CONffIDENCE: Contaminants in food and feed: Inexpensive detection for control of exposure

Sample extraction procedure for PFCs in milk, fish tissues and fish feed

P. Hradkova, J. Poustka, V. Hlouskova, J. Pulkrabova, J. Hajslova

Department of Food Chemistry and Analysis, Institute of Chemical Technology, Prague, Technická 3, 166 28 Prague 6, Czech Republic

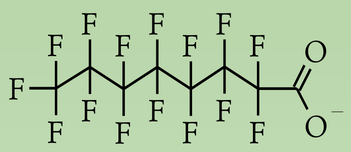
Work package WP1b - Perfluorinated compounds

WP leader: Marinella Farre (CSIC) **WP deputy:** Jan Poustka (ICT Prague)

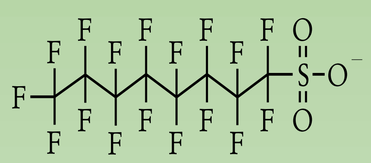
Introduction

- PFCs belong to one of the most important group of “emerging” contaminants. To assess health risks associated with dietary intake, in 2008, EFSA (European Food Safety Authority) recommended to member states to monitor two major representatives of this group – PFOS (perfluorooctane sulfonate) and PFOA (perfluorooctanoic acid) in food stuff. These two chemicals, together with PFOS precursor perfluorosulphonamide (FOSA), are usually used as indicator substances for potential occurrence of other PFCs. The fish feed, fish and dairy products were selected since these are suspected to be main inputs to human.
- Work package WP1b is focused on development of the analytical methods for the three main perfluorinated compounds (PFOS, PFOA and FOSA) in milk and dairy products, fish and fish feed. Subsequently, determination of PFCs level in these matrices.

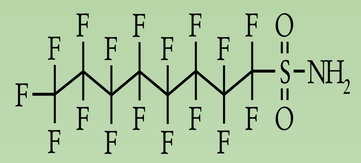
Target analytes:



Perfluorooctanoic acid (PFOA)



Perfluorooctanesuphonate (PFOS)



Perfluorosulphonamide (FOSA)

Target matrices:

- **Dairy products** (milk; 1.5% fat content)
- **Animal tissues** (fish muscles and liver)
- **Fish feed**

Optimisation of analytical procedure

Sample preparation

SAMPLE (milk, fish, fish feed)

Optimised parameters:

EXTRACTION (methanol)

A) Concentration and volume of formic acid (milk)
B) Sonication/vortexing

CLEAN – UP (activated charcoal)

C) Amount of activated charcoal added

CENTRIFUGATION & FILTRATION (10000 rpm; 5 min) & (0.2 µm centrifuge filter; 5000 rpm; 2 min)

IDENTIFICATION & QUANTIFICATION LC-MS/MS

LC–MS/MS

Alliance 2695 (Waters, USA)
Quattro Premier XE (Waters/Micromass, USA/UK)
Separation column: Atlantis T3 (100mm x 2.1mm; 3 µm) (Waters, USA)
Column temperature: 30 C
Gradient of mobile phase: A: methanol; B: 2 mM NH_4OAc in water
Injection volume: 10 µL
Injection temperature: 10 C

Results

Sample preparation

Table 1 Results of optimised method parameters for milk, fish and fish feed

Matrix	A	B	C
Milk	2 mL of 0.1M	Only vortexing	100
Fish muscle/liver	-	Only vortexing	340
Fish feed	-	Only vortexing	340

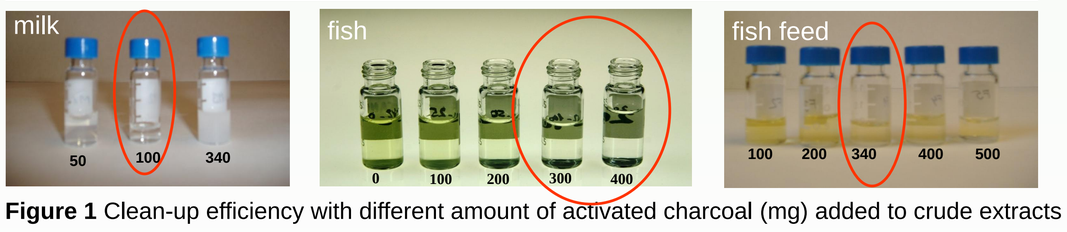


Figure 1 Clean-up efficiency with different amount of activated charcoal (mg) added to crude extracts

Performance characteristics

Table 2 Performance characteristics of analytical methods for tested matrices (n=5)

	MILK*			FISH TISSUE			FISH FEED		
	PFOS	PFOA	FOSA	PFOS	PFOA	FOSA	PFOS	PFOA	FOSA
Recovery (%)	92	91	114	77	92	90	90	95	98
RSD (%)	9	6	4	4	2	5	4	3	4
LOD (µg/kg)	0.5	0.5	0.3	0.6	0.6	0.3	0.6	0.6	0.3
LOQ (µg/kg)	2	2	1.5	2	2	1.5	2	2	1.5

*** - results in µg/L**

Figure 2 LC-MS/MS chromatogram of spiked milk (30 µg/L) and matrix matched standard (30 µg/L)

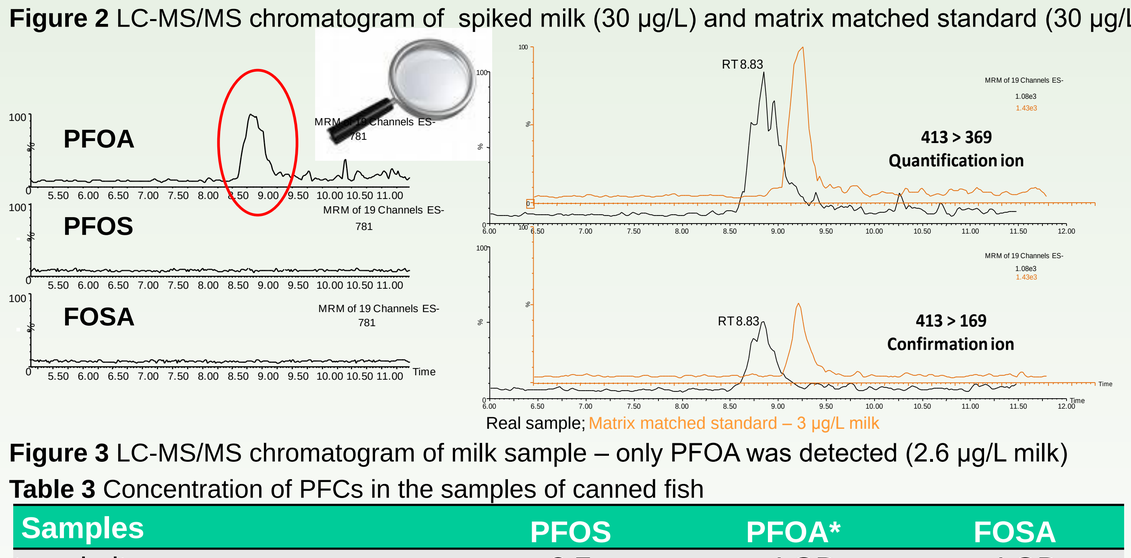


Figure 3 LC-MS/MS chromatogram of milk sample – only PFOA was detected (2.6 µg/L milk)

Table 3 Concentration of PFCs in the samples of canned fish

Samples	PFOS	PFOA*	FOSA
tuna in its sauce	3.7	< LOD	< LOD
tuna fillets in oil	< LOD	< LOD	< LOD
sardine in its sauce	29.6	0.69	< LOD
sardine in olive oil	< LOD	0.63	< LOD
sardine in seed oil (Baltic)	117.1	0.79	< LOD
cod liver in oil	59.4	< LOD	< LOD
cod liver in oil (smoked)	19.1	0.72	< LOD
herring fillets in oil	< LOD	< LOD	< LOD
mackerel in seed oil	73.8	< LOD	< LOD
sprats in oil (smoked)	65.3	< LOD	< LOD

*- LOQ > concentration in the samples > LOD

Conclusions

- The extraction procedures for all target PFCs (PFOS, PFOA and FOSA) included in project Con*ff*idence have been validated for all tested matrices (fish tissues, milk and fish feed) and several canned fish were analysed. The PFOS was the dominant contaminant in canned fish samples and its concentrations ranged from 0.6 to 117 µg/kg sample. Following the validation, 5 milk samples of milk from retail stores were examined. Only in one sample, the presence of PFOA was detected (2.6 µg/L).

Future work

- In year 2010 the first intralaboratory study on PFCs analysis in food and feed samples will be organised and evaluated, subsequently the interlaboratory test will be carried out.

The research leading to these results has received funding from the European Community's Seventh Framework Programme (*FP7/2007-2013)* under *grant agreement* n 211326.