Methanolic extraction of poly- and perfluorinated alkyl acids from biota

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Introduction

Perfluoroalkyl acids (PFAs) and their chemical precursors are a group of compounds with unique chemical and biological properties that have been detected in virtually every environmental compartment. The anionic species can be sub-divided into carboxylic [*e.g*., perfluorooctanoic acid (PFOA)] and sulfonic acids [*e.g*., perfluorooctane sulfonate (PFOS)].

The extraction of PFAs such as PFOA (and homologues) and PFOS from biota is done by an ion pair method developed by Hansen *et al*.[1] This method was initially developed for the analysis of human blood samples and was adopted for biotic samples without modification. Our studies on PFAs using the ion pair method have revealed that there can be a suppression of the ion intensity for some PFAs[2]. This effect was also found to be matrix dependent. This phenomenon is not unique to these compounds and other analytical methods that rely on LC/MS suffer similar drawbacks[3-6]. If residual lipid material is present in the extract because of co-extraction then a competition for an ion charge in ESI ion source could result in lower ion yields for PFAs.

Our objective in this study was to develop an improved method for the extraction of PFAs, PFOS-precursors and fluorotelomer acids from biotic samples. By way of a series of controlled experiments, we will illustrate the benefits of using methanol to extract these compounds from biota.

Material and Methods

Chemicals. 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), N-ethyl-PFOSA (NEtPFOSA), d_5-ethyl-PFOSA and d_3-methyl-PFOSA were obtained from Wellington Laboratories (Guelph, ON). The nomenclature of the FTUCAs was been described elsewhere[7]. 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 narwhal liver (Pond Inlet, NE Baffin Island), previously analyzed and found to have small concentrations of PFAs was prepared using a polytron blender and stored in a polypropylene tube. Livers of five burbot (*Lotalota*), captured in the Canadian Arctic, were pooled and homogenized in a similar manner. Burbot liver was selected as a matrix to compare the ion-pair (IP) against the methanol method.

Spiking solutions. Prior to extractions of narwhal 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), PFOSA, N-EtPFOSA and d_5-NEtPFOSA were spiked to tissue samples at a low (20 ng each) and high (100 ng each) dose. Tissue samples and blanks were also spiked prior to LC-injection with constant amounts of, ^{13}C-FTUCA (8:2, 50 ng), d_3-NMePFOSA (50 ng) and ^{13}C-PFOA (20 ng) as instrument performance internal standard (IPIS-mix).

Methanolic extractions. 0.1 g of homogenized narwhal liver was placed into a polypropylene tube and spiked with a low (*n=4*) and high (*n=4*) dose of PFA-mix. Two tissue blanks for each dose were spiked with 20 ng of each of the following: 4H-PFOS, ^{13}C-PFDA, 13C-FTUCA (6:2, 8:2 and 10:2), d_5-NEtPFOSA and analyzed concurrently to the

tissue spiked samples. Extractions with Optima grade methanol took place 24 hrs after spiking and were done by adding 2 mL of methanol, vortexing for 1 minute and centrifuging at 3,500 *rpm* for 5 minutes. This was repeated 2 more times and each time the methanol supernatant was removed and combined. The combined extract (6 mL total) was then solvent reduced to 0.5 mL, transferred to a microcentrifuge vial and ultracentrifuged at 13,500 *rpm*. The methanol extract was carefully removed and transferred to a HPLC injection vial. IPIS-mix was added and injections were made by an autosampler. LC/MS/MS analysis and detection has been described elsewhere[2;8].

Ion-pair (IP) versus methanolic extraction. Subsamples (~0.1 g) of homogenized burbot liver (*n=3*) were extracted using the IP method as described by Hansen *et al*. and by the methanol method (*n=3*) described above. 20 ng of 4H-PFOS, ^{13}C-PFDA, 13C-FTUCA (6:2, 8:2 and 10:2) were spiked into tissue samples and into solvent blanks prior to extraction; Optima grade water and methanol were used as blanks for the IP and methanol method, respectively. IPIS-mix was added and injections were made by an autosampler. Final sample volumes were 0.5 mL.

Results and Discussion

Narwhal liver recoveries. Mean recovery values (*n=4*) for the ionic PFAs that were spiked into liver and extracted using the methanol method are presented in Figure 1. The amount of PFAs in the narwhal liver ranged from 0.05 (PFOA) to 2 ng (PFOSA) which was much smaller than the fortified amount in our low dose; nevertheless, corrections for amounts in the tissue blanks were applied to the fortified samples. Recoveries of all the ionic PFAs were greater than 85% in both dosing experiments. For the neutrals, mean recoveries (*n=4*) of PFOSA were significantly greater than N-EtPFOSA and d_5-NEtPFOSA in both dosing experiments. For the high dose, mean recoveries of 99, 43 and 33% were observed for PFOSA, N-EtPFOSA and d_5-NEtPFOSA, respectively. Mean recoveries of the neutral compounds for the low dose were similar to the high dose.

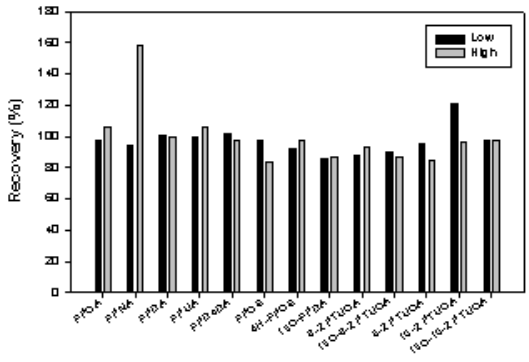

Figure 1. Mean corrected recoveries of PFAs in narwhal liver spiked at low (20 ng) and high (100 ng) dose and extracted using the methanol method (see text for details).

The effect of the matrix of the ion intensity was determined by fortifying extracts with the IPIS prior to LC-injection and comparing the signal in the extract to that of an external standard solution prepared in methanol injected in the same manner. ^{13}C-PFOA was on average (*n=8*, low and high dose treated similarly) 15% higher than the signal from the ^{13}C-PFOA in the external standard solution suggesting a small ion enhancement. ^{13}C-FTUCA (10:2) was an average 5% lower than the signal from the ^{13}C-FTUCA (10:2) in the external standard. d_3-NMePFOSA showed the greatest ion suppression: its signal in the fortified extract was 20% lower than that of the external standard.

Ion-pair (IP) versus methanolic extraction. To date, burbot livers have presented us with the biggest challenge in the analysis of these compounds. Severe matrix related effects have consistently been observed with this matrix when extracted by the IP method. The only PFA that could be detected in these samples using the methanol extraction method was PFOS (mean = 12 ng/g); all PFAs were below detection limits when the IP method was applied. Table 1 presents the average recoveries (*n=3*) of PFAs from burbot liver when both methods were applied.

Table 1. Recoveries of internal standards fortified (20 ng) into burbotliver.[a]

	^{13}C-PFDA	4H-PFOS	^{13}C-FTUCA (6:2)	^{13}C-FTUCA (10:2)
MeOH	66	128	61	64
IP	9[b]	27[b]	62	27

[a] n=3; [b] detected in 1 of 3 samples

Except for ^{13}C-FTUCA (6:2), PFA recoveries using the MeOH method were consistently higher than those found using the IP method. ^{13}C-PFDA and 4H-PFOS recoveries were very small and only were only detectable in 1 of the 3 samples. This latter observation was thought to be indicative of ion suppression. To test that hypothesis, ^{13}C-PFOA and ^{13}C-FTUCA (8:2) were intentionally spiked into the sample extract derived from the methanol and IP extractions and re-injected. The ion signals were then compared to their respective ion signals from an external standard solution prepared in methanol injected in a similar manner. ^{13}C-PFOA signal in the extract derived from the methanolic extraction was 34 % smaller than in the external standard; in the IP extract the ^{13}C-PFOA signal was smaller by 98%. This strongly suggests ion suppression due to the matrix. Diluting the sample to 1 mL resulted in significant reductions in the signal intensity from the methanolic extract (from 34 to 5%) while the IP extract showed a much smaller reduction (98 to 85%). Compared to the external standard, ^{13}C-FTUCA (8:2) in the methanolic extract was only 3% smaller than in the external standard solution while in the IP extract the signal was 53% smaller.

In conclusion, the methanolic extraction method described in this work gives quantitative recoveries of ionic PFAs as well as PFOSA, the dominant neutral PFOS-precursor identified to date in marine mammals. It was demonstrated that in burbot liver, the methanolic method gives better recoveries than the IP method and also significantly less ion suppression.

References

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