DEVELOPMENT OF A SOLID-PHASE EXTRACTION TECHNIQUE FOR THE ANALYSIS OF PBDES AND NEW BROMINATED FLAME RETARDANTS IN HUMAN SERUM

Butt CM[1*], Stapleton HM[1]
[1]Nicholas School of the Environment, Duke University, Box 90328, Levine Science Research Center, Room A220, Durham, North Carolina 27708, USA

Introduction

Brominated flame retardants (BFRs) are widely used in plastics, foams and textiles. Their common usage has resulted in nearly ubiquitous detection in humans and the environment. Polybrominated diphenyl ethers (PBDEs) were once commonly used as additive flame retardants. However, the penta- and octa-BDE commercial mixtures have been phased out or banned in most of the world. Further, the DecaBDE mixture is scheduled for phase-out by 2013. However, due to their persistence, PBDEs are still commonly detected in the environment, such as in house dust and human blood. Further, PBDEs are metabolized to hydroxylated BDEs (OH-BDEs) in the human body. In addition to PBDEs, several alternative BFRs have been developed and used, some of which were designed as PBDE replacements. These include hexabromocyclododecane (HBCD), tris (1,3-dichloroisopropyl) phosphate (TDCPP) and the Firemaster 550 mixture which contains two BFRs, tetrabromobenzoate (TBB) and tetrabromphthalate (TBPH). Further, recent work from our laboratory has shown that TBB is metabolized to the tetrabromobenzoic acid (TBBA) in human liver tissues [1].

PBDEs have been routinely measured in human serum using either liquid-liquid extraction (LLE) or solid phase extraction (SPE) techniques. SPE techniques are advantageous since they use much less solvent that the traditional LLE techniques. SPE techniques have been applied to measuring PBDEs in serum [2], but there have been few efforts to measure the alternative BFRs and their metabolites.

The present study developed a SPE extraction technique for the analysis of PBDEs, OH-PBDEs, HBCDs, TBB and TBPH in human serum. The developed technique was used to measure BFRs in human serum. Further work will measure BFRs in placental tissue and will investigate the association between BFRs in the maternal serum and placenta with measurements of deiodinase activity in the placenta. Preliminary work from our laboratory showed that BDE 47 in the maternal serum was negatively associated with type III DI activity in the placenta (unpublished work).

Materials and methods

Sample Collection

Serum samples were analyzed from a cohort of pregnant women that was previously reported on by our laboratory for the association between PBDEs, OH-BDEs, and thyroid hormones [3]. The present study investigates BFR levels in serum from the same cohort, but were collected during the second trimester (September 2008 to June 2010). Full details regarding the participant recruitment and sample collection have been described previously[3]. In brief, all participants were residents of central North Carolina, USA. Blood was collected, serum was isolated by centrifuging (5 min, 3500 rpm) and kept frozen (-20^{o} C) until analysis.

Sample Extraction and Analysis

Serum was allowed to thaw and gravimetrically transferred (~3.5-4.0 g) to a glass test tube (n=8). Internal standards were spiked (5 ng of F-BDE 69 and ^{13}C-BDE 209; 2.5 ng of ^{13}C-6-OH BDE 47, ^{13}C-6'-OH BDE 100 and ^{13}C-α HCBD) prior to the serum addition. Formic acid (2 ml) and water (6 ml) was added to denature the serum proteins and the mixture was sonicated for 20 min. The analytes were isolated on a Waters Oasis HLB column (500 mg, 6 ml). The column was conditioned with 5 ml dichloromethane (DCM), 5 ml methanol (MeOH) and 5 ml water, the sample was loaded and the column was washed with 5 ml water. The analytes were eluted with 10 ml of DCM:ethyl acetate, the eluent blown to dryness and reconstituted in 1 ml of hexane. The analytes were cleaned and fractionated using a 1 g silica column (Waters, Sep-Pak) that was initially conditioned with 10 ml of hexane. The first fraction was eluted with 10 ml of hexane, reduced in volume to ~100 µl and

analyzed by GC-MS for a suite of 26 PBDEs. Prior to analysis 10 ng of ^{13}C-CDE 141 was spiked to assess F-BDE 69 recovery. The second fraction was eluted with 10 ml of DCM:hexane, blown to dryness, reconstituted in ~100 µl MeOH and analyzed by LC-MS/MS for the HBCDs and OH-PBDEs. After LC-MS/MS analysis, the MeOH fractions were solvent switched in to hexane and analyzed for TBB and TBPH by GC-MS.

Instrumental analysis was performed using previously developed methods by our laboratory [3, 4]. The GC-MS was operated in electron capture negative ionization mode. The LC-MS/MS conditions were altered slightly and are briefly described. Analytes were separated under gradient conditions and mobile phase was acetonitrile and water (5 mM acetic acid). The column temperature was 40^{o}C and the injection volume was 20 µl. The internal standards were ^{13}C-α HCBD for the HBCDs, ^{13}C-6-OH BDE 47 for the OH-tetraBDEs and ^{13}C-6'-OH BDE 100 for the OH-pentaBDEs. The MRMs monitored for the OH-tetraBDEs were 500.7>418.8, 500.7>265.9 and 500.7>78.9. MRMs monitored for the OH-pentaBDEs were 580.7>498.8, 580.7>343.8 and 580.7>78.9. We found that the position of the hydroxyl group gave diagnostic MRMs, as has been shown by other researchers [5]. For example, the 500.7>418.8 and 580.7>498.8 (formation of $[M\text{-}Br]^-$) was unique to meta-OH BDEs. Also, the 500.7>265.9 and 580.7>343.7 (formation of the bromobenzoquinone) was indicative of para-OH BDEs. Finally, the ortho-OH BDEs showed only the 500.7>78.9 transition (formation of Br^-). In addition to retention time, these diagnostic MRMs were used to positively identify the OH-BDEs. The following OH-BDEs were quantified: 4'-OH BDE 49, 6-OH BDE 47, 6'-OH BDE 99 and 5'-OH BDE 99.

Samples were blank-corrected using the mean blank value (n=4). Method detection limits were calculated as three times the standard deviation of the blanks. Values were normalized to the serum sample mass and an assumed lipid content of 0.5%.

Results and discussion

Quality Control

Fetal bovine serum was used as the surrogate during method development to assess analyte recovery. Approximately 5 ng of PBDEs (suite of 26 congeners), 1.25 ng of 6-OH BDE 47 and 6'-OH BDE 99, and 2.5 ng of α-, β- and γ-HBCD were spiked in to 2 ml of fetal bovine serum (n=3) and were left to equilibrate overnight. The samples were carried through the developed extraction methods and analyte recovery was measured. PBDE recovery was ranged from 64%-113%. TBB recovery was 79%, but TBPH showed very low recovery (2%). HBCD recovery was 68-87%, 88-95% and 89-100% for α-, β-, γ-HBCD, respectively. OH-BDE recovery was 104-116% for 6'-OH BDE 99 and 83-87% for 6-OH BDE 47, respectively. Further, the extract was clean and did not appear to result in matrix enhancement or suppression in the LC-MS/MS.

In addition, SRM 1958 (Fortified Human Serum, National Institute of Standards and Technology, Gaithersburg, MD) was analyzed using the developed method to assess method accuracy for PBDEs only (n=3). PBDEs were 73-97% of the reported values with the exception of BDE-154 (158%) and BDE-99 (56%). Polybrominated biphenyl 153 co-elutes with BDE-154, and is a known constituent of SRM 1958, and presumably is the cause for the elevated recovery.

During extraction of the maternal serum samples, minor laboratory contamination was only observed for BDE 47 (0.06-0.14 ng) and BDE 99 (0.03-0.09 ng). Recovery of F-BDE 69 in the maternal serum samples was 72.2% ± 5.4 (mean ± standard deviation).

Maternal Serum Samples

Eight maternal serum samples, collected during the second trimester of pregnancy, were analyzed using the SPE extraction method. PBDEs were detected in 7 out of 8 samples with ΣBDE ranging from <MDL-95.5 ng/g lw. The BDE congeners most commonly detected were BDE 28/33, 47, 100, 99, 154 and 153 (Figure 1). These congeners were detected in 7 of the 8 serum samples with the exception of BDE 99 which was detected in only 2 samples. BDE 47 was the dominant congener (geometric mean = 22.3 ng/g lw), followed by BDE 99 (9.4 ng/g lw), BDE 153 (7.3 ng/g lw) and BDE 100 (5.5 ng/g lw). The PBDE levels in the current study are similar to those from the same cohort that were collected during the third trimester [3]. This result indicates that the newly developed SPE method is an accurate extraction technique for the analysis of PBDEs from human serum.

OH-BDEs were detected in 6 out of 8 samples at levels that were ~10- to 100-fold lower than the PBDEs. The 5'-OH BDE 99 ranged from 0.10-2.0 ng/g lw (n=4 detections) and the 6'-OH BDE 99 was only detected in one sample at 0.14 ng/g lw. The 4-OH BDE 49 serum levels ranged from 0.02-0.13 ng/g lw (n=4 detections) and the 6-OH BDE 49 was only detected in one sample at 0.02 ng/g lw. A representative chromatogram is shown in Figure 2. The chromatogram also shows the detection of an unknown OH pentaBDE that was not in our calibration standard mixture. Based on the observed MRM transition, this compound is likely meta-substituted. Future efforts will expand the analyte set to identify this compound. The 4'OH BDE 49 was identified based on the diagnostic 500.7 >265.9 transition, indicating a para-OH tetraBDE, and the retention time. Our chromatographic gradient program was capable of resolving the 13C-6-OH BDE 47 and 4'-OH BDE 49.

HBCDs were not detected in any serum sample. Considering the mass of serum extracted (~4 ml), the MDL for the HBCDs is 0.5 ng/g lw. Similarly, TBB and TBPH were not detected in any sample.

Conclusions

An extraction method for BFRs and metabolites was developed using SPE techniques. The developed extraction technique was applied to 8 maternal serum samples. PBDEs were observed in nearly all samples and several OH-BDEs were also detected. Future research will also analyze BFRs in paired serum and placental samples from a large cohort in Durham, North Carolina. In addition, the association between BFRs in the maternal serum and the deiodinase activity of the placenta will be investigated.

Acknowledgements

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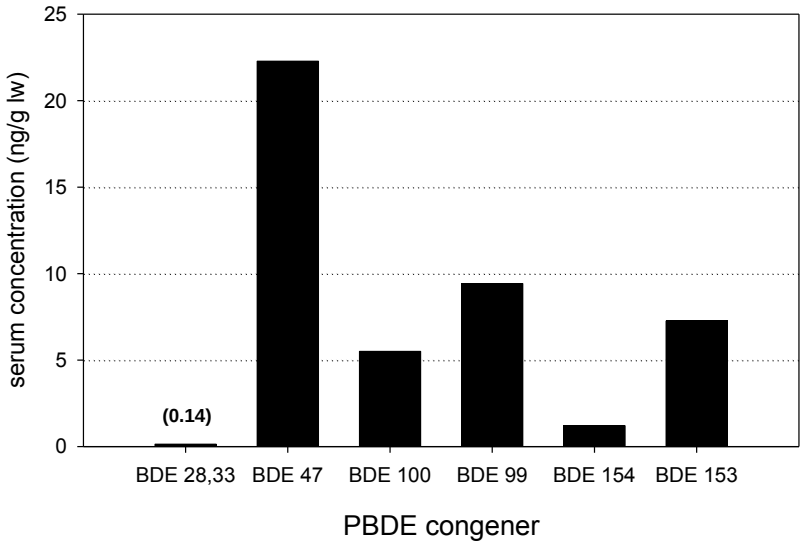

Figure 1. Geometric mean (ng/g lw) PBDE concentration in maternal serum (n=8).

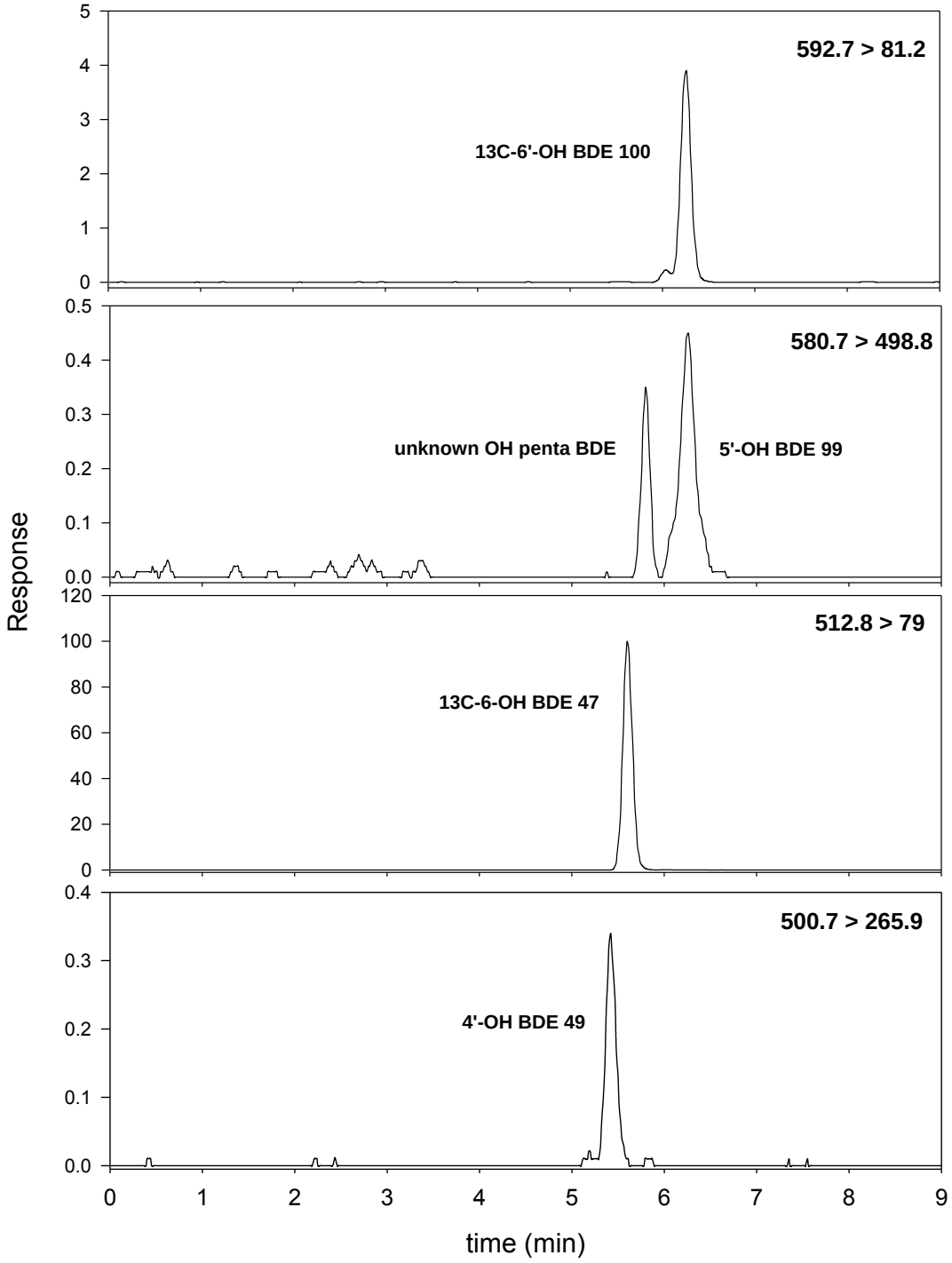

Figure 2. MRM transitions for OH-BDES from a representative serum sample.