Analysis of sub-ppb levels of perfluorooctanoic acid (PFOA) and perfluorooctanesulfonate (PFOS) in food and fish

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Abstract
The exposure of humans to perfluorinated compounds (PFC) and especially to perfluorooctanoic acid (PFOA) and perfluorooctanesulfonate (PFOS) is complex. However, there are indications, that food may play a mayor role for the non-occupationally exposed population.
Data on PFC in food is limited and do not build a base for a reliable daily intake estimation of the general population. Therefore, the authors developed an analytical method for the determination of PFOS, PFOA and related compounds in diet duplicate samples, collected in a recent diet duplicate study (INES) conducted in Bavaria, Germany. The method was intended also for single food items as well as whole fish and fish tissues.
The developed method comprises of a pressurized liquid or ultrasonic extraction, respectively, followed by a clean-up on a weak anion exchange solid phase extraction cartridge and subsequent identification and quantification by HPLC-ESI-MS/MS. It proved to be robust and sensitive (LODs of 0.05-0.1 ppb), produced good recovery rates (72-108%) and was applicable to a wide range of food and fish samples. Typical concentration ranges are given and it is demonstrated that the method allowed the separation of PFOS from a severe co-eluting interference, taurodeoxycholate.

Introduction
During the last decade perfluorinated compounds (PFC) including perfluorooctanoic acid (PFOA) and perfluorooctanesulfonate (PFOS) have been identified in huge amount of environmental and wildlife samples throughout the world.[1, 2] Furthermore, these substances have been detected in human blood and tissue samples from occupationally but also from non-occupationally exposed human populations worldwide.[3, 4]

Widespread application and distribution of PFC as well as their degradation and metabolism to PFOS and PFOA lead to a complex exposure situation to humans. Contribution of single sources and pathways to the total exposure is currently not well understood. However, as apparent from a recently published Canadian study, food is considered as a mayor source of the non-occupationally exposed population.[5]

With respect to chemical analysis of PFOS and PFOA in complex matrices two different extraction approaches were reported in the literature. Hansen et al.[6] describe an ion-pair extraction into non-polar solvents, whereas Taniyasu et al. recommend a more polar extraction with methanol followed by a clean-up on solid phase extraction (SPE) columns[7]. However, both methods report limits of detection (LOD) in the low ppb range. The recent Canadian food study applied a methanol extraction without SPE cleanup and reached LODs of 0.5 to 1 ng/g wet weight.[5]

The aim of this study was to develop a robust analytical method for the analysis of perfluorinated carboxylates and perfluorinated sulfonates for different kinds of food samples including fish muscle and fish liver. Due to the low concentrations of these substances in food[6] it was a key target to reduce the LOD to sub-ppb levels.

Materials and Methods
Samples: Frozen whole fish samples were delivered from contaminated and not contaminated sites in Germany. Samples of muscle tissue and liver were dissected from the animal. For method performance tests, whole fish samples were prepared by homogenization in a blender.

Food samples were taken from the Bavarian Integrated Exposure Assessment Study (INES) conducted in the southern part of Germany in 2005.[8] Participants of this study collected daily diet duplicate samples over 7 consecutive days. Solid and liquid foods were collected separately and delivered to the laboratory, where both

samples were blended and homogenized. A 30 g aliquot of the homogenized sample was filled in a PP tube and kept frozen until analysis. For method application tests single food items (potatoes and cereals) were subjected to the method. To study the recovery, a diet duplicate sample, which was shown not to contain detectable levels of PFC, was spiked to concentration levels between 0.5 and 50 ng/g fresh weights before analysis.

Chemicals and Materials: PFOS (as tetra ethyl ammonium salt, molecular weight 629,38 g/mol; 98% purity), PFOA (MW 414.17; > 96%), perfluorohexanesulfonate (PFHxS, MW 422.10; >98%), and perfluorohexanoic acid (PFHxA, MW 314,04, > 98) were purchased from Fluka Chemie AG (Buchs, Switzerland), Sigma-Aldrich (Schnelldorf, Germany), and ABCR GmbH& Co. (Karlsruhe, Germany), respectively. The internal standards, $^{13}C_4$-PFOA and $^{13}C_4$-PFOS, were purchased from Wellington (Ontario, Canada), whereas $^{13}C_4$-PFOA were used for quantification of PFOA and PFHxA and $^{13}C_4$-PFOS for PFOS and PFHxS.

Other reagents and solvents, i.e. methanol, water, ammonia hydroxide (25% in water), ammonia acetate, glacial acetic acid and tert. butyl methyl ether, were of HPLC grade and were purchased from Fluka and Merck (Darmstadt, Germany). 50 ml PP centrifuge tubes and 0.8 µm syringe filters (CME) were obtained from Roth (Karlsruhe, Germany). Weak anion exchange SPE cartridges (Oasis® Wax, 150 mg, 6 ml, 30 µm) were delivered by Waters (Eschborn, Germany).

Sample preparation: Fish samples (homogenized whole fish, liver and muscle tissue), potatoes and cereals were extracted by accelerated solvent extraction (ASE 200, Dionex). Wet samples were mixed with silica, spiked with both internal standards and filled into an ASE cartridge. Extraction was performed with methanol/water (1/1;v/v) at 100°C and 100 bar in three static cycles of 15 minutes each. Extracts were diluted with the 3-fold amount of water, passed through a syringe filter and subjected to SPE cleanup. Diet duplicate samples were filled in PP centrifuge tubes and fortified with internal standards. After addition of 2 ml of water and 10 minutes of ultrasonic extraction, the samples were centrifuged for 10 minutes at 4500 rpm and the supernatant was collected in a PP vessel. The sediment was twice re-extracted with methanol in an ultrasonic bath. The combined extracts were diluted with the fivefold amount of water, filtrated through a syringe filter and subjected to SPE.

Weak anion exchange SPE cartridges were preconditioned with 2 ml of methanol and water, respectively, and the extracts were passed through the preconditioned cartridges. The cartridges were then washed with methanol/water (1/1; v/v) and eluted with 1% NH_4OH in methanol. SPE eluates were evaporated under a gentle stream of nitrogen and diluted with water to a final volume of 1 ml.

HPLC-ESI-MS/MS: Identification and quantification of perfluorinated substances was performed on a Surveyor Plus HPLC connected to a Quantum Ultra AM mass spectrometer (both Thermo, Dreieich, Germany). Chromatographic separation was achieved by a Fusion RP phase (20 x 2 mm, 2 µm, Phenomenex, Aschaffenburg, Germany). Gradient HPLC was performed with methanol and 5mM ammonia acetate in water (pH 3.5), increasing methanol from 20 to 100% within 10 minutes.

Mass spectrometry was performed by electron spray ionisation (ESI) in the negative ion mode and subsequent single reaction monitoring. The following MS/MS transitions were monitored: PFOS (499 -> 99), $^{13}C_4$-PFOS (503 -> 99), PFOA (413 -> 369), $^{13}C_4$-PFOA (417 -> 372), PFHxS (399 -> 99), PFHxA (313 -> 269), PFOSA (498 -> 78).

Results and Discussion

The described method proved to be a robust and sensitive means to analyze PFOS, PFHxS, PFOA and PFHxA in food and fish samples. Less than 0.1 ng/g analyte were detected in method blank samples. In fish and food samples, limits of detection were between 0.05 for PFOS and PFOA and 0.1 for the other two target compounds.

The sensitivity of the method is displayed in figure 1. Peaks in the MS/MS fragmentograms exhibit a convincing signal to noise ratio and reflect 0.5 ng/g of each of the four target compounds in a spiked diet duplicate sample.

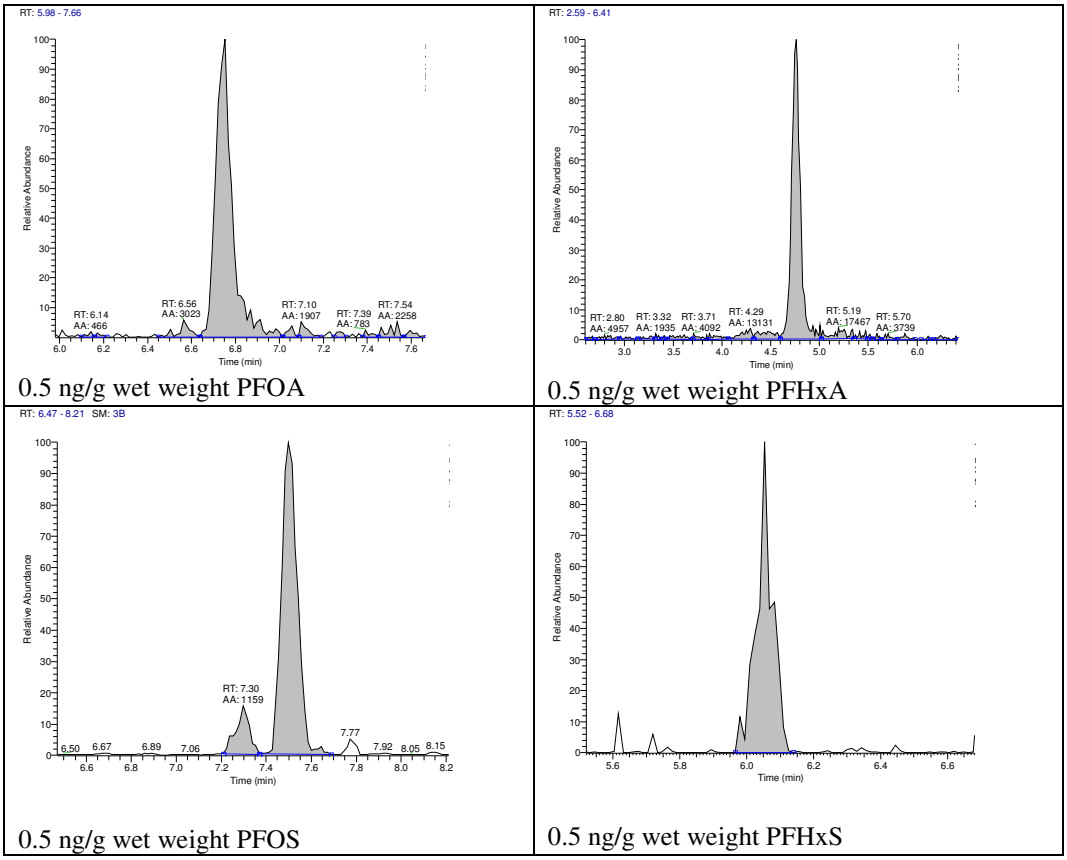

Figure 1: Sensitivity of the method is shown by MS/MS fragmentograms of PFOA, PFOS, PFHxA, and PFHxS reflecting 0.5ng/g wet weight, respectively, in a diet duplicate sample.

Recovery was investigated by one diet duplicate sample, which was shown not to contain detectable levels of PFC and which was spiked to five concentration levels between 0.5 and 50 ng/g fresh weights. As listed in Tab. 1, satisfying recovery rates were calculated for PFOS, PFHxS, PFOA, and PFHxA. In addition, recovery rates were constant over the investigated concentration range.

Table 1: Mean recoveries of PFOS, PFHxS, PFOA, and PFHxA obtained from spiked diet duplicate samples

PFOS	108 ± 11 %	PFOA	72 ± 6 %
PFHxS	97 ± 15 %	PFHxA	85 ± 19 %

Table 2 lists results of typical concentrations ranges of PFOS and PFOA detected in the investigated fish and food samples. Daily intake estimations have been published elsewhere.[9] It is apparent that sensitive analytical methods are required to detect the very low concentrations in food and diet duplicate samples. In contrast, PFOS levels in fish liver and muscles tissue from areas with distinct PFT sources can be detected much easier.

Table 2: Typical concentrations ranges of PFOS and PFOA in ng/g wet weight for fish from Germany (areas with PFT sources) and food samples

	PFOS	PFOA
Fish (muscle tissue)	5-60	< 0.2-5
Fish (liver)	100-900	< 0.2-9
Diet duplicates	< 0.05 - 1.0	< 0.05 - 118
Potatoes	< 0.2 – 3	< 0.2 – 3

However, even with HPLC-ESI-MS/MS interferences can disturb the quantification of PFOS significantly. As shown in Fig. 2, taurodeoxycholate, which coelutes with PFOS isomers, produces significant interference on the most sensitive MS/MS transition of PFOS, 499 → 80. The interference can be avoided by monitoring the more specific transition 499 → 99.

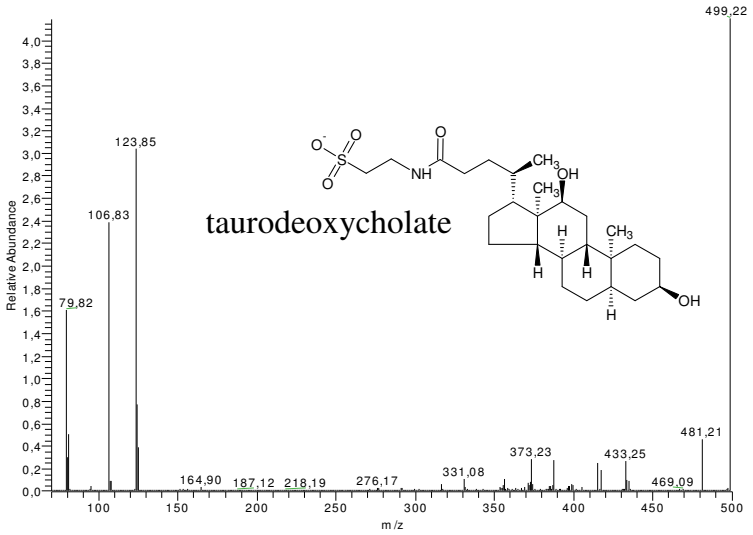

Fig. 2: HPLC-ESI-MS spectrum of taurodeoxycholate, a coeluting interference disturbing the sensitive MS/MS transition 499 → 80 of PFOS.

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References

1. Giesy JP, Kannan K. *Environ Sci Techno* 2001; 35:1339.
2. Yamashita N, Kannan K, Taniyasu S, Horii Y, Petrick G, Gamo T. *Marine Pollution Bulletin* 2005; 51:658.
3. Olsen GW, Church TR, Miller JP, Burris JM, Hansen K.J, Lundberg JK, Armitage JB, Herron RM, Medhdizadehkashi Z, Nobiletti JB, O'Neill EM, Mandel JH, Zobel LR. *Environ Health Perspect* 2003; 111:1892.
4. Kannan K, Corsolini S, Falandysz J, Fillmann K, Kumar KS, Loganathan BG, Mohd MA, Olivero J, Van Wouwe N, Yang JH, Aldous KM. *Environ Sci Technol* 2004; 38:4489.
5. Tittlemier S, Pepper K, Seymour C, Moisey J, Bronson R, Cao X-L, Dabeka R. *J Agric Food Chem* 2007 ; 55: 3203.
6. Hansen KJ, Clemen LA, Ellefson ME, Johnson HO. *Environ Sci Technol* 2001; 35:766.
7. Taniyasu S, Kannan K, So, MK, Gulkowska A, Sinclair E, Okazawa T, Yamashita N. *J. Chromat. A* 2005; 1093:89.
8. Fromme H, Albrecht M, Angerer J, Drexler H, Gruber L, Schlummer M, Parlar H, Korner W, Wanner A, Heitmann D, Roscher E, Bolte G. *Int J Hyg Environ Health* 2007; 210:345.
9. Fromme H, Schlummer M. Möller A, Gruber L, Wolz G, Ungewiß J, Böhmer S, Dekant W, Mayer R, Twardella D. *Environ Sci Techn.* Submitted.