DEVELOPMENT OF METHODS FOR THE ANALYSIS OF PBDES IN HUMAN SERUM REFERENCE MATERIALS AND WILDLIFE BLOOD SAMPLES

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Introduction

Polybrominated diphenyl ethers (PBDEs) have been identified as nearly ubiquitous contaminants in environmental samples including human serum and in many wildlife species. Methods for the extraction and cleanup of solid and tissue samples for PBDE analysis are generally similar to organochlorine compounds such as the polychlorinated biphenyls. However, serum and plasma samples have proved to be more challenging due to the high protein content and low PBDE levels characteristic of this matrix. Most methods for extraction of plasma and serum involve liquid/liquid extraction (LLE) or solid phase extraction (SPE); however, as will be shown, these techniques may suffer from poor equilibration of matched labeled PBDEs with native PBDEs. This paper evaluates several extraction alternatives including microwave assisted extraction (MAE), open focused microwave extraction (OFM), and pressurized fluid extraction (PFE). In addition, fluorinated PBDEs[1] are also evaluated for use as internal standards in analysis by gas chromatography mass spectrometry with negative chemical ionization (GC/NCI-MS) and gas chromatography inductively coupled plasma mass spectrometry (GC/ICP-MS) as matched ^{13}C analogs are generally unsuitable for these techniques. GC/ICP-MS has been shown to be a potentially useful analytical technique for the determination of PBDEs by Vonderheide et al.[2]

Materials and Methods

Standard Reference Material (SRM) 1589a Human Serum was used for initial method development. This material has been value assigned for several PBDEs including PBDEs 47, 99, 100, 153 and 154. Aliquots SRM 1589a were prepared according to the certificate instructions and split into 2-5 mL aliquots depending on method; 2 mL for OFM and SPE and 5 mL for MAE, LL, and PFE. A recovery solution containing ^{13}C PCB 28, 52, 118, 180, 206, PBDEs 47, 99, 209 and deuterated DDT and related compounds was added to the samples. This solution was allowed to equilibrate (generally 2 hours) with the serum. Prior to extraction, an equal volume of formic acid was added to the serum mixture. An additional 2 mL of water was added to the serum for SPE following the method of Sandau et al.[3] Acetonitrile as also evaluated as a denaturant for one set of analyses by PFE. Extraction details are listed in Table 1. Extracts were reduced in volume with pressured nitrogen (Turbovap, Caliper Life Science), the solvent exchanged to hexane then passed through a 34 mm bed height alumina column with 9 mL of 35% dichloromethane in hexane (V:V). The extract was reduced in volume then passed through a 30 cm x 7.5 mm ID; 10 µm particle size, 100 Å pore size size exclusion column (Polymer Laboratories Inc.) using dichloromethane as the mobile phase at 1 mL/minute. Prior to analysis, samples were spiked with a solution containing ^{13}C-PCB 47, ^{13}C-PCB 155, ^{13}C-dieldrin and ^{13}C-hexachlorobenzene in order to track the recovery of the ^{13}C and deuterated compounds listed above.

Samples were analyzed by GC/electron ionization (EI)-MS the for the native and labeled organohalogen compounds. Twenty microliters of each extract were introduced into the GC/MS using a PTV inlet in the solvent vent mode cooled to 10 ^{o}C then ballistically heated to 250 ^{o}C. The column used was a 60 m x 0.25 mm x 0.25 µm 5% phenyl methylpolysiloxane column (DB-5ms, Agilent Technologies). Data were acquired in the selected ion monitoring mode.

Table 1: Details on methods used for the extraction of SRM 1589a Human Serum.

Technique	Instrumentation	Solvent System	Conditions
LLE	--	10-20 mL 1:4 dichloromethane:hexane (V:V); repeat three times	Rotate extraction tubes for 10 min each time, vortex then transfer organic phase
SPE	Rapid Trace (Caliper Life Sciences)	3 mL rinse solution (0.1 M HCH in methanol) followed by 12 mL of dichloromethane	Oasis HLB (Waters) loaded with samples, dried with N_2 prior to elution
MAE	Mars Xpress (CEM Corporation)	25 mL 1:4 (V:V) dichloromethane: hexane; repeat three times	Ramp to 90 °C; hold for 10 min; transfer organic phase
OFM	Discover (CEM Corporation)	3 mL 1:4 (V:V) dichloromethane: hexane; repeat three times	Ramp to 90 °C; hold for 3 min
PFE	ASE (Dionex Corporation)	1:3 acetone:dichloromethane (V:V)	100 °C; 13.8 MPa; three cycles of 5-10 minutes each

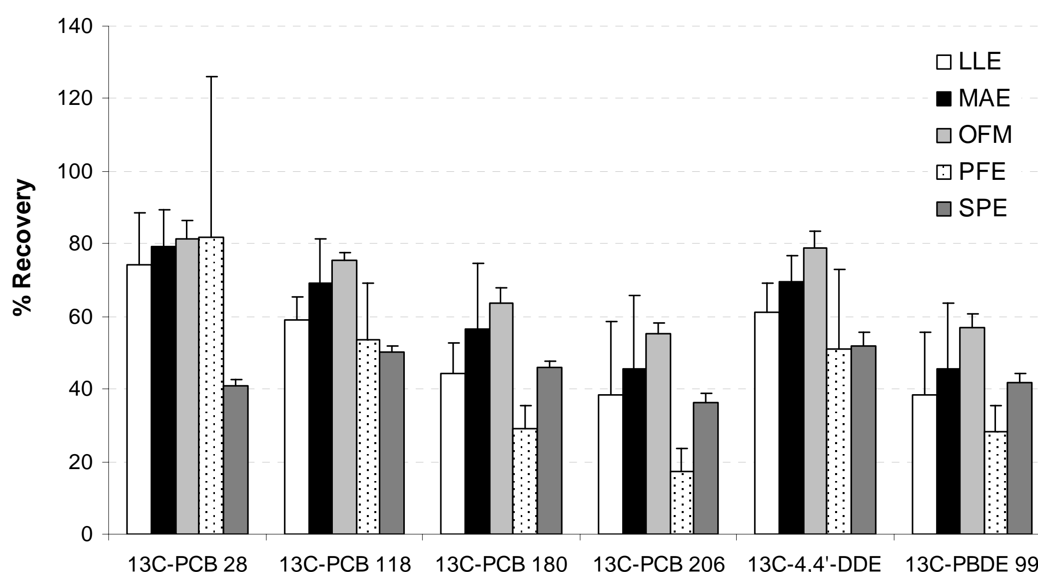

Figure 1: Recovery of labeled compounds from SRM 1589a Human Serum.

Aliquots of SRM 1589a and bottlenose dolphin plasma were also extracted using the OFM method then analyzed by GC/NCI-MS using on-column sample introduction (2 µL sample volume). The column used was a 15 m x 0.25 mm x 0.25 µm DB-5 ms column. Data were acquired in the selected ion monitoring mode using m/z 79/81. For this set of analyses, 6-F PBDE 47, 4'-F PBDE 208, 4'-F PBDE 160, and PBDE 104 were used as internal standards. Extracts were also analyzed by GC/ICP-MS. Samples were introduced in the pulsed splitless mode onto the same column (15 m DB-5ms) listed above.

Results and Discussion

The five methods all differed in their ability to recover the labeled compounds amended at the beginning of each extraction (Figure 1). Generally, recovery declined with degree of halogenation with the highest recoveries observed for the OFM extracted samples. The value of an individual PBDE calculated using ^{13}C PBDE 99 was dependent on the extraction method. This suggests that certain methods had a better ability to liberate native PBDEs from the matrix than other methods. In other words, the ratio of ^{13}C PBDE 99 to native PBDE was method dependent. A relationship between the calculated concentration of PBDE 47, for example, and the recovery of ^{13}C PBDE 99 was observed for LL, SPE, MAE but not for PFE and OFM. So far, of the methods evaluated, OFM appears to provide the highest recovery and most stable ratio between native and labeled PBDEs extracted.

GC/NCI-MS is a common technique for analysis of PBDEs that is especially useful for the analysis of PBDEs at low levels due to its greater sensitivity than GC/EI-MS. GC/ICP-MS is an alternative to this technique and provides good sensitivity for the reference solution tested (Figure 2). Based on initial comparisons with GC/NCI-MS, GC/ICP-MS is roughly ten-fold more sensitive.

Work continues on the evaluation of methods for extraction of PBDEs from serum. Current evaluations include optimization of PFE for extraction of PBDEs and an evaluation of acetonitrile as a reagent to remove protein from serum prior to PFE. Dolphin plasma, additional aliquots for SRM 1589a, and a sediment reference material will be analyzed by GC/ICP-MS to better evaluate this technique as an alternative to GC/NCI-MS.

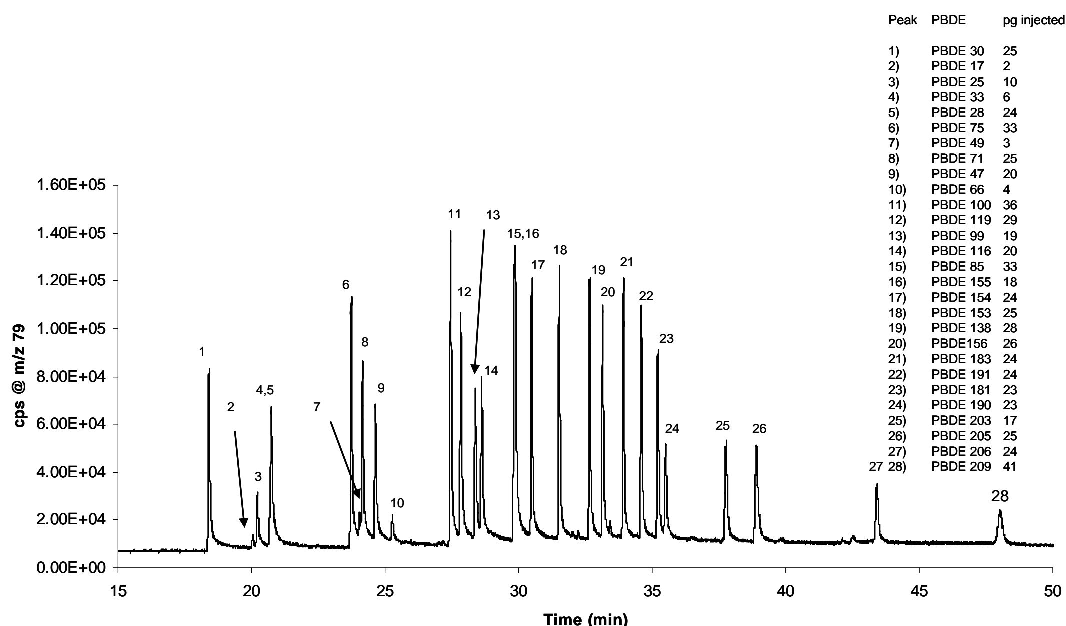

Figure 3: GC/ICP/MS chromatogram of a 28 congener PBDE solution.

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