

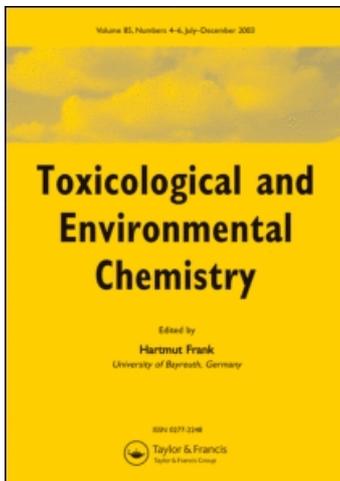
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Standard purity and response factors of perfluorinated compounds

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Standard purity and response factors of perfluorinated compounds

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It has been reported that the relative response factors of isotopically labeled standards and unlabeled standards of the same perfluorinated compounds could be different. Individual (100 ng mL⁻¹) solutions of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) were analyzed using high-performance liquid chromatography tandem mass-spectrometry under negative-ion electrospray to detect any impurities present down to 0.5%–0.1% relative to the major component. Purity of the standards ranged from approximately 86% to $\geq 97\%$. Standard solutions of unlabeled and isotopically labeled materials were analyzed to compare the response factors of isotopically labeled analytes *versus* their nonlabeled counterparts in three different matrices at equivalent concentrations: organic solvent (methanol), serum extract, and water present individually and concurrently. Not all labeled analytes have the same response factor as their nonlabeled complement, and in at least one case the matrix in which the standard is present may cause significant suppression of response. Standard solutions of electrochemical fluorination produced PFOA and PFOS were quantified under multiple reaction monitoring mode, using calibration curves prepared from standards consisting primarily of linear standards only. The use of linear only standards may cause under-prediction of concentrations, and that the working range of these standards may be limited.

Keywords: calibration; PFCs; PFOS; PFOA; LC-MS/MS

Introduction

Perfluorinated compounds (PFCs) have been produced in large quantities since the 1950s for a wide range of applications ranging from carpet coatings to fire retardants (Paul et al. 2009; Prevedouros et al. 2006). They have been found to be ubiquitous in both remote and urban environments (Giesy and Kannan 2001). They have been measured in various matrices including human blood, plasma, serum,

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sediments, water, and wildlife tissue (Giesy and Kannan 2001; Higgins et al. 2005; Kannan et al. 2004). The most widely distributed, and also the most studied PFC is perfluorooctane sulfonate (PFOS). Production of PFOS-based products was voluntarily halted by North America's largest producer, the 3M company, in 2000 (3M 2000).

Although PFCs had been produced on a large scale for more than 40 years, it was not until the late 1990s that researchers started detecting PFCs in the environment (Giesy and Kannan 2001). This was due to a number of factors including the lack of accurate and sensitive methods for extraction, the lack of standards, especially isotopically labeled ones, and the lack of instrumentation with sufficient sensitivity (Martin et al. 2004; Van Leeuwen et al. 2006). With the advent of high-performance liquid chromatography (HPLC) coupled to an electrospray-ionization tandem mass spectrometry (ESI-MS/MS), scientists could begin to accurately and routinely measure PFCs in the environment (Hansen et al. 2001).

Although in recent years there have been many advances in the quantification of PFCs, many issues still remain, such as matrix and isomer effects and impure standards (Arsenault et al. 2008b; Jacoby 2008; Reagen et al. 2008a). Production of PFOS-based products by electrochemical fluorination (ECF) that was commonly used by the 3M Company, results in a complex mixture of PFCs with a given purity of approximately 86% (Reagen et al. 2008b; Seacat et al. 2003). This ECF-produced PFOS is comprised of 11 different isomers, of which approximately 70% is the linear isomer and approximately 30% is made up of different branched isomers (Arsenault et al. 2008a; Benskin, Batatineh, and Martin 2007; Martin et al. 2004; Reagen et al. 2008b). These issues become increasingly important as scientists strive to measure PFCs in the environment at lower concentrations (Yamashita et al. 2004), which can often result in significant analytical bias.

This study was conducted to address three questions relevant to the quantification of PFCs. The first objective was to determine the concentrations of other PFCs present in specific lots of standard solutions of PFOS and perfluorooctanoic acid (PFOA). The second objective was to determine the relative response factors of isotopically labeled analytes relative to their nonlabeled counterparts. Lastly, the feasibility of using linear PFOS and PFOA standards to quantify ECF-produced, branched PFOS and PFOA was investigated. Individual 10 ng mL^{-1} solutions of PFOS and PFOA were diluted from original stock standards supplied by the 3M Company and were analyzed using HPLC-MS/MS with negative ion electrospray to detect any impurities present at proportions down to 0.1%, relative to the major components. Standard solutions of isotopically and nonlabeled materials were analyzed to compare response factors of isotopically labeled analytes *versus* their nonlabeled counterparts in three different matrices at equivalent concentrations: organic solvent (methanol), serum extract, and water. Standards were also re-analyzed, with an isotopically labeled analyte concurrently present in the solvent, serum extract, and water with its nonlabeled counterpart at the same concentration. This allowed for the determination of suppression or the enhancement of response due to possible interactions. Lastly, standard solutions of ECF-produced PFOS and PFOA were quantified using calibration curves prepared from standards consisting of linear only PFOS and PFOA.

Experimental

Chemicals and standards

Omni-Solv grade methanol was purchased from EMD Chemicals (Gibbstown, NJ, USA). HPLC grade ammonium acetate was purchased from J.T. Baker (Phillipsburg, NJ, USA). The 3M Company provided ECF-produced PFOS (potassium salt, 86.9%, Lot #217) and PFOA (ammonium salt, 95.2%, Lot #332). Other PFC standards were obtained as follows: L-PFOA (Linear, free acid, 99.51%, Oakwood Products), L-PFOA (free acid, >98%, Wellington Laboratories), L-PFOSNa (sodium salt, >98%, Wellington Laboratories), L-PFOSK (potassium salt, >98%, Wellington Laboratories), PFOA[1,2,3,4 ¹³C] (>98%, Wellington Laboratories), PFOS[¹⁸O₂] (RTI International), PFOS[1,2,3,4 ¹³C] (>98%, Wellington Laboratories), perfluorobutyric acid (PFBA) (99.2%, Sigma-Aldrich, USA), perfluoropentanoic acid (PFPA) (99.2% Alfa Aesar), perfluorohexanoic acid (PFHxA) (97.7%, Oakwood Products), perfluoroheptanoic acid (PFHpA) (98.2%, Sigma-Aldrich, USA), perfluorononanoic acid (PFNA) (98% Oakwood Laboratories), perfluorodecanoic acid (PFDA) (98%, Oakwood Laboratories), perfluoroundecanoic acid (PFUnA) (96.4%, Oakwood Laboratories), perfluorododecanoic acid (PFDoA) (99.65%, Oakwood Laboratories), perfluorobutane sulfonate (PFBS) (97.3%, 3M Lot #2), perfluorohexane sulfonate (PFHxS) (98.6%, 3M NB 120067-69), and linear perfluorodecane sulfonate (PFDS) (98%, Wellington Laboratories). All standards were purchased by the 3M Company which were then supplied to the University of Saskatchewan for instrumental analyses.

HPLC-MS/MS conditions

Analytical methods were optimized to allow for simultaneous detection of all target analytes during a single run. Isomer separation was accomplished by the use of an Agilent 1200 HPLC fitted with a Thermo Scientific Betasil C18 (100 mm × 2.1 mm, 5 μm particle size) analytical column operated at 35°C. Gradient conditions were a 300 μL min⁻¹ flow rate, starting with 60% A (2 mM ammonium acetate) and 40% B (100% methanol). Initial conditions were held for 2 min and then ramped to 20% A at 18 min, held until 20 min, decreased to 0% A at 21 min, increased to 100% A at 22 min, held until 22.5 min, returned to initial condition at 23 min, and finally held constant until 26 min.

Mass spectra were collected using an Applied Bioscience SCIEX 3000 (Foster City, CA) tandem mass spectrometer, fitted with an ESI source, operated in negative ionization mode. Chromatograms were recorded using MRM mode, where at least two transitions per analyte were monitored. The following instrument parameters were used: desolvation temperature (450°C), desolvation (curtain) gas 6.0 arbitrary units (AU); nebulizer gas flow 5 AU; ion spray voltage – 3500 V; collision gas 12 AU; and a dwell time of 40 ms. The optimal settings for collision energies and declustering potential were determined for each analyte's transitions to enhance detection and improve the signal to noise ratio. Quantification using these transitions was performed using Analyst 1.4.1 software provided by Sciex (Applied Bioscience, Foster City, CA).

Quality control

To reduce instrument background contamination coming from the HPLC or solvents, a ZORBEX (Thermo Scientific, 50 mm × 2.1 mm, 5 μm particle size) column was inserted directly before the injection valve, as adapted from Benskin, Bataineh, and Martin (2007). Blanks were run every four to five samples to check for carryover and background contamination, and at all times during analysis the blanks were found to be below the limit of quantification. Teflon coated lab-ware was avoided during all steps of standard solution preparation to minimize the contamination of samples. Samples were stored and analyzed in polypropylene auto-sampler vials fitted with polypropylene septa (Canadian Life Science, Peterborough, ON, Canada), as it has been shown that glass vials and polytetrafluoroethylene septa may cause the loss of analyte and increased contamination, respectively (Yamashita et al. 2004).

Data and statistical analysis

Differences in PFOS and PFOA response factors were investigated using a one-way analysis of variance (ANOVA). If differences were found, a Tukey's *post hoc* test was also performed to determine where the differences existed. All statistical analyses were performed using the statistical software SYSTAT[®] 17.0 Package (SPSS Software Inc., Chicago, IL, USA).

Purity verification of standards

Individual 100 ng mL⁻¹ solutions of each of the PFOS and PFOA standards were prepared in a 50% methanol, 50% 2 mM ammonium acetate solution and were analyzed using HPLC-MS/MS under negative-ion electrospray to detect any impurities present down to 0.1%, relative to the major components. PFOS and PFOA materials that were analyzed for purity verification are listed in Table 1. The target impurities which were monitored in each of the PFOS and PFOA standard solutions are given in Table 2. Calibration curves made from individual standard reference materials were used to quantify each of the target impurities.

Unlabeled versus isotopically labeled materials

Response factor verification

It has been reported that one of the major uncertainties in measurement of PFCs are the problems of ion suppression or enhancement as a result of matrix or co-elution effects (Arsenault et al. 2008b, Martin et al. 2004, Reagen et al. 2008a). For this reason, an experiment was designed to measure the response factors of isotopically labeled and native PFOS and PFOA standards present individually at a concentration of 100 ng mL⁻¹ in methanol, serum extract, and reagent grade water. The same set of experiments were repeated, wherein the native standards were present concurrently with the isotopically labeled counterparts to see if there were any synergistic or antagonistic effects.

Individual standard solutions of unlabeled and isotopically labeled materials (Table 1) were prepared to compare the response factors of isotopically labeled analytes *versus* their nonlabeled counterpart in three different matrices at equivalent 100 ng mL⁻¹ concentrations: organic solvent (methanol), bovine serum extract,

Table 1. PFOA and PFOS standards evaluated for target impurities.

Name	Synonym or acronym	Formula	Vendor source	Reported purity
Perfluorooctanoic acid (ammonium salt)	PFOA (C8 acid)	$C_7F_{15}COO^-NH_4^+$	3M Lot #332	95.20%
Perfluorooctanoic acid (free acid) ^a	PFOA (C8 acid)	$C_7F_{15}COOH$	Oakwood Products	99.51%
Perfluorooctanoic acid (free acid) ^a	PFOA (C8 acid)	$C_7F_{15}COOH$	Wellington	99.51%
Perfluorooctane sulfonate (potassium salt)	PFOS	$C_8F_{17}SO_3^-K^+$	3M Lot #217	86.90%
L-Perfluorooctane sulfonate (sodium salt) ^a	PFOS	$C_8F_{17}SO_3^-Na^+$	Wellington	>98%
Perfluorooctane sulfonate (potassium salt) ^a	PFOS	$C_8F_{17}SO_3^-K^+$	Wellington	>98%
Isotopically labeled perfluorooctanoic acid	PFOA [1,2 ^{13}C]	$C_6F_{13}[^{13}C]F_2[^{13}C]OOH$	Perkin Elmer	97.60%
	PFOA [1,2,3,4 ^{13}C]	$C_4F_9[^{13}C]F_2[^{13}C]F_2[^{13}C]OOH$	Wellington	>98%
Isotopically labeled perfluorooctane sulfonate	PFOS [$^{18}O_2$]	$C_8F_{17}S[^{18}O_2]O^-NH_4^+$	RTI International	>98%
	PFOS [1,2,3,4 ^{13}C]	$C_4F_9[^{13}C]F_2[^{13}C]F_2[^{13}C]SO_3^-Na^+$	Wellington	>98%

Note: ^aPredominantly linear isomer.

and 18 M Ω water. The solvent, serum, and water were extracted and checked for contamination prior to usage, and all samples were found to have PFC concentrations below the limit of quantification. The response factors were calculated as follows:

$$\text{Response Factor} = \frac{\text{Analyte Area Counts}}{\text{Analyte Amount}}$$

The response factor comparisons were done on equivalent mass transitions for the labeled and unlabeled PFCs (listed in Table 3).

Synergistic effects

After determining the response factor for the labeled materials present as individual analytes, the same set of experiments was repeated, where the isotopically labeled analyte was concurrently present in the solvent, serum extract, and water with its unlabeled counterpart at the same concentration, to determine if any synergistic effects were present.

Accuracy of isomer quantification

Standard solutions of 3M ECF PFOS (Lot #217) and 3M ECF PFOA (Lot #332) were quantified using calibration curves prepared from standards consisting

Table 2. Compounds determined as target impurities.

Name	Synonym or acronym	Formula	Vendor source	Given purity
Perfluorobutyric acid	PFBA (C4 acid)	C ₃ F ₇ COOH	Sigma-Aldrich	99.20%
Perfluoropentanoic acid	PFPA (C5 acid)	C ₄ F ₉ COOH	Alfa Aesar	99.20%
Perfluorohexanoic acid	PFHxA (C6 acid)	C ₅ F ₁₁ COOH	Oakwood Products	97.70%
Perfluoroheptanoic acid	PFHpA (C7 acid)	C ₆ F ₁₃ COOH	Sigma-Aldrich	98.20%
Perfluorooctanoic acid (ammonium salt)	PFOA (C8 acid)	C ₇ F ₁₅ COO ⁻ NH ₄ ⁺	3M Lot 217	95.20%
Perfluorononanoic acid	PFNA (C9 acid)	C ₈ F ₁₇ COOH	Oakwood Products	98.02%
Perfluorodecanoic acid	PFDA (C10 acid)	C ₉ F ₁₉ COOH	Oakwood Products	98.01%
Perfluoroundecanoic acid	PFUnA (C11 acid)	C ₁₀ F ₂₁ COOH	Oakwood Products	96.40%
Perfluorododecanoic acid	PFDoA (C12 acid)	C ₁₁ F ₂₃ COOH	Oakwood Products	99.65%
Perfluorobutane sulfonate (potassium salt)	PFBS	C ₄ F ₉ SO ₃ ⁻ K ⁺	3M Lot#2	97.30%
Perfluorohexane sulfonate (potassium salt)	PFHxS	C ₆ F ₁₃ SO ₃ ⁻ K ⁺	3M NB 120067-69	98.60%
Perfluorooctane sulfonate (potassium salt)	PFOS	C ₈ F ₁₇ SO ₃ ⁻ K ⁺	3M Lot#217	86.90%
L-Perfluorodecane sulfonate; (predominantly linear)	PFDS	C ₁₀ F ₂₁ SO ₃ ⁻ Na ⁺	Wellington	>98.00%

Table 3. Mass transitions of isotopically labeled and unlabeled.

Isotopically labeled material	Unlabeled counterpart	Isotopically labeled transition(s)	Unlabeled transition(s)
PFOA [1,2 ¹³ C]	PFOA (C8 acid)	415 > 370	413 > 369; 413 > 219; 413 > 169
PFOA [1,2,3,4 ¹³ C]	PFOA (C8 acid)	417 > 372	413 > 369; 413 > 219; 413 > 169
PFOS [¹⁸ O ₂]	PFOS (C8 sulfonate)	503 > 103; 503 > 84	499 > 99; 499 > 80; 499 > 130
PFOS [1,2,3,4 ¹³ C]	PFOS (C8 sulfonate)	503 > 99; 503 > 80	499 > 99; 499 > 80; 499 > 130

primarily of the linear isomer only (linear standards commercially obtained from Oakwood Products and Wellington Labs).

In this experiment we used primarily linear ($\geq 98\%$) PFOS and PFOA standards to generate calibration curves in order to quantify 3M ECF-produced multiple branched materials. When the linear standards used were present in salt form, great care was taken to insure that the concentration used were salt corrected, as many commercially available standards do not correct for the salt form that is present. Known concentrations of branched PFOS and PFOA standards were analyzed as unknowns and quantified against their linear standard calibration curves. These results were then compared to their known concentrations.

Results and discussion

Purity verification of PFOS and PFOA standards

The use of impure standards and the problems it presents to the analysis for environmental samples have been well documented (Arsenault et al. 2008b; Jacoby 2008; Martin et al. 2004; Reagen et al. 2008b; Seacat et al. 2003; Taniyasu et al. 2005). The manufacturers reported that purities of PFOS and PFOA standards ranged from 86% to over 98%, but as Arsenault et al. (2008b) reported these measurements are often based on an acid/base titration method after an ion exchange with a strong acid resin, which is not specific to PFOS or PFOA. Using these impure standards could not only result in improper measurement of the target analyte, but it could also result in a significant contamination of an environmental sample with other PFCs that may be present such that they cannot be accurately quantified. The latter issue is becoming increasingly important as the sensitivity of newer more powerful instruments has allowed for the detection of PFCs at lesser concentrations.

Impurities are in fact an issue with some PFC standards in that the relative amount of each impurity can vary greatly depending on the source of the standard (Table 4). In general, our results matched the reported purity of the standards, with a few notable exceptions. Our results also closely agree with a recent study that used calibration curves from standards of known purity to quantify PFOS standards of "unknown" purity (Arsenault et al. 2008b). The largest difference between a reported and measured purity was for the isotopically labeled PFOA produced by Perkin Elmer, which had relatively high levels of the C10 acid (Table 4).

The biggest potential issue that could result from impurities being found in standards is the potential to introduce multiple PFCs into an environmental sample that was spiked with an impure internal standard, such as for a lab matrix or a field matrix spike sample. Based on the results of our study, under the worst case scenario, one could introduce approximately 14 ng (sum of impurities) of various PFC impurities from intentionally spiking 100 ng of 3M ECF-produced PFOS, whereas the worst case scenario using supplier grade standards could introduce approximately 7 ng of various PFC (sum of impurities). This 14 and 7 ng, respectively, could easily be enough to result in reporting erroneous quantities of PFCs in environmental matrices, where often the concentrations reported are in the picogram per millilitre range. For example, if a researcher spiked 100 ng of 3M ECF-produced PFOS into a 100 mL water sample, the concentration of the impurities unknowingly added could be 10–100-fold greater than those in the unadulterated sample (Yamashita et al. 2004). This makes the choice of standards and sources of those

Table 4. Purity verification of PFOS and PFOA materials (ng mL⁻¹).

Compound	Branched										Branched		
	PFBA	PFPA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoA	PFBS	PFHxS	PFOS	PFDS
ECF PFOA (3M)	≤0.25	≤0.5	0.50	1.29	NA ^a	≤0.1	≤0.5	0.66	≤0.25	≤0.25	≤0.5	0.13	≤0.1
L-PFOA (Oakwood)	0.52	≤0.5	≤0.1	0.74	≤0.1	≤0.1	≤0.5	0.31	≤0.25	≤0.25	≤0.5	≤0.1	≤0.1
L-PFOA (Wellington)	≤0.25	≤0.5	≤0.1	≤0.1	≤0.1	≤0.1	≤0.5	0.22	≤0.25	≤0.25	≤0.5	≤0.1	≤0.1
ECF PFOS (3M)	1.21	0.82	1.28	0.59	0.59	0.11	≤0.5	0.24	≤0.25	1.72	7.31	NA	≤0.1
L-PFOS Na (Wellington)	≤0.25	≤0.5	≤0.1	≤0.1	≤0.1	≤0.1	≤0.5	0.32	≤0.25	≤0.25	≤0.5	≤0.1	≤0.1
L-PFOS K (Wellington)	≤0.25	≤0.5	≤0.1	≤0.1	≤0.1	0.14	≤0.5	0.29	≤0.25	≤0.25	≤0.5	≤0.1	≤0.1
PFOA [1,2- ¹³ C] (Perkin Elmer)	≤0.25	≤0.5	≤0.1	1.19	0.18	≤0.1	3.96	0.26	≤0.25	≤0.25	≤0.5	≤0.1	≤0.1
PFOA [1,2,3,4- ¹³ C] (Wellington)	≤0.25	≤0.5	≤0.1	≤0.1	0.21	≤0.1	≤0.5	0.33	≤0.25	≤0.25	≤0.5	0.85	≤0.1
PFOS [¹⁸ O ₂] (RTI)	0.38	≤0.5	1.57	≤0.1	0.32	≤0.1	≤0.5	0.24	≤0.25	≤0.25	≤0.5	0.85	≤0.1
PFOS [1,2,3,4- ¹³ C] (Wellington)	1.83	0.94	0.89	≤0.1	≤0.1	≤0.1	≤0.5	0.26	≤0.25	≤0.25	≤0.5	0.10	≤0.1
Instrument detection limit (ng mL ⁻¹)	0.25	0.5	0.1	0.1	0.1	0.1	0.5	0.1	0.25	0.25	0.5	0.1	0.1

Note: NA – not applicable.

standards very important, as using standards whose purity has only been verified using a nonspecific acid/base titration technique could result in the overestimation of several different PFCs.

Response factors of labeled and native PFOS and PFOA standards

It should be noted that when discussing the issue of response factor comparisons for any analytes determined by any chromatographic separation technique, the subsequent results are only valid for that particular chromatographic separation method and detection technique. This arises from the issue of signal suppression or enhancement which can result from the presence of co-eluting, or closely eluting, interferents.

The individual response factors for PFOS and PFOA are presented in Figures 1 and 2. In general, all of the PFOS and PFOA standards (both labeled and nonlabeled) followed the same pattern of relative response factors regardless of the matrix, although significant differences were seen when comparing standards within the same matrix, and more importantly differences were seen when comparing the same standard present in different matrices. For the PFOS standards, response factors were the greatest in serum extract, then methanol, and were the lowest in water. For the PFOA standards, the effects of each of the three matrices on response factors were less profound, although there were significant differences between PFOA in methanol and PFOA present in serum *versus* PFOA present in water. The most drastic difference seen between response factors as a result of matrix effects was the reduced response factor of native PFOS in water when compared to either methanol or serum. Native PFOA had a consistently lower response factor than either of its isotopically labeled standards in all three matrices. The response factor of the $^{13}\text{C}_4$ -labeled PFOA was also significantly higher than the $^{13}\text{C}_2$ -labeled standard. For the PFOS standards, the $^{18}\text{O}_2$ -labeled standard had a consistently greater response factor than either the $^{13}\text{C}_4$ -labeled PFOS or the native standard in all three matrices. However, unlike the PFOA standards where both labeled analytes response factors were greater than the nonlabeled standard, the $^{13}\text{C}_4$ -PFOS standard was consistently lower than the native PFOS response factor. This suggests that there can be significant differences between

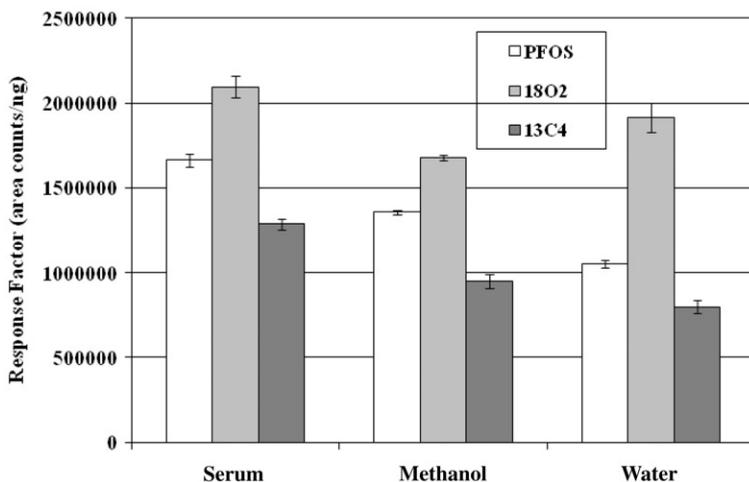


Figure 1. Individual response factors (mean \pm SD; $n = 3$) of PFOS compounds.

the effects that isotopical labelling may have on the perfluorosulfonates and the perfluorocarboxylates. Although there were many significant differences as a result of matrix effects, further studies should be done using different instruments to confirm that these effects are in fact a result of matrix effects.

The effects of combining labeled and nonlabeled standards in methanol, serum, and water resulted in consistently greater response factors for both the $^{13}\text{C}_4$ -labeled PFOA and the $^{18}\text{O}_2$ -labeled PFOS than the other standards present as mixtures, regardless of the matrix (Figures 3 and 4). However, the relative response factors were similar when comparing both the individual experiments to the mixtures, so this increase in signal does not appear to be mixture-dependent. The mixed PFOA and PFOS standards followed similar trends for all of the analytes. However, all of the combined PFOS standards present in water were significantly suppressed relative to

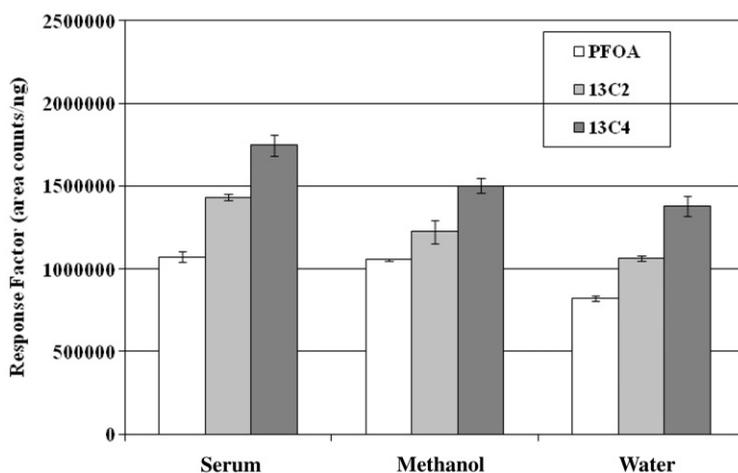


Figure 2. Individual response factors (mean \pm SD; $n = 3$) of PFOA compounds.

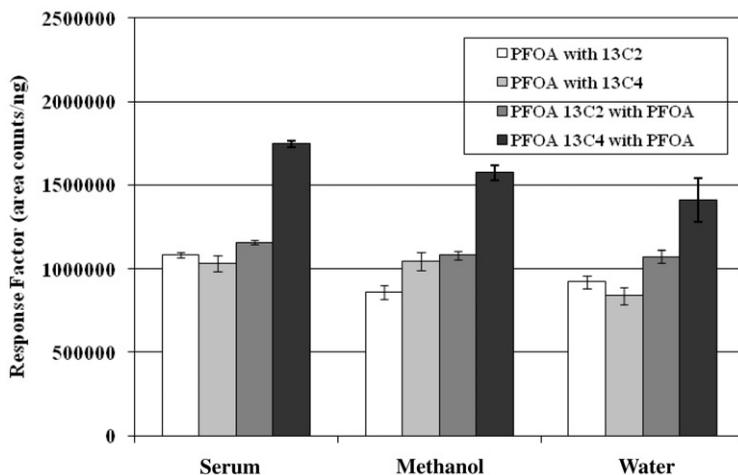


Figure 3. Response factors (mean \pm SD; $n = 3$) of PFOA compounds present concurrently with other PFOA standards.

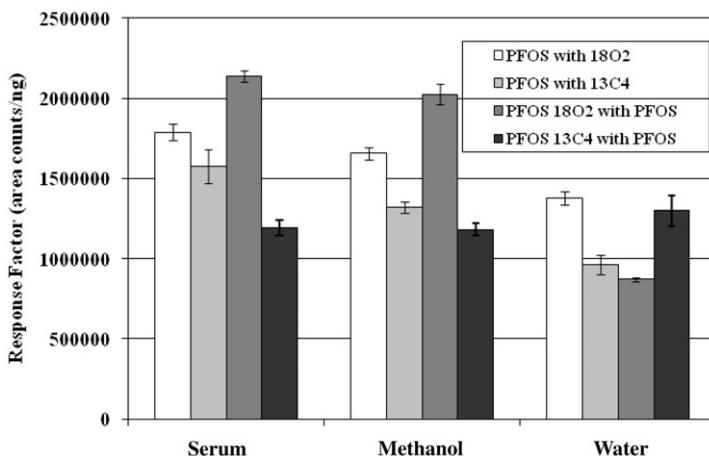


Figure 4. Response factors (mean \pm SD; $n = 3$) of PFOS compounds present concurrently with other PFOS standards.

the other matrices. The effect of having either $^{13}\text{C}_2$ -PFOA or $^{13}\text{C}_4$ -PFOA present with native PFOA appears to cause no significant effects on response factors, compared to the response of the native PFOA present individually.

The results of these studies demonstrate that native PFOS and PFOA standards behave differently compared to their isotopically labeled counter parts, and this was especially true for $^{13}\text{C}_4$ -PFOA and $^{18}\text{O}_2$ -labeled PFOS standards; however, it should be noted that additional experiments using multiple HPLC-MS/MS instruments should be done to confirm that these differences are seen on all ESI-MS/MS instruments. This study also shows that, in general, there is little difference in the response factors of both labeled and nonlabeled standards when they are present concurrently or independently. For both PFOS and PFOA the type of matrix in which standards were prepared had the most profound effects on comparing serum to both methanol and water, but significant differences were also seen on comparing methanol with water. These differences in response factor as a result of matrix effects would be especially problematic if a researcher was using a methanol-based calibration curve to measure the concentrations of PFOS and/or PFOA present in water and serum, as it would cause an overestimation in the response to the serum samples and a underestimation in response to the water samples relative to the methanol-based curve. This difficulty in accurately reporting concentrations of PFCs in water samples has been reported before (Taniyasu et al. 2005), and the present study further supports the need for better extraction methods and the use of matrix-matched calibration curves.

Accuracy of isomer quantification against linear PFOS and PFOA standards

It is known that PFOS and PFOA exist in the environment as a mixture of multiple isomers (Benskin, Bataineh, and Martin 2007; De Silva and Maybury 2006; Furdui et al. 2008), and that these isomers might behave differently both analytically and toxicologically (De Silva and Maybury 2006). However, most standards that are used to quantify environmental samples consist of a primarily linear standard. It has

been assumed that these linear standards will behave similarly under HPLC-MS/MS conditions, and that using them to quantify multiple branched isomers will result in accurate measurements.

The use of linear PFOS or PFOA to quantify branched materials (sum of integration from all isomer peaks) resulted in an underestimation of approximately 21% for PFOS (average of both linear standards) over a concentration range of 0.5–100 ng mL⁻¹ and 12% for PFOA over a concentration range of 0.1–100 ng mL⁻¹ (Figures 5 and 6). Calibration curves made using L-PFOSNa resulted in concentrations that were closer to the actual known concentrations, than to which were reported using calibration curves generated using L-PFOSK, although the difference was relatively small; approximately 2%, which is within the method uncertainty. The overall difference seen between the linear standards for both PFOS and PFOA, compared to that of the multiple branched compounds, is not likely due to the counter ion present, as the 3M ECF PFOS material had the same counter ion present as the linear Wellington standard. The implications of this under-prediction of multiple-branched isomer measurement in environmental samples is significant

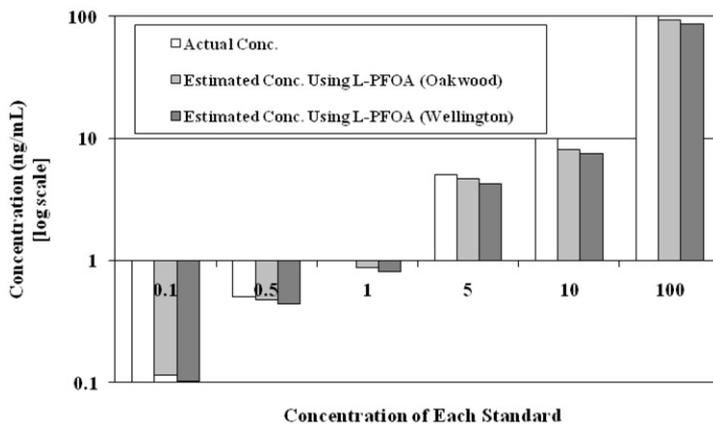


Figure 5. Accuracy of isomer quantification using linear only PFOA standards.

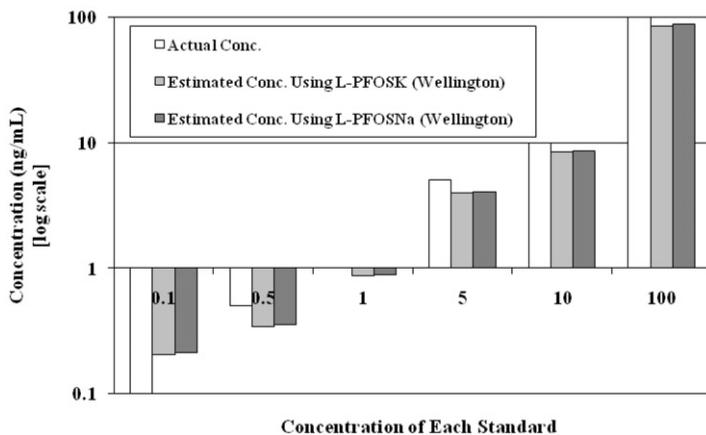


Figure 6. Accuracy of isomer quantification using linear only PFOS standards.

and seems to be increasingly important at either end of the calibration curves, which adds uncertainty to the already more uncertain ends of the calibration curve, suggesting that there may be an optimal working range for these standards. This under-prediction alone may not be enough to cause gross misrepresentation of an analyzed environmental sample, but it is another one of the many issues that hamper accurate PFCs research.

Conclusions

It was shown that depending on the sources, both PFOS and PFOA standards may contain significant amounts of impurities, which could potentially cause a significant over-estimation of many environmentally relevant PFCs. We have also shown that not all reported purities of standards are necessarily accurate and that the choice of suppliers is therefore, very important. The response factors of isotopically labeled and native standards were compared, and it was observed that not all labeled analytes have the same response factor as their nonlabeled complements, and that depending on the matrix there may be significant suppression or enhancement of response relative to a methanol-based standard. Linear standards were used to quantify branched PFOS and PFOA standards which showed that, in general, the use of linear standards may cause a marked underprediction of concentrations, and that the working range of these standards may be limited. In general, these results show that unless great care is taken to ensure proper standard selection and use, researchers may generate data that could be significantly inaccurate.

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References

- 3M 2000. Phase-out plan for PFOS-based products. *US EPA Docket AR 226-0588*.
- Arsenault, G., B. Chittim, J. Gu, A. McAlees, R. McCrindle, and V. Robertson. 2008a. Separation and fluorine nuclear magnetic resonance spectroscopic (^{19}F NMR) analysis of individual branched isomers present in technical perfluorooctanesulfonic acid (PFOS). *Chemosphere* 73: S53-9.
- Arsenault, G., B. Chittim, A. McAlees, R. McCrindle, N. Riddell, and B. Yeo. 2008b. Some issues relating to the use of perfluorooctanesulfonate (PFOS) samples as reference standards. *Chemosphere* 70: 616-25.
- Benskin, J.P., M. Bataineh, and J.W. Martin. 2007. Simultaneous characterization of perfluoroalkyl carboxylate, sulfonate, and sulfonamide isomers by liquid chromatography-tandem mass. *Analytical Chemistry* 79: 6455-64.

- De Silva, A.O., and S.A. Maybury. 2006. Isomer distribution of perfluorocarboxylates in human blood: Potential correlation to source. *Environmental Science & Technology* 40: 2903–9.
- Furdui, V.I., P.A. Helm, P.W. Crozier, C. Lucaciu, E.J. Reiner, C.H. Marvin, D.M. Whittle, S.A. Mabury, and G.T. Tomy. 2008. Temporal trends of perfluoroalkyl compounds with isomer analysis in lake trout from Lake Ontario (1979–2004). *Environmental Science & Technology* 42: 4739–44.
- Giesy, J.P., and K. Kannan. 2001. Global disiribution of perfluorooctane sulfonate in wildlife. *Environmental Science & Technology* 35: 1339–42.
- Hansen, K.J., L.A. Clemen, M.E. Ellefson, and H.O. Johnson. 2001. Compound-Specific, Quantitative Characterization of Organic Fluorochemicals in Biological Matrices. *Environmental Science & Technology* 35: 766–70.
- Higgins, C.P., J.A. Field, C.S. Criddle, and R.G. Luthy. 2005. Quantitative determination of perfluorochemicals in sediments and domestic sludge. *Environmental Science & Technology* 39: 3946–56.
- Jacoby, C.B. 2008. A thorough understanding of the characteristics of perfluorinated acid reference materials is paramount to generate high quality analytical data. To 35th Annual Society of Environmental Toxicology and Chemistry, November 11–15, in Milwaukee, USA. WP174.
- Kannan, K., S. Corsolini, J. Falandysz, G. Fillmann, K.S. Kumar, B.G. Loganathan, M.A. Mohd, J. Olivero, N. Van Wouwe, J.H. Yang, and K.M. Aldaous. 2004. Perfluorooctanesulfonate and Related Fluorochemicals in Human Blood from Several Countries. *Environmental Science & Technology* 38: 4489–95.
- Martin, J.W., K. Kannan, U. Berger, P. Pim de Voogt, J. Field, J. Franklin, J.P. Giesy, et al. 2004. Analytical Challenges Hamper Perfluoroalkyl Research. *Environmental Science & Technology* 38: 248A–55A.
- Paul, A.G., K.C. Jones, and A.J. Sweetman. 2009. A first global production, emission, and environmental inventory for perfluorooctane sulfonate. *Environmental Science & Technology* 43: 386–92.
- Prevedouros, K., I.T. Cousins, R.C. Buck, and S.H. Korzeniewski. 2006. Sources, fate and transport of perfluorocarboxylates. *Environmental Science & Technology* 40: 32–43.
- Reagen, W.K., M.E. Ellefson, K. Kannan, and J.P. Giesy. 2008a. Comparison of extraction and quantification methods of perfluorinated compounds in human plasma, serum, and whole blood. *Analytica Chimica Acta* 628: 214–21.
- Reagen, W.K., K.R. Lindstrom, C.B. Jacoby, R.G. Purcell, T.A. Kestner, R.M. Payfer, and J.W. Miller. 2008b. In environmental characterization of 3m electrochemical fluorination derived perfluorooctanoate and perfluorooctanesulfonate, To 35th Annual Society of Environmental Toxicology and Chemistry, November 11–15, in Milwaukee, USA. 455.
- Seacat, A., P.J. Thomford, K.J. Hansen, L.A. Clemen, S.R. Eldridge, C.R. Elcombe, and J.L. Butenhoff. 2003. Sub-chronic Dietary Toxicity of Potassium Perfluorooctanesulfonate in Rats. *Toxicology* 183: 117–31.
- Taniyasu, S., M.K. So, A. Gulkowska, K. Kannan, P.K.S. Lam, J.P. Giesy, and N. Yamashita. 2005. Trace level analysis for per and poly-fluorinated compounds. International Symposium of Fluorinated Alkyl Organics in the Environment, August 18–20, Toronto, Canada. ANA032.
- Van Leeuwen, S.P.J., A. Karrman, B. Van Bavel, J. De Boer, and G. Lindstrom. 2006. Struggle for Quality in Determination of Perfluorinated Contaminants in Environmental and Human Samples. *Environmental Science & Technology* 40: 7854–60.
- Yamashita, N., K. Kannan, S. Taniyasu, Y. Horii, T. Okazawa, G. Petrick, and T. Gamo. 2004. Analysis of Perfluorinated Acids at Parts-Per-Quadrillion Levels in Seawater Using Liquid Chromatography-Tandem Mass Spectrometry. *Environmental Science & Technology* 38: 5522–28.