METHOD DEVELOPMENT OF ANALYSING PERFLUORINATED PHOSPHONIC ACIDS, POLYFLUOROALKYL PHOSPHORIC ACIDS DIESTERS, PERFLUORINATED CARBOXYLIC ACIDS AND PERFLUORINATED SULPHONIC ACIDS IN FISH SAMPLES

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Introduction

Perfluorocarboxylicacids (PFCAs) and perfluorinated sulfonic acids (PFSAs) have created significant concern because of their toxicity, persistence and worldwide occurrence in the environment and human sera. Recently additional perfluorinated compounds besides the PFCAs and PFSAs have aroused researchers' interest. Polyfluoroalkyl phosphoric acid diesters (diPAPs) and perfluorinated phosphonic acids (PFPAs) are commercial fluorinated surfactants used as leveling and wetting agents in food-contact paper products[1-4] . These chemicals can leach from food packaging into food and have been observed in human serum, Canadian surface waters and wastewater treatment plant effluent[6].

In order to effectively monitor these compounds it is necessary to develop a simple, efficient method to analyze PFCAs, PFSAs, diPAPs, and PFPAs in environmental samples. However, current methods are not able to extract these compounds simultaneously from fish samples because of low extraction efficiency and high relative standard deviation[5]. The purpose of the present study is to develop a method to extract PFCAs, PFSAs, diPAPs and PFPAs from fish samples simultaneously.

Materials and methods

Chemicals:

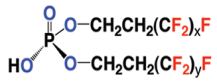
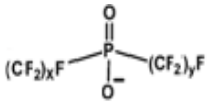
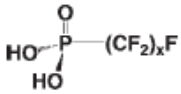
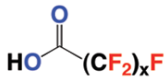
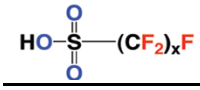
Analytes including C5-C13-PFCAs, C6, C8, C10-PFSAs and PFPAs standards were obtained from Wellington Laboratories (Guelph ON, Canada). The 6:2, 8:2, 10:2 diPAPs were purchased from Chiron (Trondheim, Norway). Table 1 lists the structure, compound abbreviation, and description of the analytes measured by this method.

Extraction procedure:

Fish samples weighing 0.5g were spiked with 1 ng mass-labeled PFCs, and then were homogenized in 2mL of acetonitrile. After centrifugation, the supernatant was transferred to a new polypropylene tube. Another 2 mL of acetonitrile was added to each fish samples. After shaking and centrifugation, the supernatants were combined and then evaporate to dryness under a gentle nitrogen stream. A 1mL aliquot of 0.5M tetrabutyl ammonium hydrogen sulphate (TBAS) was added and pH was adjusted to 4. Two 5 mL aliquots of methyl tert-butyl ether (MTBE) was added. These MTBE aliquots were combined, evaporated to dryness under nitrogen and then

reconstituted in 1 mL methanol.

Table 1 Structure, abbreviation, and description of the congeners monitored for the analytes of interest

Structure	Congeners monitored	Abbreviation	MRM transition
	x=6, 8, 10, y=x	6:2 , 8:2 , 10:2 diPAPs	789>78.9, 989>78.9, 1189>78.9
	x=6,8 y=6, 8	6:6 PFPi, 6:8 PFPi, 8:8 PFPi	701>401, 801>401, 901>501
	x=6, 8, 10	PFHxPA, PFOPA, PFDPA	399>79, 499>79, 599>79
	x=5-13	PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnA, PFDoA, PFTrDA, PFTeA	313>269, 369>319, 413>369, 469>413, 513>469, 569>513, 613>569, 663>613, 713>669
	x=6,8,10	PFHxS, PFOS, PFDS	399>80, 499>80, 599>80

Quality control

PFCAs and PFSAs were quantified using the following mass-labeled internal standards: ^{13}C2-PFOA (>99%), ^{13}C5-PFNA (>99%), ^{13}C2-PFDA (>99%), ^{13}C2-PFUnA (>99%), ^{13}C2-PFDoA (>99%), ^{18}O2-PFHxS (>94%) and ^{13}C4-PFOS (>99%). A matrix-matched standard curve was used for the quantifications of PFPAs and diPAPs in the absence of mass-labeled internal standards.

Results and discussion

DiPAP stability

Usually, in order to obtain good peak shape, the samples were diluted with water or methanol/water to a solvent strength less than the initial mobile phase solvent strength prior to the LC-MS[2]. However, as to diPAPs, it is difficult to obtain linear calibration curve in this solvent mixture and the relative standard deviation (RSD) of instrument response is higher than 50%. DiPAPs standard solution were injected consecutively in 18 hours in different solvent mixtures including ACN/H2O

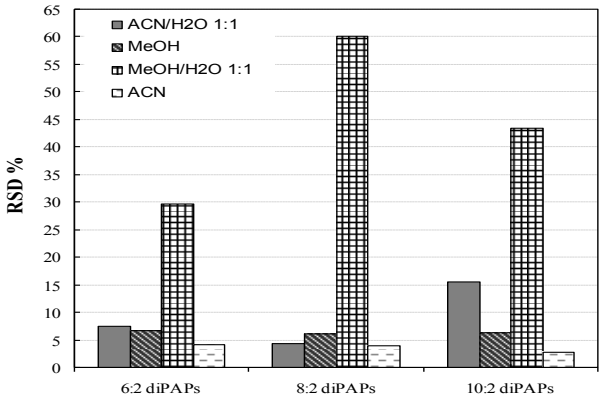


Figure 1 RSD of diPAPs instrument response in solvent

1:1, methanol:water 1:1, pure acetonitrile and pure methanol. Figure 1 showed the RSD of the instrument response in four solvent mixtures. High RSD of instrument response were obtained when diPAPs were solved in methanol:water 1:1, indicating diPAPs are not stable in the solvent mixture. RSD of instrument response was lower than 20% in mixture of acetontrile and water, pure acetonitrile and methanol. Considering the extract was solved in pure methanol, we chose pure methanol as the solvent in the later experiment.

Protein precipitation

Initial results indicated that it is necessary to add a protein precipitant to fish tissue samples as PFHxPA, PFOPA, PFDPA are likely bound to fish proteins. Without using a protein precipitant, the recoveries of PFHxPA, PFOPA, and PFDPA were 40% lower. After adding the protein precipitant, the recoveries of these compounds increased significantly. Acetonitrile was selected since it is widely used as protein precipitant.

TBAS pH optimization

The TBAS pH effect on extraction efficiency was studied as shown in Figure 2. At both pH 3 and pH 4 TBAS can be used to efficiently extract PFPAs from fish samples. However, from pH 5 to pH 9, the recovery of PFPAs decreased dramatically. A value of pH 4 was chosen to extract diPAPs, PFCAs and PFCAs from fish samples. Figure 3 shows the recovery of PFPAs. diPAPs, PFCAs and PFSAs TBAS at pH 4. For PFPAs and diPAPs, the recovery ranged from 91-102% and 69-84%, respectively. For PFCAs and PFSAs, the recovery varied between 71 and 91%.

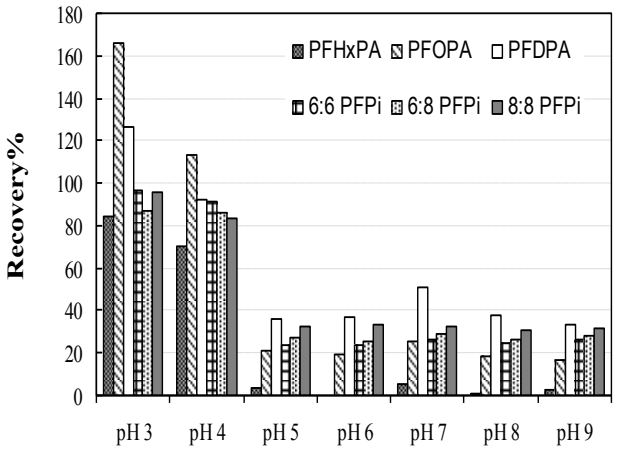


Figure 2 pH effect on recovery of PFPAs

For the analysis of fish sample, two factors affected extraction efficiency. One was the use of a protein precipitant and the other is pH of the TBAS solution. Without protein precipitatation, PFFxPA, PFOPA and PFDPA couldn't be extracted effectively. At neutral pH, we expect the phosphonate moiety of the PFPAs to be dianionic, because the addition of fluorine to an alkyl chain adjacent to an acidic functional group tends to stabilize the negative change, resulting in a significant decrease in pKa[7]. Considering the bulk structure of TBAS, it is difficult to form stable

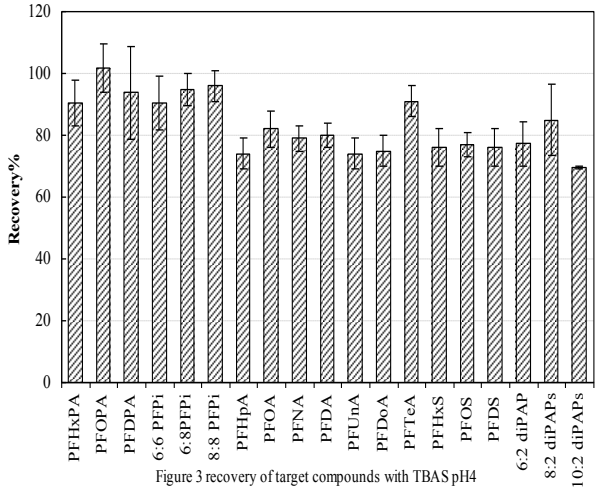


Figure 3 recovery of target compounds with TBAS pH4

di-ion pairs at neutral pH. At low pH, PFPAs tend to be single anionic, which allows them to form an ion pair with TBAS.

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References:

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