0071	0.0046	0.0082	0.0064	0.005	0.005	0.0056	0.0041	0.0048	0.0035	0.0019
	b(DDI)	4	0.07	0.44	0.15	0.1	0.11	0.3	0.088	0.093	0.23	0.096	0.047
	b(sheep)	4	0.0046	0.1	–0.0096	–0.022	–0.0011	0.047	–0.011	–0.016	–0.018	–0.011	–0.0076
	b(human)	6	0.018	0.29	0.026	0.0024	0.042	0.14	0.014	0.011	0.018	0.025	0.0061
	r(2)[DDI]	4	0.967	0.9	0.921	0.853	0.899	0.895	0.898	0.833	0.451	0.788	0.798
	r(2)[SHEEP]	4	0.964	0.99	0.988	0.99	0.995	0.993	0.992	0.987	0.988	0.99	0.985
	r(2)[HUMAN]	6	0.997	0.566	0.994	0.992	0.988	0.881	0.997	0.994	0.988	0.998	0.996

All calibration parameters taken from ChemStation printouts
N - number of calibration points used in the ordinary least squares (OLS) regression; m is the slope and b is the y-intercept of the OLS regressed calibration curve;
r(2) is the coefficient of determination (square of correlation coefficient r) for each OLS regressed line
BDE# 28-2,4,4′-TBDE; 47-2,2′,4,4′-TBDE;77-3,3′,4,4′-TBDE;100-2,2;,4,4′,6-PBDE;99-2,2′,4,4′,5-PBDE 85-2,2′,3,4,4′-PBDE;154-2,2′,4,4′,5,6′-HBDE;153-2,2′,4,4′,5,5′-HBDE;183-2,2′,3,4,4′,5′,5-HBDE 66-2,3′4,4′-TBDE(IS)

chromatograms. Most targeted BDE congeners are seen while both BB congeners are absent. BDE-47 and -99 peak abundances exceed 55,000 counts (data not shown). The same Twister was then placed in 1–2 mL toluene and subsequently bath sonicated and then thermally desorbed without reconditioning at elevated temperature. Peak broadening is seen for the BDE-47 and -99 contaminants. After reconditioning, the narrowed peak widths that are typical of capillary gas chromatography for the BDE congener contaminants have returned. The BDE-47 and -99 peaks abundances have been reduced by more than one-half when compared to conditioning without extraction.

The efficiency of toluene as an extractant could not be fully realized until we first soaked with polar solvent as mentioned earlier, then immediately extracted 3x with 1–2 mL toluene. The Twister was then reconditioned at 325°C (a recommended temperature from Gerstel, Inc.) for 1 h. Peak abundances are reduced by more than two-thirds.

We applied SBSE to spiked DDI using uncoated Twisters supplied by Gerstel and found detectable abundances when compared to a coated Twister that was spiked with the same amount of BDE/BB congeners. This limited experiment suggests that because both ends of a Twister is uncoated and made of glass, the potential exists for adsorption of congeners onto the glass surface.

Table X. Comparison of Triplicate ICVs for BDE/BB Congeners using SBSE from 0.1 mL of Spiked Hserum

	#ppt		
Congener #	ICV-1	ICV-2	ICV-3
Expect	50	50	50
28	50.1	55.5	45.8
47	47	176	43.4
77	50.5	47.3	46.8
155	57.9	53.3	45.0
100	53	60	48
99	55.8	105	44.6
85	55.2	47.7	46.2
154	59.4	47.7	46.7
153	61.2	48.3	45.4
153	60.0	42.9	39.6
183	60.6	40.4	43.6

Instrument and method detection limits

SBSE using Twister for sample preparation and automated TD-Cryo-C-GC-MSD-ECNISIM combines a microextraction technique with a highly sensitive and highly selective determinative technique. Calibration data generated for the case where < 45 pg each BDE/BB congener could be extracted, was sufficient enough to use confidence band calibration statistics to yield instrument detection limits (IDLs). The confidence band calibration statistical approach in contrast to the blank statistics approach has been gaining popularity (19,20). We programmed equations into Excel (Microsoft, Redmond, WA) to find the Hubaux-Vos type decision level, y(C), and decision limit, 6 x[C], for BDE / BB congeners (15). To calculate IDLs for each congener, we programmed in a non-iterative equation for X(D). This equation, according to the authors (21), for the case of m = 1, is algebraically equivalent to the formula developed by Currie (22). Table XII lists IDLs for all 11 BDE / BB congeners studied. For SBSE coupled to TD-Cryo-C-GC-MSD-ECNI-SIM, the method detection limit (MDL) equals the IDL since internal standard was added to samples prior to SBSE. Again, blank Twisters used to generate calibration data were contaminated and the unusually high x[D] reflects this. IDLs are conservative estimates of detection limts when applying confidence band calibration statistics since false negative as well as false positive errors are accounted for. Table XII reveals IDLs as low as 7.1 ppt (BDE-153) and as high as 20.4 ppt (BB-153) ignoring IDLs for BDE 47 and 99 due to blank Twister contamination as discussed earlier.

Conclusion

The feasibility of isolating PBDEs and PBBs via SBSE from sheep and human serum has been demonstrated. Recovery of BDE/BB congeners from thermally desorbed congeners off of Twister was maximized by optimizing the final TDU hold times. Pretreatment of serum with either formic acid or methanol significantly increased congener relative recovery while reducing precision among replicate SBSEs. Octanol–water partition coefficients were either found or estimated for the nine BDE congeners studied. The effect of changing CIS-4 inlet glass liners for TD-Cryo-C-GC-MSD-ECNI-SIM was studied. The finite capacity of Twister to extract selected BDE / BB congeners from serum was established. Calibration and verification results are pre-

Table XI. Unspiked (Blank) and Four Spiked Replicate Bovine Calf Serum Samples: Precision SBSE Study

		#ppt										
Date	Lsquares	BDE-28	BDE-47	BDE-77	PBB-155	BDE-100	BDE-99	BDE-85	BDE-154	PBB-153	BDE-153	BDE-183
5/30/08	Blank	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	BCS-1	19.7	C	19.2	20.7	16	C	21.9	20	19.3	17.4	24.8
	BCS-2	20.9	C	19.2	19.3	15	C	20.2	19.4	18.6	16.6	24.4
	BCS-3	17.6	C	16.5	17.5	13.2	C	19.2	18.5	16.5	13.7	17.4
	BCS-4	20	C	18.8	20.8	15.5	C	21.5	19.2	19.9	17	20
	mean	19.6		18.4	19.6	14.9		20.7	19.3	18.6	16.2	21.6
	s	1.4		1.3	1.5	1.2		1.2	0.62	1.5	1.7	3.7
	RSD(%)	7.1		7	7.9	8.2		6	3.2	8	10.4	17.1

ND-not detected; C-contaminated calibration standards

Table XII. Decision levels, Limits and Detection Limits for BDE / BB Congeners Isolated and Recovered by SBSE

BDE/BB#	y[C] A(#)/A(66)	x[C] (ppt)	x[D] (ppt)
28	0.0451	4.14	8.1
47	0.563	59.3	174.7
77	0.0837	7.5	14.6
155	0.0427	7.8	15.0
100	0.0788	7.9	15.3
99	0.251	22.5	42.7
85	0.0479	5.95	11.6
154	0.0344	6.54	12.7
153	0.0621	10.67	20.4
153	0.0360	3.64	7.1
183	0.0206	7.26	14.0

sented for the isolation and recovery of BDE / BB congeners from serum. A procedure that effectively eliminates memory effects in reused Twisters was developed. Method detection limits for each BDE/BB congener based on applying confidence band calibration statistics were found to be at the low ppt level.

The cost of adding thermal desorption / cryotrapping automation to existing GC–MS instruments is considerable. These costs, however, are offset due to the significant decreases in sample prep time, analyst labor and solvent consumption.

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References

1. C. Naert, M. Piette, N. Bruneel, and C. Van Peteghem. Occurrence of polychlorinated biphenyls and polybrominated diphenyl ethers in Belgian human adipose tissue samples. *Arch. Environ. Contam. Toxicol.* **50:** 290–296 (2006).
2. H. Aalm, B. Scholz, C. Fischer, K. Kultima, H. Viberg, P. Eriksson, L. Dencker, and M. Stigson. Proteomic evaluation of neonatal exposure to 2,2′,4,4′5pentabromodiphenyl ether. E*nviron. Health Perspect.* **114(2):** 254–259 (2006).
3. A. Sjödin, R.Jones, J.Focant, C. Lapeza, R. Wang, E. McGhee III, Y. Zhang, W. Turner, B.Slazyk, L. Needham, and D. Patterson, Jr. Retrospective time-trend study of polybrominated diphenyl ether and polybrominated and polychlorinated biphenyl levels in human serum from the United States. *Environ. Health Perspect.* **112(6):** 654–658 (2004).
4. A. Sjödin, L. Wong, R. Jones, A. Park, Y. Zhang, C. Hodge, E. Dipietro, C. McClure, W. Turner, L. Needham, and D. Patterson, Jr. Serum concentrations of polybromnated diphenyl ethers (PBDEs) and polybrominated biphenyl (PBB) in the United States population: 2003-2004. *Environ. Sci. Technol.* **42(4):** 1377–1384 (2008).
5. J. Dye, M. Venier, L. Zhu, C. Ward, R. Hites, and L. Birnbaum. Elevated PBDE levels in pet cats: sentinels for humans? *Environ. Sci. Technol.* **41(18):** 6350–6358 (2007).
6. P.R. Loconto, D. Isenga, M. O'Keefe, and M. Knotterus. Isolation and recovery of polybrominated diphenyl ethers from human 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. *J. Chromatogr. Sci.* **46:** 53–60 (2008).
7. P.R. Loconto. Selectivity and sensitivity improvements for selected polybrominated dipheny ethers and polybrominated biphenyls using capillary gas chromatography / electron capture negative ion mass selective detection: a cost effective approach to biomonitoring. *LC-GC NA* **26(11):** 1118–1130 (2008).
8. A. Prieto, O. Telleria, N. Etxebarria, L.A. Fernández, A. Usobiaga, and O. Zuloaga. Simultaneous preconcentration of a wide variety of organic pollutants in water samples: Comparison of stir bar sorptive extraction and membrane-assisted solvent extraction. *J. Chrom. A* **1214(1-2):** 1–10 (2008).
9. E.Baltussen, P. Sandra, F. David, and C. Cramers. Stir bar sorptive extraction (SBSE), a novel extraction technique for aqueous samples: theory and principles. *J. Micro Sep.* **11:** 737–747 (1999).
10. F. David, B. Tienpont, and P. Sandra. Stir-bar sorptive extraction of trace organic compounds from aqueous matrices. *LC-GC.* **21(2):** 1–7 (2003).
11. B. Tienpont, F. David, K. Desmet, and P. Sandra. Stir bar sorptive extraction-thermal desorption-capillary GC-MS applied to biological fluids. *Anal. Bioanal. Chem.* **373:** 46–55 (2002).
12. P. Serôdio and J.M.F. Nogueira. Multi-residue screening of endocrine disrupters chemicals in water samples by stirbar sorptive extraction-liquid desorption-capillary gas chromatography-mass spectrometry detection. *Anal. Chim. Acta.* **517:** 21–32 (2004).
13. G. Fries. The PBB episode in Michigan: an overall appraisal. *CRC Crit. Rev. Toxicol.* **16:** 105–156 (1985).
14. EPI Suite K_{OW} WINNT.exe, Downloaded from the Environmental Protection Agency. Few log K_{OW} values PBDEs are found in the database. The author calculated all K_{OW} values for BDE congeners not found using the SMILES algorithm.
15. P.R. Loconto. Trace Environmental Quantitative Analysis, 2nd Ed., CRC Press, Taylor and Francis, Boca Raton, FL, 2006, Chapters 2,3, and Appendix A.
16. A. Pauwels, D. Wells, A. Covaci, and P. Schepens. Improved sample preparation method for selected persistent organochlorine pollutants in human serum using solid-phase disk extraction with gas chromatographic analysis. *J. Chrom. B* **723:** 117–125 (1999).
17. P. Sandra, E. Baltussen, F. David, and A. Hoffman. "Stir bar sorptive extraction (SBSE) applied to environmental aqueous samples". App. Note 2/2000, Gerstel GmbH & Co. KG.
18. EPI Suite K_{OW} WINNT.exe, Downloaded from the Environmental Protection Agency. Few log K_{OW} values for PBDEs are found in the database. The author calculated all K_{OW} values for BDE congeners not found using the SMILES algorithm. To download this software go to: http://www.epa.gov/oppt/exposure/pubs/episuitedl.htm
19. P. Loconto. IDLs: will we ever let 3s go? *Am. Lab. News Ed.* **37(4):** Feb 27, 2005, pp. 36–40.
20. D. Coleman and L. Vanatta. Statistically derived detection limits (concluded). *Am. Lab.* **40(12):** 34–37 (2008). This reference is from a series started in 2002.
21. J. Burdge, D. MacTaggart, and S. Farwell. Realistic Detection Limits from Confidence Bands. *J. Chem. Educ.* **76:** 434–439 (1999).
22. L. Currie and G. Svehla. Nomenclature for the presentation of results of chemical analysis. *Pure & Appl. Chem.* **66(3):** 595–608 (1994).

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