Sequential extraction of poly- and perfluorinated alkyl acids and brominated flame retardants from biological matrices

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Introduction

Growth in research activities on perfluorinated alkyl acids (PFAs) and bromine-based flame retardants (BFRs) appear to have risen exponentially. The birth of liquid chromatography/mass spectrometry as an analytical tool has been the driving force spurring PFA research endeavours while synthesis of external standards have partially fuelled much of the BFR research.

The dissimilar chemical properties between both classes of compounds suggest that their partitioning in solvents will be different. Extraction methods for BFRs rely on apolar solvents like hexane or dichloromethane. PFAs have been extracted by first forming an ion-pair and then extracting with an apolar solvent like methyl t-butyl ether (MTBE)[1]. More recently however, our group developed an extraction method for PFAs employing methanol as the extracting solvent[2]. We found that methanol was not only an excellent solvent for the extraction of PFAs in biota and but that it also greatly mitigated ion suppression that can sometimes plague the ion pairing method. This latter finding led us to hypothesize that PFAs and BFRs could be co-extracted from biological matrices by simply making use of their different chemical properties.

Our objective in this study was to devise an experimental approach to simultaneously extract BFRs and PFAs in biota using solvents of different polarities. Hexabromocyclododecane (HBCD) and polybrominateddiphenyl ethers (PBDEs) were chosen as representative BFRs while perfluorocarboxylic acids (PFCAs, C_8-C_{12}), perfluorooctanesulfonate (PFOS), perfluorooctanesulfonamide (PFOSA) and unsaturated telomer acids (FTUCAs) were selected for study.

Materials and Methods

Mass labelled ^{13}C-perfluorodecanoic acid (^{13}C-PFDA) and ^{13}C-PFOA, the suite of labelled and native unsaturated fluorotelomer acids (6:2, 8:2 and 10:2 FTUCAs), perfluorooctanesulfonamide (PFOSA), native and labelled (^{13}C and d_{18}) diastereoisomers of hexabromocyclododecane (HBCD) and BDE -71, -126, -156, -197 and -207 were obtained from Wellington Laboratories (Guelph, ON). FTUCAs nomenclature has been described elsewhere[3]. Tetrahydro-PFOS (4H-PFOS), PFOS, PFOA and the analogous suite of carboxylic acids: perfluoro-nonanoic (PFNA), -decanoic (PFDA), -undecanoic (PFUA) and –dodecanoic (PFDoDA) acids were obtained from SynQuest Laboratories (Alachua, FL). Optima grade methanol and water were obtained from Caledon Laboratories (Georgetown, ON).

Samples. A homogenized composite (~ 20 g) of walrus liver (Igloolik, Nunavut), previously analyzed and found to have small concentrations of PFAs and BFRs was prepared using a polytron blender and stored in a 12 mL polypropylene tube.

Spiking solutions. Prior to extractions of walrus liver, a PFA-mixture (PFA-mix) containing native PFCAs (C_8-C_{12}), ^{13}C-PFDA, PFOS, 4H-PFOS, FTUCAs (6:2, 8:2, 10:2), ^{13}C-FTUCAs (6:2 and 10:2) and PFOSA were spiked to tissue samples at a low (20 ng each) and a high dose (100 ng); HBCD-mix containing native and ^{13}C-α, -β, and -γ diastereoisomers were spiked to tissue samples at a low (10 ng each) and high (100 ng each) dose and a PBDE-mix containing BDE-71 (2,3',4',6), -126 (3,3',4,4',5), -156 (2,3,3',4,4',5), -197 (2,2',3,3',4,4',6,6') and -207 (2,2',3,3',4,4',5,6,6') were spiked into tissue at a low (10 ng each) and a high (100 ng each) dose. Tissue sample and blank PFA extracts were spiked prior to LC-injection with constant amounts of ^{13}C-FTUCA (8:2, 50 ng) and ^{13}C-

PFOA (20 ng); HBCD Florisil fraction (*see* below) with d_{18}-HBCD (20 ng each) as labelled instrument performance internal standards.

Sequential extraction method. 0.2 g of homogenized walrus liver was placed into a 12 mL polypropylene tube and spiked with a low (*n=3*) and high (*n=3*) dose of PFA-, HBCD- and PBDE-mix. Three tissue blanks for each dose were spiked with 20 ng of each of the following: 4H-PFOS, ^{13}C-PFDA, ^{13}C-FTUCA (6:2, 8:2 and 10:2), ^{13}C-HBCD (α, β, and γ) and BDE-156 and analyzed concurrently to the tissue spiked samples. The first extraction to capture PFAs was done with Optima grade methanol and took place 24 hrs after spiking. Details of the methanolic extraction procedure can be found elsewhere[3]. Once all the methanol extract was removed, tissue was then spiked with hexane (5 mLs), vortexed (30 seconds), sonicated for 10 minutes and centrifuged at 3,500 *rpm*. The extraction with hexane was repeated. The combined hexane extract (10 mLs) was carefully removed and then reduced in volume and spiked onto a column (300 mm x 10.5 mm i.d.) of deactivated Florisil (1.2% deactivated (w/w), 8 g, 60-100 mesh size, Fisher Scientific). PBDEs were eluted using 40 mL of hexane followed by 30 mL of hexane:DCM (85:15, F1); HBCDs were eluted using 7 mL of hexane:DCM (85:15) followed by 50 mL of hexane:DCM (50:50, F2). F2 was reconstituted in isopropanol and reduced in volume 200 µL; F1 was reduced to 100 µL. F1 was then spiked with aldrin (20 ng), and F2 was spiked with d_{18} α-, β-, and γ-HBCD (20 ng) labelled instrument performance matrix internal standard. HBCD was analyzed by LC/MS/MS and PBDEs by GC-MS. Details of the analysis of PFAs, HBCD and PBDEs can be found elsewhere[4-6].

Results and Discussion

Our first attempt at method development involved using a two phase mixture of methanol and hexane to simultaneously extract PFAs and BFRs. Although it is stated that both solvents are immiscible with each other we found that when added together the volume of the methanol layer (bottom) always increased. This suggested that some hexane was partitioning into the methanol. Adding a saturated solution of sodium chloride only partially recovered some of the hexane out of the methanol. In the end, our recoveries using the biphasic mixture were consistently low for both classes of compounds. Because we were met with limited success, we chose to do our extractions sequentially.

The walrus tissue selected was previously analyzed in our laboratory for PFAs and BFRs. The amount of PFAs [(range 0.06 (PFOA) to 0.9 ng (PFOS)] and HBCD [total HBCD (sum of α, β, γ) was 0.2 ng] in liver was well below the fortified amount in our low dose experiment; the BDE congeners selected for study are not environmentally relevant and were not detected in the sample. Corrections for amounts in the tissue blanks were still applied to the fortified samples. Mean recovery values (*n=3*) for the ionic PFAs and BFRs that were spiked into liver and extracted are presented in Figure 1. Overall, no consistent differences were observed between recoveries and dosing amount for either class of compounds. Recoveries of all the ionic PFAs were greater than 70% in both dosing experiments which is consistent with our earlier findings[2]. Consistent with our earlier findings, mean PFOSA recoveries from both dosing experiments were greater than 95%.

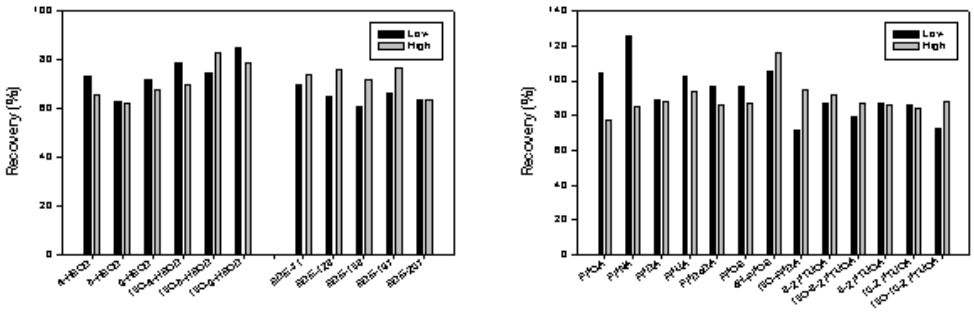

Figure 1. Mean corrected recoveries of PFAs (left panel) and BFRs (righ panel) in walrus liver spiked at a low and high dose and extracted sequential (see text for details).

In general, recoveries of the BFRs were smaller than PFAs but were still greater than 60%. The recoveries of BDE congeners were considered to be acceptable and consistent with BDE congener recoveries reported in the open literature. The HBCD recoveries were slightly smaller than those we have observed with other studies on HBCD in our laboratory. Admittedly, our extraction methods for HBCD in the past have been based on a more aggressive extraction procedure, accelerated solvent extraction, and used a mixture of hexane and

dichloromethane. Nevertheless, our observations in this current study suggest that perhaps some partitioning of HBCD isomers into the methanol extract containing the PFAs had occurred and/or that HBCD is adsorbing to the walls of the polypropylene tube. The latter hypothesis was tested by spiking a 12 mL polypropylene tube with HBCD and analyzing 24 hrs after dosing by rinsing the tube with 10 mL of hexane. More than 95% of HBCD was recovered suggesting that adsorption to the walls of the polypropylene tube is negligible. The former hypothesis of HBCD partitioning into methanol was tested by analyzing the extracts derived from the methanol extraction for HBCD. Less than 2% of ΣHBCD was detected in the methanol extracts (both low and high) strongly suggesting that partitioning of HBCD to methanol was negligible.

Effect of the matrix extract on ion signal suppression was assessed by spiking extracts with the IPIS-mix prior to LC-injection and comparing the signal in the extract to that of an external standard solution prepared in methanol and injected in the same manner. Small signal enhancements and suppression were observed for ^{13}C-PFOA and ^{13}C-FTUCAs, respectively, which is consistent with our earlier observations. No differences between the mean d_{18}-HBCD (*n=6*, low and high dose treated similarly) responses in the sample to those from an external standard solution were observed for either three d_{18}-isomer strongly suggesting that signal attenuation is absent.

In summary, the sequential extraction method gives excellent recoveries for PFAs (>70 %) and greater than 60 % recoveries of BFRs. Work is ongoing in our laboratory to improve upon recoveries of HBCD (and to a lesser extent on PBDEs) by using different solvent mixtures. Regardless, the work presented here represents an important first step in streamlining the extraction of PFAs and BFRs from biota.

References

1. Hansen, Kris J; Clemen, Lisa A; Ellefson, Mark E; Johnson, Harold O *Environ.Sci.Technol.* **2001,** *35*, 766-70.

2. Tomy, Gregg T; Halldorson, Thor; Tittlemier, S.A. *Anal. Chem*. **2005**, in preparation

3. Dinglasan, Mary Joyce A; Ye, Yun; Edwards, Elizabeth A; Mabury, Scott A *Environ.Sci.Technol.* **2004,** *38*, 2857-64.

4. Tomy, Gregg T; Budakowski, Wes R; Halldorson, Thor. H. J.; Helm, Paul A; Stern, Gary A; Friesen, Ken J; Pepper, Karen; Tittlemier, Sheryl A; Fisk, Aaron T *Environ.Sci.Technol.* **2004,** *38*, 6475-81.

5. Budakowski, Wes R; Tomy, Gregg T *Rapid Communications in Mass Spectrometry* **2003,** *17*, 1399-404.

6. Tomy, Gregg T; Palace, Vince P; Halldorson, Thor. H. J.; Braekevelt, Eric; Danell, Robert W.; Evans, Bon; Brinkworth, Lyndon; Fisk, Aaron T *Environ.Sci.Technol.* **2004,** *38*, 1496-504.