Development of extraction and cleanup approaches for PFASs analysis in fish tissue by HPLC-MS/MS technique

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Abstract. The history of PFASs emergence and current status in the context of the Stockholm Convention on POPs, their environmental distribution and toxicity are discussed. Various approaches to PFASs analysis in fish tissues are discussed. An original method of sample preparation is described, which allows quantitative PFASs determination at 0.2 – 100 ppb levels.

1. Introduction

Perfluorinated compounds (PFASs), classified according to the recommended terminology [1], are a large family of technogenic contaminants. The family consist of different compound classes, such as carboxylates, sulfonates, sulfonamides, alcohols and etc., of which carboxylates and sulfonates are most often analysed in laboratories. PFASs started to emerge into the environment in the late 40s as by-products of Teflon development by DuPont. Teflon was approved by FDA for kitchenware coating in 1962, and Zonyl was approved for food packaging in 1967. At present, PFASs are used in textile industry, paper production, as components in various resins, foams, etc. Accordingly, perfluorooctanoic acid (PFOA) (Fig. 1) was first detected in the blood samples of factory personnel in 1978, and in the ground water in 1984. Due to toxicity concerns, some companies stopped producing 8-carbon PFAS since 2000.

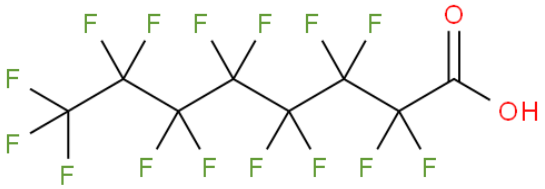

Figure 1. Structure of perfluorooctanoic acid (PFOA).

PFASs are stable contaminants capable of bioaccumulation, they are detected in animal tissues. At present, the worldwide production of PFASs has been reduced due to information about their potential health risks, including risks of cancer promotion. According to the European Union data on PFAS in food collected in 2006 – 2012, perfluoroalkanesulfonates (PFSAs) and perfluoroalkylcarboxylicacids

(PFCAs) are the most widespread food contaminants. PFSAs were mostly found in fish, meat, drinking water and fruits. The highest levels were detected in liver samples.

High levels of PFCAs and PFSAs were found in marine mammals feeding on fish in such industrially developed areas as the Baltic Sea, Mediterranean, the Great Lakes, and along the South East Asia coast, but also in such remote areas as Alaska and the Antarctic (Table 1).

Table 1. Detectable concentration of PFSAs and PFCAs in different objects.

Object	Mean data for PFSAs (Sum, ppb)	Mean data for PFCAs (Sum, ppb)
Fish	230	22.9
Fish liver	540	53
Milk	0.852	0.527
Meat	2.6	0.203
Eggs	0.195	0.008

Recently, perfluorooctanesulfonic acid (PFOS), its salts and perfluorooctanesulfonyl fluoride (PFOS-F) (Fig. 2) were listed in Annex B (SC-4/17) of the Stockholm Convention on Persistent Organic Pollutants (POPs).

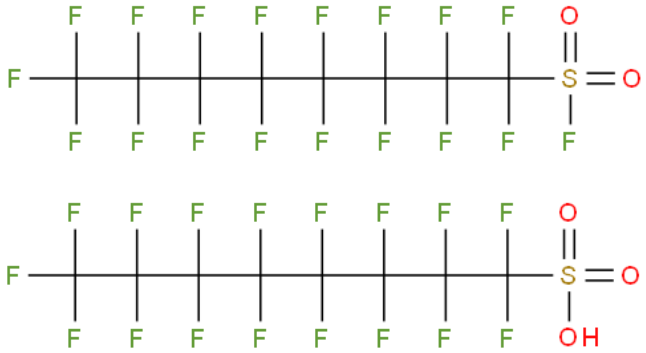

Figure 2. Structure of general PFASs listed in Annex B of Stockholm Convention.

Most common approaches to PFASs extraction and cleanup involve methanol, acetonitrile, and their mixtures with acidic or alkaline modifications followed by SPE cleanup on commercial sorbents. Here we report on the determination of perfluorinated carboxylates and sulfonates in medium-fat fish tissues using an alternative method of lipids removal described below.

2. Materials and Methods

2.1. Chemicals and Standards

All chemicals were of analytical grade and obtained from Sigma-Aldrich, Fluka and Merck companies. The PFASs standards (PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUdA, PFDoA, PFTrDA, PFTeDa, PFHxDA, PFODA, PFBS, PFPeS, PFHxS, PFHpS, PFOS, PFNS, PFDS, PFDoS) and labeled standards were obtained from Wellington Laboratories. Blank fish samples were obtained from a local commercial source. Oasis WAX cartridges (3 cc, 60 mg) were obtained from Waters. The Reacti-Therm (heating and stirring module, Thermo) was used for concentration step.

2.2. LC-MS-MS Conditions

HPLC separation was carried out in gradient mode on ACQUITY BEH Shield RP 18 column, 1.7 µm 2.1*50 mm (Waters). Mobile phases were: “A” – 2 mM ammonium acetate in deionized water, “B” 2

mM ammonium acetate in methanol. Separation program for HPLC at 45 0C and 0.55 µl/min was as follows: 1 minute 10 % of "A", from 1 to 6.5 minutes to 50 % of "B", then, up to 100 % "B" to 18 minutes. After 1 minute at 100 % "B", equilibration at 10 % of phase "B" till 25 minutes.

Detection was carried out on Q-TOF systems: Maxis (Bruker) and Xevo G2 (Waters) in negative ionization mode with fragment ions registration. The mass-spectrometer was adjusted for best intensity. For Xevo G2, leucine enkephalin in concentration 2 ng/µl was used in each run for mass accuracy achievement. Temperature in source and capillary voltage were set on 120 0C and 2.7 kV respectively; sampling cone, extraction cone, LM and HM resolutions parameters were set on 40, 3.0, 15, 15 respectively. Cone gas was set at 30 l/h, desolvation gas at 750 l/h. Temperature of desolvation gas was 500 0C. Collision energy were in gradient mode (10-35 eV). RT and MS/MS parameters listed in table 2. Working solutions for routine analysis were prepared in methanol and stored at -20 0C.

Table 2. Retention times and accurate masses of the analytes.

Name	RT (min.)	Molecular ion (m/z)	Product ion (m/z)
PFPeA	6.54	262.975	219.1
PFHxA	6.32	312.972	268.9
PFHpA	6.89	362.969	319.0
PFOA	7.32	412.965	369.0
PFNA	7.67	462.962	419.0
PFDA	7.95	512.959	469.0
PFUdA	8.20	562.956	519.0
PFDoA	8.41	612.953	569.0
PFTrDA	8.59	662.949	619.0
PFTeDa	8.76	712.946	669.0
PFHxDA	9.02	812.940	796.8
PFODA	9.25	912.933	868.8
PFBS	5.71	298.942	98.9
PFPeS	6.43	348.939	79.9
PFHxS	6.93	398.936	99.0
PFHpS	6.75	448.932	99.0
PFOS	7.68	498.929	99.0
PFNS	7.95	548.926	98.9
PFDS	8.19	598.923	99.0
PFDoS	8.58	698.916	99.0

2.3. Sample Preparation

Samples were homogenized and stored at -200C. To 1 g of a fish sample in a centrifuge polypropylene tube were added IS and Std solutions (in case of calibration samples). 5 ml of acetonitrile were added to the sample and the tube was shaken for 30 minutes followed by ultrasonic bath (10 min) and centrifugation at 4750 rpm for 20 min. The first cleanup stage was performed by passing the extract through 2 g of neutral alumina oxide, packed inside of 5 cc syringe equipped with removable Nylon 0.22 µm filter in to a new tube. Clean extract was mixed on vortex and concentrated to 1 ml in a stream of nitrogen at 40 0C. Water was added up to 3.5 ml followed by centrifugation at 4750 rpm.

The second cleanup stage by SPE was done by the following scheme. Activation of sorbent was performed with 2 ml of methanol and 2 ml of deionized water. Sample extract was loaded on the sorbent bed, and the cartridge was rinsed with 2 ml of 1% formic acid in deionized water and vacuum dried. Analytes were eluted to a new tube from sorbent with addition of 3 ml of 1 % ammonium hydroxide in methanol. Cleaned extract was mixed and concentrated till 0.5 ml by nitrogen stream at 40°C. 0.5 ml of deionised water was added in to concentrated extract followed by mixing and filtration on Nylon 0.22 µm filter in to HPLC vial.

3. Results and Discussion

Several approaches to extraction and cleanup were tested: extraction with methanol, 50% of methanol in deionized water, acetonitrile and their formulations with acidic and basic additives, liquid-liquid cleanup with hexane and heptane, 10-fold dilution and alumina oxide. It was found that usage of acetonitrile as the extraction solvent was most optimal in our conditions for all analytes described above. This allowed to obtain quite clean samples on a last stage in the HPLC vial and decrease system pressure while gradient elution. Usage of neutral alumina oxide decreased matrix effects in the ion source of the mass-spectrometer and increased recovery of the analytes by 5-10 % compared with ordinary approach.

Using this method, we achieved 0.2 ppb LOQ in the 0.2 to 100 ppb range. PFASs recoveries ranged from 51 % (PFBS) to 108 %. Specificity of the method was confirmed by analysis of 20 blanks where no interferences were observed. Correlation coefficients of calibration curves were greater than 0.98 during validation experiments. Stability of the analytes was confirmed for overnight storage. Maximum and minimum RSD values were 31 and 11 % respectively. Robustness was confirmed during validation procedure with variation of the following factors: matrix (type of fish), analysts (at sample preparation stage), and storage time (injection on the same day or after overnight storage).

4. Conclusion

HPLC-MS is the most optimal analytical method for PFASs determination in complex matrices, such as animal tissues and environmental samples. However, HPLC-MS results are sensitive to matrix effects which may affect detection limits. We have developed a new approach for sample preparation with additional cleanup stage and optimal solvent scheme for HPLC separation. The use of Q-TOF MS detection increased the analytical quality of the results up to 4,5 identification points (according to 2002/657/EC) in comparison with LR-MS. The method was developed using atlantic cod, but gave satisfactory results with other fish matrices as well.

References

[1] Buck RC, Franklin J, Berger U, et al. Perfluoroalkyl and polyfluoroalkyl substances in the environment: terminology, classification, and origins. *Integr Environ Assess Manag*. 2011;**7(4)**:513-41.