FOLLOW-UP STUDIES ON THE FIRST INTERLABORATORY STUDY ON PERFLUORINATED COMPOUNDS IN HUMAN AND ENVIRONMENTAL MATRICES

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Introduction

The first worldwide interlaboratory study on determination of per- and poly-fluorinated compounds (PFCs) was conducted in 2005 and was organized jointly by RIVO (on behalf of PERFORCE) and Őrebro University[1]. The objective of the study was to assess between-lab reproducibility for PFCs in a variety of human and environmental matrices. Thirty-eight labs from 13 countries participated to some extent. However, a number of prominent laboratories reporting environmental monitoring data did not participate. The level of agreement decreased with increasing matrix complexity. The poorest agreement was obtained for the water sample and the fish tissue sample. While the fish tissue result was not surprising, the water sample contained relatively high levels of at least some of the analytes and should not have posed serious problems. Follow-up studies were performed in order to provide guidance for a second interlaboratory study and to improve laboratory performance in general. The studies focused on sources of bias and variability including calibration, background due to contamination, variations in methodology and sample storage.

Materials and Methods

PFC standards were obtained commercially. The stable isotope internal standard ($^{13}C_2$-PFOA) was synthesized in-house. All determinations were done using LC/MS/MS, with an electrospray interface operating in the negative ion mode[2]. Most sample preparation was done using acetonitrile or methanol extractions, optionally followed by an Envi-Carb cleanup procedure described by Powley[2]. The alternative method trials were conducted using either the ion-pairing/methyl *tert*-butyl ether extraction described by Hansen[3], or the solid-phase extraction procedure described by Kuklenyik[4].

Results and Discussion

Accurate and precise measurement of trace-level compounds requires a significant amount of analytical rigor. The usual steps that must be taken include ensuring that the instrumentation is performing within specifications and that it is properly calibrated over the range of analyte concentrations to be measured. Use of one or more internal standards is recommended, and stable isotope analogs of many of the perfluorinated analytes are now available. However, if an internal standard is used, steps must be taken to ensure that its use does not cause any interference with the analytes, either from chemical impurities or its signal being inadequately mass-resolved from that of an analyte ("crosstalk").

Contamination also must be evaluated and potential sources eliminated if it could occur. In addition to the usual precautions, such as isolating concentrated standards from the sample preparation area, many of the perfluorinated compounds are to be considered as ubiquitously occurring in a laboratory environment because they can be extracted from common labware or even instrument components. Therefore, procedural and instrumental blanks must be prepared and analyzed with every set of samples. Instrumental contamination, if present, can be reduced or even eliminated by replacing poly(tetrafluoroethylene) components of the system with parts made of other materials. Alternatively, a pre-column can be inserted between the pump and injector to trap any contaminants before they can collect on the analytical column.

Human and environmental samples can contain mixtures of branched and linear isomers of some of the PFCs, most notably PFOA and PFOS. Most commercially available standards of these compounds totally consist of the linear isomer. Most laboratories sum the areas of any branched and linear isomer peaks and quantitate all as linear. In Table 1, the slopes of calibration curves constructed using a branched/linear standard of PFOA (manufactured using the ECF process) and a purely linear standard of PFOA are compared. It is apparent that the branched isomers have a lower response than the linear isomers, so the practice of calibrating branched responses using linear standards is not strictly correct. However, for the purpose of interlaboratory comparison, it is important that all participants follow the same convention.

Table 1. Comparison of slopes of PFOA calibration curves constructed using different standards

Standard used for quantification – Quantified peaks	Calibration Curve slope (area counts ng^{-1} mL)
Branched/linear standard - branched isomer peaks	0.0204
Branched/linear standard - linear isomer peak	0.129
Sum of slopes of branched and linear peaks	**0.149**
Purely linear standard – linear peak	**0.171**

The three methods most commonly used for determination of PFCA's in blood were compared for PFOA determinations. All three methods gave results that agreed to within ± 20% for a wide range of PFOA concentrations. The first Interlab study did provide reasonably good agreement among the participants, who used a variety of methods. Since the levels in the samples provided were towards the lower limit of most methods for blood, careful attention to issues such as background, contamination, and interference are essential.

Very poor agreement was obtained among the participants for the water sample. This was quite surprising, as the levels of both PFOA and PFOS were approximately 20 ng/mL each in the sample as it was originally sent out. Many labs failed to detect any of these compounds in the sample. Table 2 shows results of various experiments conducted to determine the cause of the non-detects. Overspiking sample aliquots with PFOA and PFOS and aging for a week under ambient conditions resulted in significant losses, even when the sample was simply direct injected. Fresh fortifications yielded quantitative recoveries, which led to the hypothesis that the compounds were adsorbed into the container walls as a result of the formic acid treatment applied to minimize microbial growth. Adsorption was verified by extraction of the container with methanol, where some of the analytes were recovered. It can be concluded that possible adsorption to the container will certainly contribute to the variety in the analytical results.

Table 2. Results of fortification experiments conducted to investigate losses of PFOA and PFOS in the Interlab surface water sample

	PFOA result	PFOS result
Direct injection of water sample	ND	ND
Overspike of water sample (100 ng/mL), direct injection	99% recovery	99% recovery
Water sample through SPE procedure	0.07 ng/mL	ND
Overspike of water sample (100 ng/mL), through SPE procedure	110% recovery	96% recovery
Overspike of water sample (10 ng/mL), aged 1 week in original container (HDPE), ambient conditions	46% recovery	14% recovery
Overspike of water sample (10 ng/mL), aged 1 week in polypropylene container, ambient conditions	110% recovery	72% recovery

The fish liver extract sample had undergone a silica column purification procedure before it was shipped to the participants, so most laboratories did not perform additional cleanup. The agreement among laboratories was poor, with most of the error attributed to matrix effects, especially for PFOS. Table 3 shows the results of follow-up studies on this matrix. Results obtained by direct analysis of the matrix without further purification are compared with those obtained using an added Envi-Carb cleanup. In all cases, standards in solvent were used, with only one internal standard used for all analytes ($^{13}C_2$-PFOA). The results are comparable, and matrix effects appear to be minimal for either procedure. The recommendation is that matrix effects should always be evaluated by overspiking a sample extract just prior to injection with a known amount of analyte, and then determining the net recovery. If matrix effects are shown to be present, standard should be prepared in sample extract, standard addition should be used, or stable isotope internal standards should be used if they are available.

Table 3. Comparison of results obtained for fish liver extract, with and without additional cleanup

	ng/mL found					
	PFHxA	PFOA	PFDA	PFUnA	PFDoA	PFOS
Direct analysis of fish liver extract (no cleanup)	1.2	13	13	3.6	29	29
Envi-Carb cleanup added	1.2	12	11	3.1	20	25
Assigned value from organizers[1]	1.7	12	8.9	-	18	19
	Net recovery (%)					
	PFHxA	PFOA	PFDA	PFUnA	PFDoA	PFOS
Overspike of fish liver extract (10 ng/mL), direct injection (matrix spike)	119	108	135	130	140	109
Overspike of fish liver extract (20 ng/mL), Envi-Carb cleanup	98	94	110	101	96	93
Overspike of fish liver extract *after* Envi-Carb cleanup (10 ng/mL) (matrix spike)	123	111	110	120	122	100

Poor agreement among the participating labs was also obtained for the fish tissue sample. Most labs used the ion-pairing/MTBE extraction. In Table 4, the results obtained using this method are compared to those obtained using the acetonitrile extraction with the Envi-Carb cleanup. Both methods gave comparable results for the PFCAs and PFOS, except for PFUnA where we observed severe matrix suppression where the ion-pairing method was used. Even high-level (200 ppb) fortifications made at the beginning of the procedure and to the final extract did not have detectable responses. A significant matrix enhancement was also observed for PFHxA in the ion-pairing method. The overall quality control data from fortifications made directly to the sample as well as to the final extracts was noticeably better for the acetonitrile/Envi-Carb method than it was for the MTBE/ion-pairing method. The learning from this matrix was that complex matrices require effective purification, especially if stable isotope internal standards are not available for each analyte.

Table 4. Comparison of results obtained for fish tissue, using two different methods

	ng/g found				
	PFHxA	PFOA	PFDA	PFUnA	PFOS
Fish tissue sample, acetonitrile extraction with Envi-Carb cleanup	ND	11	3.0	1.0	110
Fish tissue sample, MTBE/ion-pairing method	ND	9.8	1.6	ND	73
Assigned value from organizers[1]	1.0	10	2.2	-	36
	Net recovery (%)				
	PFHxA	PFOA	PFDA	PFUnA	PFOS
Overspike of fish tissue (200 ng/g), acetonitrile extraction with Envi-Carb cleanup	100	100	105	100	91
Overspike of fish tissue (200 ng/g) *after* acetonitrile extraction with Envi-Carb cleanup (matrix spike)	100	103	107	91	85
Overspike of fish tissue (200 ng/g), MTBE/ion-pairing method	220	119	79	ND	80
Overspike of fish tissue (200 ng/g) *after* MTBE/ion-pairing method (matrix spike)	147	122	124	ND	106

References

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