SUPPORTING INFORMATION

Method and Validation for the Analysis of Perfluorinated Compounds in Water by Pre-Sampling Isotope Dilution-Direct Injection- LC/MS/MS.

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Sample preparation and analyte adsorption

During the validation of the method, it was observed that the internal standard (IS) and target analyte area counts were decreasing during the course of the run for PFDA, PFUnA, and PFDoA, when using clear 2-mL borosilicate glass autovials. This was the first time this was observed, since the method had been in use for several months with only occasional area response losses noticed for PFDoA. After reviewing all aspects of the analysis to determine the possible source of decreasing response, it was determined that the change in the IS area count response for these analytes was not instrument or solvent related, but had to be the result of a change in the preparation of the samples. Since the only sample preparation step for this method involved 1:1 dilution of the sample with methanol, the 2mL glass autovial used in the laboratory was investigated.

The autovials that were investigated included 2-mL borosilicate clear glass autovials manufactured by Agilent (stock number 5182-0543), silanized 2-mL borosilicate clear glass autovial from Agilent (stock number 5183-4495) and 1-mL polypropylene autovials from National Scientific (stock number C4011-14). To evaluate the possible effects due to autovial type, the method validation samples, prepared in laboratory reagent water (Milli-Q™ water generated from a Milli-Q™ water system from Millipore Corporation, containing hardness at 165 mg equivalent $CaCO_3$ L^{-1}) were diluted 1:1 with methanol into each of the three vial types. A comparison of the IS area counts for the four different sample types; calibration standards, continuing calibration verification samples, method blanks, and method validation samples, were evaluated during the course of a 36 hour analytical run. An RSD <20% was considered acceptable, based on method acceptance criteria regarding precision, with similar results observed for the target analyte area response. The RSD results are shown in Table 1 and demonstrate that the autovial type; borosilicate glass, silanized glass or polypropylene, has an effect on area count stability for PFDA, PFUnA, and PFDoA when analyzing a well-mixed water sample aliquot diluted 1:1 with methanol in an autovial.

Similar observations were made for samples diluted 1:1 with methanol into amber 2-mL borosilicate glass autovials and analyzed for PFOS. A series of seventy-eight samples were analyzed in amber 2-mL borosilicate glass autovials with the [13*C4*]PFOS surrogate recovery standard (SRS) having a peak area RSD of 49% while the [13*C8*]PFOS IS peak area RSD was 48%. When these same samples were diluted 1:1 with methanol into 1-mL polypropylene autovials analyzed for PFOS, the peak area RSD for the [13*C4*] PFOS surrogate was 17% while the [13*C8*]PFOS IS peak area RSD was 10%.

Table 1. Evaluation of autovial types for the analysis of water samples diluted 1:1 with methanol for PFDA, PFUnA, and PFDoA.

Samples analyzed during a 36 hour analytical run	Internal Standard Area Count RSD (%)		
2-mL clear borosilicate glass vial	**[13*C6*]PFDA**	**[13*C7*]PFUnA**	**[13*C2*]PFDoA**
RSD of initial calibration curve (n=15)	17	**39**	**73**
RSD of CCV samples (n=4)	**42**	**132**	**186**
RSD of method blank (n=15)	**73**	**129**	**153**
RSD of control samples (n=23)	**44**	**64**	**88**
	Internal Standard Area Count RSD (%)		
2-mL silanized glass vial	**[13*C6*]PFDA**	**[13*C7*]PFUnA**	**[13*C2*]PFDoA**
RSD of initial calibration curve (n=15)	8.7	11	13
RSD of CCV samples (n=4)	11	18	12
RSD of method blank (n=15)	9.5	15	**27**
RSD of control samples (n=23)	12	13	**24**
	Internal Standard Area Count RSD (%)		
1-mL polypropylene vial	**[13*C6*]PFDA**	**[13*C7*]PFUnA**	**[13*C2*]PFDoA**
RSD of initial calibration curve (n=15)	11	14	12
RSD of CCV samples (n=4)	7.2	10	10
RSD of method blank (n=15)	4.7	5.9	7.5
RSD of control samples (n=23)	8.5	7.3	**21**

Matrix Effect

Early efforts at developing a direct injection analysis method for local municipal drinking water found that samples were often not meeting the method acceptance criteria for FMS recovery (100%±30%) for PFOS when analyzed against an IS calibration curve prepared in laboratory Milli-Q™ water. It was found that there was an approximate 30% difference in the peak area response for PFOS in a 1ppb control sample prepared in carbon filtered municipal drinking water as compared to a 1ppb standard of PFOS in laboratory Milli-Q™ water. Based on limited information on the water chemistry (i.e. hardness, alkalinity, sulfate, iron, potassium), an investigation into the effect of anions and cations present in groundwater matrices was evaluated. Control samples were prepared in laboratory Milli-Q™ water containing various salts of Mg^{2+}, Ca^{2+}, sulfate (SO_4^-), carbonate (CO_3^-), and chloride (Cl^-) to evaluate matrix effects. The results of the evaluation showed that the cations Mg^{2+} and Ca^{2+} appear to suppress the analyte signal for PFOS by ~30-40% when analyzed against a calibration curve prepared in Milli-Q™ water. Data from this investigation was presented in a poster titled "Development and Utilization of a Large-Volume Direct Injection LC/MS/MS Method for the Analysis of PFCs in Water" at the SETAC North America 29Th Annual Meeting in Tampa bay Florida in 2008.

Laboratory reagent water was prepared by adding 25 mg L^{-1} Mg^{2+} (as magnesium acetate) and 25 mg L^{-1} Ca^{2+} (as calcium acetate) to Milli-Q™ water to produce a calculated water hardness of 165 mg L^{-1}, similar to what was observed in local municipal drinking water.[1] The use of laboratory reagent water for the preparation of calibration standards improved the recoveries for target analyte FMS samples for groundwater matrices when analyzed by direct injection (DI) analysis. The development of the validated DI-LC/MS/MS analytical method presented herein included the use of 1:1 laboratory reagent water and methanol, which stabilized the low level calibration standards for the long chain PFCAs, extending the use of the calibration curve.

To demonstrate the effects that matrix matched calibration standards, the use of IS, and solvent dilution, has on PFOS recovery in environmental water samples, a series of control samples were prepared in chlorinated (1 mg L^{-1}), pH 8 water for organic analysis (water harness of 100 mg L^{-1}), as specified in ANSI-NSF Standard 61 section B.9.6.[2] The control samples were analyzed against a calibration curve prepared in laboratory Milli-Q™ water containing IS, and prepared with and without 1:1 dilution with methanol. Control samples were evaluated by both IS calibration and external calibration. The effectiveness of matrix dilution and IS use on accuracy and precision can be found in Table 2. The data shows that when samples containing 100 mg L^{-1} hardness are analyzed for PFOS by external standard calibration against a curve prepared in Milli Q water, recoveries for PFOS range from 43.8% - 70.6%. The use of IS brings the recovery for PFOS to 119-124%. By diluting the samples 1:1 with methanol, the matrix effects associated with a water harness of 100 mg L^{-1} are eliminated regardless of whether the samples are quantitated by internal or external standard calibration, with recoveries ranging from 90.3 – 109%. In addition to 1:1 dilution with methanol, the use of an IS specific for PFOS, in this case [13*C8*]PFOS, appears to correct for these matrix effects.

Table 2. Mean recovery and precision for PFOS to evaluate matrix effects [a].

		Direct Inject IS calibration		Direct Inject External calibration		1:1 dilution with methanol IS calibration		1:1 dilution with methanol External calibration	
Analyte	Spike level ng mL^{-1}	Mean Recovery (%)	RSD (%)	Mean Recovery (%)	RSD (%)	Mean Recovery (%)	RSD (%)	Mean Recovery (%)	RSD (%)
Chlorinated NSF 61, pH 8 water (100 mg L^{-1} hardness) with sodium thiosulfate addition									
PFOS	0.0922	124	3.3	51.8	12	104	4.0	90.3	2.8
PFOS	0.922	119	2.1	58.5	8.1	101	1.1	97.1	2.7
PFOS	9.22	119	0.48	70.6	3.1	102	0.0	106	4.1
$^{13}C_4$-PFOS	0.0950	123	1.6	46.4	16	108	4.6	92.2	4.5
$^{13}C_4$-PFOS	0.950	118	2.6	52.2	8.2	101	2.8	96.9	3.3
Chlorinated NSF 61, pH 8 water (100 mg L^{-1} hardness) without sodium thiosulfate addition									
PFOS	0.0922	122	1.7	43.8	3.7	98.8	1.1	90.8	2.7
PFOS	0.922	122	1.3	54.6	15	100	2.2	94.1	4.2
PFOS	9.22	122	0.47	64.8	12	101	0.57	109	5.6
$^{13}C_4$-PFOS	0.0950	122	3.1	38.7	5.9	103	3.4	93.3	0.81
$^{13}C_4$-PFOS	0.950	121	0.83	48.7	17	101	1.1	95.0	3.4

[a] Control samples were prepared in triplicate (n=3) at each spiking level in ANSI-NSF Standard 61 pH 8 water with hardness = 100 mg L^{-1} as calcium carbonate and chlorinated at 1 mg L^{-1}. Control samples were analyzed against a calibration curve prepared in laboratory Milli-Q™ water and evaluated by IS calibration and external calibration. Control samples were analyzed with and without 1:1 dilution with methanol.

The use of laboratory reagent water was added to the method as a means to address the signal suppression that was observed due to matrix effects on PFDA, PFUnA, and PFDoA and the signal enhancement that was observed for FOSA. Table 3 demonstrates these matrix effects in a side by side comparison of the analyte peak area of fortified Milli-Q™ water and laboratory reagent water. Control samples were prepared at three concentration levels in triplicate in high density polyethylene (HDPE) containers and diluted 1:1 with methanol into 1 mL polypropylene autovials. Peak area suppression in hard water relative to Milli-Q™ water was observed for PFDA (16%), PFUnA (40%), [13*C2*]PFUnA SRS (38%) and PFDoA (53%). The same observation trends were made for the IS peak area for [13*C2*]PFDA (15%), [13*C7*]PFUnA (40%), and [13*C2*]PFDoA (55%). Peak signal enhancement was observed for PFOSA (25%) and the IS peak [13*C8*]PFOSA (30%). What this demonstrates is that the use of isotopically-labeled analogues of the PFC method analytes is critical for accurate quantitation of water samples that contain these matrix components. In addition to analyte suppression or enhancement, the use of these ISs can also account for analyte adsorption of longer chain PFCA analytes, as was reported by Berger et. al (2011).[3] As Berger notes, losses of more than 30% were observed for PFUnA and PFDoA after 28 days. These findings highlight that an IS, when applied incorrectly, can potentially bias analytical results. For example, it would not be appropriate to quantitate water samples for PFUnA and PFDoA that have high hardness levels, with the [13C2]PFDA IS, due to the

differences in matrix effects that would bias the samples results low.

Table 3. Effects from matrix (water hardness); suppression or enhancement on analyte response.

Control Samples[a]	Analyte	*Peak Area Milli Q water*	*Peak Area laboratory reagent water*	*% difference due to matrix* [b]
0.1 ng mL^{-1} (average n=3)	PFDA	257298	220904	-14
1 ng mL^{-1} (average n=3)	PFDA	2719337	2209074	-19
10 ng mL^{-1} (average n=3)	PFDA	24445301	20975227	-14
			Average	**-16**
0.1 ng mL^{-1} (average n=3)	[13C2]PFDA-IS	2004580	1776440	-11
1 ng mL^{-1} (average n=3)	[13C2]PFDA	2120648	1715339	-19
10 ng mL^{-1} (average n=3)	[13C2]PFDA	2057083	1767652	-14
			Average	**-15**
0.1 ng mL^{-1} (average n=3)	PFUnA	182963	123546	-32
1 ng mL^{-1} (average n=3)	PFUnA	1917541	1073464	-44
10 ng mL^{-1} (average n=3)	PFUnA	18629264	10689108	-43
			Average	**-40**
0.1 ng mL^{-1} (average n=3)	[13C2]PFUnA-SRS	143325	97434	-32
1 ng mL^{-1} (average n=3)	[13C2]PFUnA-SRS	1532816	850900	-44
			Average	**-38**
0.1 ng mL^{-1} (average n=3)	[13C7]PFUnA-IS	1307777	886504	-32
1 ng mL^{-1} (average n=3)	[13C7]PFUnA-IS	1398317	765314	-45
10 ng mL^{-1} (average n=3)	[13C7]PFUnA-IS	1458421	812577	-44
			Average	**-40**
0.1 ng mL^{-1} (average n=3)	PFDoA	154311	85238	-45
1 ng mL^{-1} (average n=3)	PFDoA	1684531	706135	-58
10 ng mL^{-1} (average n=3)	PFDoA	16006981	6876068	-57
			Average	**-53**
0.1 ng mL^{-1} (average n=3)	[13C2]PFDoA-IS	1166829	622247	-47
1 ng mL^{-1} (average n=3)	[13C2]PFDoA-IS	1280663	532713	-58
10 ng mL^{-1} (average n=3)	[13C2]PFDoA-IS	1325061	547756	-59
			Average	**-55**
0.1 ng mL^{-1} (average n=3)	PFOSA	81465	91980	15
1 ng mL^{-1} (average n=3)	PFOSA	838944	1054306	27
10 ng mL^{-1} (average n=3)	PFOSA	6429103	8511592	33
			Average	**25**
0.1 ng mL^{-1} (average n=3)	[13C8]PFOSA-IS	728501	893489	26
1 ng mL^{-1} (average n=3)	[13C8]PFOSA-IS	775091	1012923	32
10 ng mL^{-1} (average n=3)	[13C8]PFOSA-IS	607148	795496	32
			Average	**30**

[a] Control samples were prepared in triplicate in HDPE containers in either laboratory Milli-Q™ water or laboratory reagent water. Control samples were prepared at 0.1, 1, and 10 ng mL^{-1}, with all samples pre-spiked with an IS mix at 1 ng mL^{-1}. An aliquot was removed and diluted 1:1 with methanol into a 1-mL polypropylene autovial just prior to sample analysis.

[b] To determine matrix suppression, the percent difference was calculated by subtracting the average analyte peak area for the control samples in laboratory Milli-Q™ water from the average analyte peak area for the control samples in laboratory reagent water and dividing by the average analyte peak area for the control samples in laboratory Milli-Q™ water.

EPA Method 537 comparison with DI-LC/MC/MC method

Analysis of PFC method analytes in chlorinated, pH 8 drinking water was conducted to compare the recovery of control samples analyzed by the DI-LC/MS/MS method and EPA Method 537. Control samples were prepared in 200mLs of synthetic chlorinated drinking water at pH 8. Two sets of five replicate samples were fortified with PFCAs (C6-C12) and PFSAs (C4, C6, and C8), at 0.1 ng mL^{-1}. Sample containers for control samples to be analyzed by the DI-LC/MS/MS method were pre-spiked with ISs at 1 ng mL^{-1} and SRSs [$^{13}C_4$]PFOA, [$^{13}C_2$]PFUnA, and [$^{13}C_4$]PFOS at 0.1 ng mL^{-1}. Both sets of samples were held for 7 days prior to analysis by DI-LC/MS/MS and EPA Method 537. At day 7, the control samples analyzed by EPA Method 537, the SRSs were added at 0.1 ng mL^{-1} just prior to sample extraction. The concentrated extract was diluted 1:10 with methanol, fortifed with ISs 1 ng mL^{-1} and analyzed against a 96:4% (vol/vol) methanol: water curve from 0.5 – 15 ng mL^{-1}. At day 7, the control samples analyzed by DI-LC/MS/MS were diluted 1:1 with methanol and analyzed against a calibration curve prepared in 50:50 laboratory reagent water:methanol. The mean recoveries for the PFC method analytes analyzed by DI-LC/MS/MS ranged from 88.4-95.7% (77.6-87.8% for SRSs). The mean recoveries for the PFC method analytes analyzed by EPA Method 537 ranged from 91.6-105% (86.2-90.4% for SRSs) (Figure 1). The average absolute difference between the EPA Method 537 and DI-LC/MS/MS measurements for the method analytes and SRSs was 8%.

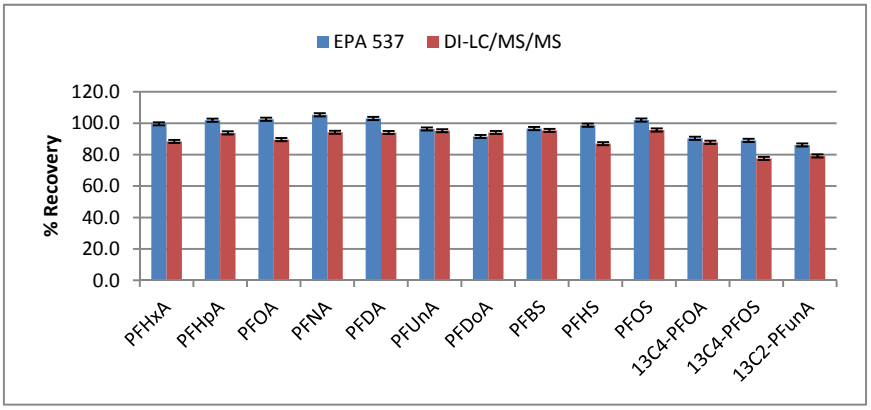

Figure 1. Mean Recovery of Control Samples (n=5) prepared at 0.1 ng mL^{-1} in Synthetic Chlorinated Drinking Water and analyzed by EPA 537 and DI-LC/MS/MS.

1 Eaton, A. D.; Clesceri, L. S.; Greenberg, A. E., Eds. Standard Methods for the Examination of Water and Wastewater, 19th ed.; American Public Health Association: Washington, DC, 1995; Physical & Aggregate Properties, Part 2340 B, p 2-36.

2 NSF International Standard / American National Standards Institute, Drinking Water System Components – Health Effects, NSF/ANSI 61, http://www.nsf.org, 2011.

3 U. Berger, M.A. Kaiser, A. Karrman, J.L. Barber, S.P.J. van Leeuwen, Recent developments in trace analysis of poly- and perfluoralkyl substances, Anal. Bioanal. Chem, 2011, 400, 1625-1635.