First worldwide interlaboratory study on perfluorinated compounds

Stefan Van Leeuwen[1], Anna Kärrman[2], Astrid Zammit[3], Bert van Bavel[2], Ike van der Veen[1], Christiaan Kwadijk[1], Jacob de Boer[1], Gunilla Lindström[2]

[1]Netherlands Institute For Fisheries Research (rivo)
[2]Man-Technology-Environment (MTM) Research Centre, Örebro University
[3]Water Services Corporation - Institute of Water Technology Laboratory

Introduction

The Netherlands Institute for Fisheries Research and Örebro University organized in 2005 the first world-wide interlaboratory study in collaboration with the EU Perforce research project. The study covers environmental matrices, a standard solution, and human plasma and blood.
Accurate and reproducible analysis of perfluorinated alkyl compounds (PFCs) in human and environmental tissues are challenging in many ways[1]. Many laboratories have developed analytical methods for the extraction, clean-up and final determination of PFCs in environmental and human matrices. Many factors, such as contamination from laboratory materials, incomplete recoveries and leakage from instrumental parts are common problems, and lead to poor accuracy, sensitivity and reproducibility. Today LC-MS electrospray is the most common detection technique used in PFC analysis. A known difficulty with this technique is ion-suppression, both from the matrix and from poorly separated PFCs. Branched isomers challenge separation further, and reference standards are needed to assure the specificity and accuracy of the data. The performance of analytical methods have so far only been tested in-house by spike experiments because up to date, no certified reference materials are available and no interlaboratory studies have been organized. The first world-wide interlaboratory study reported here was initiated to assess the current quality of the analytical techniques applied by laboratories world wide.

Method and materials

The following environmental samples were prepared for the study:

- Brackish water (North Sea Canal, The Netherlands, 1 liter, packed in a brown high density poly ethylene bottle)
- Fish liver extract (3 ml, equivalent to 1.5 gram fish liver) from flounder from the Western Scheldt, The Netherlands. The livers were homogenized and extracted by ion pair extraction. Lipids were removed by silica clean-up and the cleaned extract was spiked with all target PFCs (except PFDS) in order to adjust to the target levels.
- Pike perch (Lake IJssel, The Netherlands) muscle tissue, spiked with all target PFCs (except PFDS) several PFCs, homogenized and sterilized at 121°C, 3 bar for 30 minutes. The glass jar contained 60 g.
- Study standard, containing the PFC compounds in the range of 5-100 ng/ml (ampoule, 4 ml).

The fish tissue was tested for homogeneity by analyzing PFOS and PFOA. The homogeneity was found satisfactory.
The human matrices part of the interlaboratory consisted of a plasma sample (7 ml) and a whole blood sample (3 ml). Authentic blood samples, without addition of PFCs, representing the current levels of PFCs in the Swedish general population were used.
The samples were sent by courier to the participants (ambient temperature). Laboratories were asked to use any method they preferred, and to analyze any the PFCs mentioned below. Nineteen laboratories signed up for the human matrix part, and 17 of these have returned results. Twenty seven laboratories submitted results for the environmental matrix part. The PFCs in this study were perfluorobutanesulfonate (PFBS), perfluorohexanesulfonate (PFHxS), perfluorooctanesulfonate (PFOS), perfluorodecanesulfonate (PFDS), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA), perfluorotetradecanoic acid (PFTDA) and perfluorooctanesulfonamide (PFOSA).

Methods used by participants

Clean-up methods used included ion-pair extraction, different types of solid-phase extractions or only protein precipitation. Analysis and detection methods were for example LC-ESI-MS/MS (triple quadrupole), LC-ESI-MS/MS (ion trap), LC-ESI-MS (single quadrupole), LC-ESI-TOF-MS and GC-MS. Quantification was performed by using extracted or non-extracted standard curves with or without matrix or internal standard present.

Results and discussion

Human matrix part

Table 1 shows a summary reported from the different laboratories of the results for PFOS and PFOA in the plasma and whole blood samples. No processing of data or removal of outliers has been made, and consequently no consensus value and Z-scores are given here. Mean, median and variability (CV%) are given if a sufficient number of participants (>50%) reported values > non-detect (ND). Since not all participants reported the exact limit of detection all ND values were excluded from the calculations.

Table 1. Summary results of PFOS and PFOA for human plasma and whole blood.

	PFOS		PFOA	
	Human plasma (ng/ml)	Whole blood (ng/ml)	Human plasma (ng/ml)	Whole Blood (ng/ml)
Min	7.1	1.8	0.5	1.4
Max	34.9	24	5.2	4.06
Median	22.45	20.0	2.0	2.0
Mean	22.9	10.4	2.11	2.23
STdev	7.2	5.85	1.02	0.88
CV (%)	32	56	48	39
n*=	16	9	18	11

* No of submitted data

Almost all laboratories reported values for PFOS and PFOA. The CV for PFOS was lower (CV~30%) for plasma and higher (CV~60%) for whole blood. Corresponding results for PFOA were ~50 and ~40%. A lower number of participants reported levels for PFNA and PFHxS and the variations were higher compared to PFOS and PFOA. For PFOSA, PFBS, PFDS, PFDA, PFHpA and PFUnDA, the very low levels hampered the detection of these compounds and a considerable number of data was

Environmental matrix part

Similarly to the human samples, most results have been reported for PFOS and PFOA. Data on the other perfluorinated compounds have been reported, but will not be discussed here. Table 2 shows a summary of results of PFOS and PFOA.

For both compounds the study standard shows good. The cleaned fish extract analysis resulted in CV values for PFOS and PFOA of 57 and 79%, respectively. The pike perch results were 130 and 198% for PFOS and PFOA, respectively. The extraction and clean-up contributes ca. 2 times the variation caused by the LC-MS determination only. For the pike perch, the range between the minimum and maximum is considerable. The dataset is not normally distributed, which can be seen from the difference between the median and the mean value. The fact that outliers have not yet been removed from the dataset contributes to this. Compared to the human matrix, the CV of fish tissue is higher. This might be caused by a more complex nature of the fish tissue material which may have resulted in inaccurate extraction (and clean-up), or errors in the final LC-MS determination due to e.g. co-eluting compounds effecting the electrospray.

Table 2. Summary of results for the environmental matrices for PFOS and PFOA.

	PFOS			PFOA		
	Study standard (ng/ml)	Cleaned fish extract (ng/ml)	Fish tissue (ng/g ww)	Study standard (ng/ml)	Cleaned fish extract (ng/ml)	Fish tissue (ng/g ww)
Min	6.2	2.7	2.8	4.1	4.5	0.5
Max	59	62	295	46	77	204
Median	30	19	34	9	14	13
Mean	31	23	57	12	19	23
STdev	11	13	74	10	15	45
CV (%)	36	57	130	85	79	198
n*=	25 (1)	23 (1)	18 (1)	27 (-)	25 (-)	21 (1)

* No of submitted data (number of

Figure 1 shows the z-scores of PFOS in the study standard, calculated by the Cofino model[2]. Eighteen out of 24 laboratories arrived at a z-score of -26 or z<-6 are outlying data points, often caused by e.g. errors in the calculation.

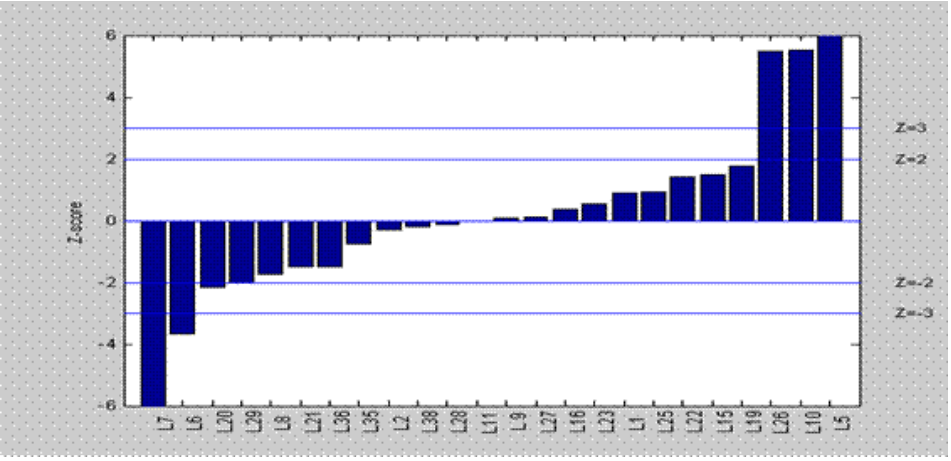

Figure 1. Z-scores of PFOS in the study standard. The x-axis indicates the lab number.

Concerning the water sample, 21 laboratories submitted their data. Fifteen or more datasets were obtained for PFOS, PFHxS, PFOA, PFNA, PFDA, PFUnA and PFDoA. The data for the water sample is scattered, showing that the methods for water analysis are less well under control. This was confirmed by the information received from participating laboratories stating that they are not very experienced. Further development of methods is required.

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