SAMPLE CLEAN-UP AND LC-MSMS METHOD DEVELOPMENT FOR POLYFLUOROALKYL PHOSPHATE MONO-, DI-, AND TRI-ESTERS

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Introduction

Fluorotelomer alcohol (FTOH) based polyfluoroalkyl phosphate esters (PAPs) are chemicals that are used in products such as water and grease proofing of food packaging materials made of paper or board. Several phosphate di-esters (diPAPs) and tri-esters (triPAPs) with various FTOH chain lengths have been identified in microwave popcorn bags[1]. In human serum from the USA, diPAPs with variable chain lengths were identified, and concentrations were in the same range as perfluorocarboxylates (PFCAs), while in wastewater treatment plant sludge concentrations of individual diPAPs were comparable to perfluorooctane sulfonate (PFOS) concentrations[2]. The presence of PAPs in the environment is of concern as these chemicals can be degraded to the persistent PFCAs[3,4].

Currently used extraction methods for PAPs are based on liquid extraction (rat blood, urine, and feces) or an ion-pairing method was used (sludge and human serum)[2,3]. No further clean up of the extracts was performed prior to LC-MSMS analysis. In order to analyze PAPs in a variety of environmental matrices, such as water, sediments, fish homogenates or liver samples, an additional clean up step is desirable in order to remove matrix compounds that could interfere with the LC-MSMS analysis.

The aim of this study was to develop an extraction and clean up method in order to determine mono, di, and triPAPs of various chain lengths in environmental samples with complex matrices.

Materials and methods

The 6:2 and 8:2 mono and 6:2 and 8:2 diPAPs were purchased from Wellington Laboratories, 10:2 monoPAP, 10:2 diPAP and 6:2 triPAP were purchased from Chiron. A technical grade PAP mixture (Zonyl-RP®) was kindly donated by Dr Jutta Tentschert (Federal Institute for Risk Assessment (BfR), Berlin, Germany).

Fish homogenate samples were extracted twice using solid-liquid extraction with acetonitrile. Tap water samples were directly extracted on the clean up column. The clean up using Waters OASIS weak anion exchange (WAX) columns was based on Chu and Letcher[5] and Gebbink and Letcher[6]. Briefly, the WAX columns were conditioned with methanol and water after which the water sample or fish sample extract was loaded. The column was washed with 1 mL 2% formic acid in water and 2 mL water. The column was eluted with 2 mL methanol (Fraction 1) and 2 mL 1% NH_4OH solution in methanol (Fraction 2).

The separation of the target compounds was carried out using an Acquity UPLC system (Waters) with a BEH C18 (1.7 µm particles, 50 × 2.1 mm) analytical column. The mobile phase consisted of solvent (A) 95% water and 5% acetonitrile and solvent (B) 75% methanol, 20% acetonitrile and 5% water. Both mobile phases contained 2 mM ammonium acetate and 5 mM 1-methyl piperidine (1-MP). A gradient elution with a flow rate of 0.3 mL/min was applied. Initial conditions were 90% A and the percentage of B increased linearly to 100% during 5 min and was kept at 100% B for another 2 min. The solvent composition reached again initial conditions at 95% A at 7.5 min and equilibration of the column was achieved at 11 min. An injection volume of 5 µL in the partial loop injection mode was used. Coupled to the UPLC was a Xevo TQ-S triple quadrupole mass spectrometer (Waters), which was operated in negative electrospray ionization (ESI^-) mode. Data acquisition was performed using multiple reaction monitoring (MRM). The capillary voltage was set at 3.0 kV, the source and desolvation temperatures were 150°C and 350°C, respectively, and the desolvation and the cone gas flows

were 650 and 150 L/h, respectively. Table 1 lists the optimized cone voltages and collision energies for the MRM transition channels for three mono and three diPAPs.

Table 1. Optimized cone voltages and collision energies for three mono and three diPAPs

Compound name	MRM transition	Cone Voltage (V)	Collision Energy (eV)
6:2 monoPAP	443.08 > 96.93	10	18
8:2 monoPAP	543.08 > 96.93	10	24
10:2 monoPAP	643.08 > 96.92	14	20
6:2 diPAP	789.03 > 96.99	50	34
8:2 diPAP	989.03 > 96.99	50	36
10:2 diPAP	1189.10 > 96.99	50	34

Results and discussion

Using the six authentic standards (6:2, 8:2, and 10:2 mono and 6:2, 8:2, and 10:2 diPAPs) chromatographic conditions were optimized for resolution and signal shape. The use of 1-MP in the mobile phase was found to improve chromatographic resolution considerably, especially for the di-anionic monoPAPs[7]. ESI^- MSMS parameters were optimized for sensitivity and are given in the Materials and methods section and in Table 1.

A solution of Zonyl-RP® was analyzed under the optimized chromatographic and MSMS conditions. Cone voltages and collision energies optimized for the six mono and diPAPs (Table 1) were also used for other chain lengths mono and diPAPs. Along with the three mono and three diPAPs, several other diPAPs were identified in Zonyl-RP®, many of which coeluted under the applied chromatographic conditions (Figure 1). The PO_4^- (*m/z* 97) product ion was the most abundant fragment for all PAPs. However, using this product ion in the MRM transition channels does not allow to distinguish between the different chain lengths diPAPs with the same molecular mass that are coeluting.

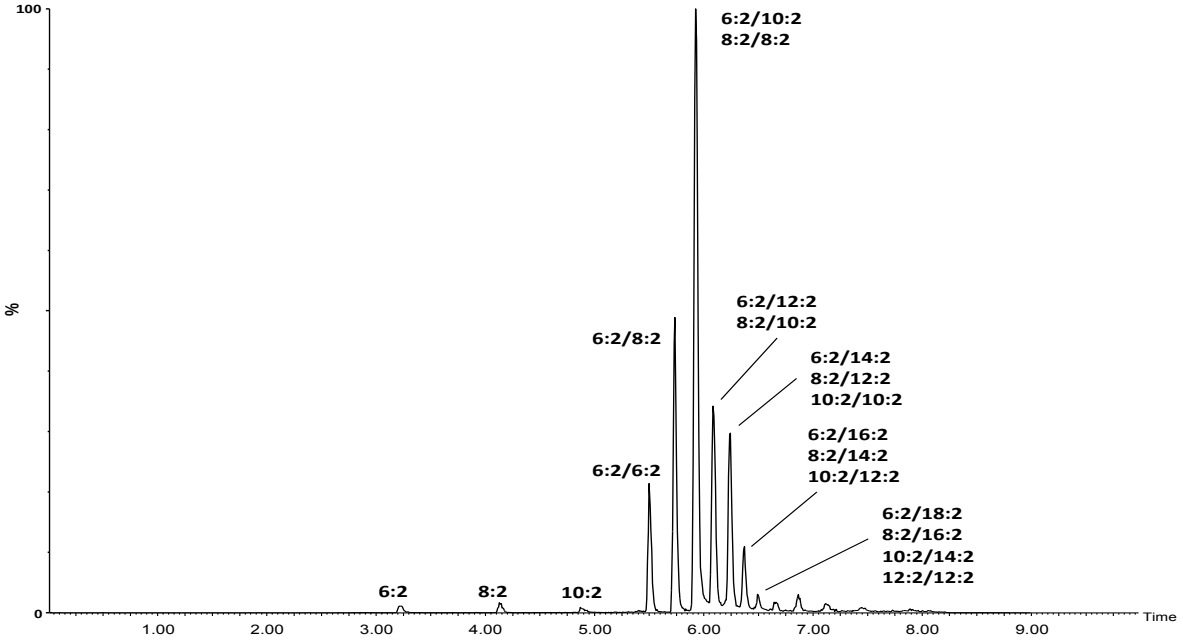

Figure 1. Identified mono and diPAPs in the technical PAP mixture Zonyl-RP®. The chromatogram shows the molecular ion to PO_4^- product ion MRM channels. However, coeluting diPAPs were identified in the more specific molecular ion to monoPAPs product ion MRM channels.

Using the monoPAP fragment on top of the PO_4^- fragment allowed for the identification of the coeluting diPAPs in this technical mixture. The Zonyl-RP® contained small amounts of triPAPs, which were analyzed using the diPAPs MRM channels after in-source fragmentation[1]. Based on the retention time of the authentic standard, 6:2 triPAP was identified in Zonyl-RP®. Further possible triPAP signals could only be identified tentatively, due to lack of authentic standards.

Repetitive standard injections of mono and diPAPs in different solvent/water compositions (25/75, 50/50, 75/25, and 100/0 of methanol or acetonitrile/water) gave the highest signals for standard solutions in 100% methanol for the mono and diPAPs. Instrumental detection limits (LOD) and quantitation limits (LOQ) were calculated for three mono and three diPAPs using standard solution in 100% methanol (Table 2). LODs and LOQs for the monoPAPs were in the sub pg/µL range, whereas the LOD and LOQ for the diPAPs were in the low fg/µL range. The method limit of quantitation (MLOQ) was calculated for the three mono and three diPAPs using a spiked fish homogenate as a matrix that underwent the extraction and clean up procedure. The MLOQ for the three mono and the three diPAPs ranged from 5 to 130 pg/g.

Table 2 Limits of detection (LOD), limits of quantitation (LOQ) and method limits of quantitation (MLOQ) for three mono and three diPAPs

Compound name	LOD (S/N 3, pg/µL)	LOQ (S/N 10, pg/µL)	MLOQ (S/N 10, pg/g)
6:2 monoPAP	0.02	0.05	64.9
8:2 monoPAP	0.11	0.36	28.5
10:2 monoPAP	0.16	0.54	132
6:2 diPAP	0.001	0.003	9.3
8:2 diPAP	0.002	0.005	4.7
10:2 diPAP	0.007	0.025	41.0

Using the methodology published by Chu and Letcher[5] for the clean up and isolation of perfluoroalkyl acids and neutral fluorinated compounds from environmental matrices, the mono, di, and triPAPs were enriched on and eluted from the WAX column. The 2 mL methanol (Fraction 1) eluted the neutral 6:2/6:2/6:2 triPAP, and the 2 mL 1% NH_4OH in methanol (Fraction 2) eluted the six anionic mono and diPAPs. Fraction 2 contained no 6:2/6:2/6:2 triPAP, indicating it was all eluted in Fraction 1. Additional elution with 1 mL 1% NH_4OH in methanol only contained <2.5% of the recovered mono and diPAPs. The total recoveries of the mono, di, and triPAPs in the clean up step ranged from 66% to 110%.

The extraction and clean up methodology was tested by spiking fish homogenate and tap water samples with a mix of native 6:2, 8:2, and 10:2 mono and 6:2/6:2, 8:2/8:2, and 10:2/10:2 diPAP standards, or with Zonyl-RP®. Recoveries of the six mono and diPAP native standards from spiked fish calculated versus a standard solution in methanol ranged from 68% to 331%. However, recoveries calculated versus a standard in a matrix matched solution were between 53% and 92%. Comparable results were obtained for the fish homogenate spiked with Zonyl-RP® (Figure 2.). Similar results were also seen when tap water was spiked with the mix of six mono and diPAPs or Zonyl-RP® and the sample was loaded onto the WAX column and eluted. Recoveries calculated using matrix matched standards instead of using solvents in pure solvent ranged from 45% to 98% for the mix of six mono and diPAPs instead of 76% to 180%. Repetitive injections of PAP standard solutions in pure solvents showed declines in signal areas, likely due to increased sorption of the PAPs to the walls of the vials over time. This was observed using glass, polyethylene, and polypropylene HPLC vials. Co-extracted matrix constituents are assumed to (partly) mitigate this sorption effect. Using standards in pure solvents is therefore problematic due to non-repeatability of the observed response and due to overestimation of the recoveries of PAPs in sample extracts containing matrix constituents. Using extracted and matrix matched standards in quantification is a possibility to correct for recovery losses and sorption effects of PAPs that lack stable isotope labeled analogues as internal standards.

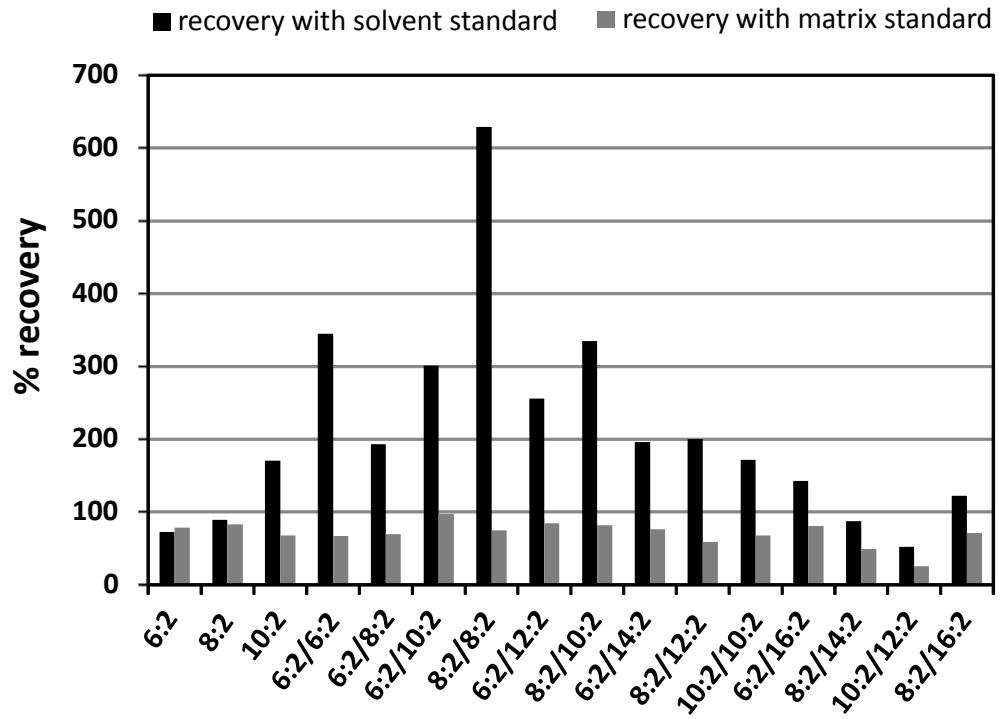

Figure 2. Recoveries of mono and diPAPs extracted from a fish homogenate matrix spiked with Zonyl-RP®. Recoveries were calculated relative to a Zonyl-RP® standard in pure methanol solution and in a matrix matched solution.

In summary, LC-MSMS parameters were optimized using authentic standards for mono and diPAPs. The developed methodology allowed for the identification of several other PAPs in Zonyl-RP®. The usability of WAX columns for additional clean up and isolation of the PAPs from different matrices was demonstrated with MLOQs being in the ~5-130 pg/g range. However, in order to accurately quantify mono and diPAPs in environmental samples, stable isotope labeled analogues as internal standards or extracted and matrix matched calibration standards are essential.

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