DETERMINATION OF PERFLUORINATED COMPOUNDS IN NIST STANDARD REFERENCE MATERIALS USING INDEPENDENT ANALYTICAL METHODS

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Introduction:
Perfluorinated compounds (PFCs) are being determined in human samples worldwide to assess the potential routes of human exposure[1-6]. Issues with the reliability and quality of PFC analysis have been discussed since 2003[7], and with the vast number of laboratories reporting data, a tool for evaluating individual laboratory performance is essential to improve the accuracy and comparability of the chemical measurements. The National Institute of Standards and Technology (NIST) provides over 1300 Standard Reference Materials (SRMs) that can be used to evaluate the overall performance of a laboratory's method.

Using previous sample preparation and instrumental analysis methods as a guide, this study's aim was to develop, implement, and compare different methods for the analysis of PFCs in NIST SRM 1950 Human Plasma. SRM 1950 is a new reference material that was developed to identify and quantify metabolites in plasma samples. SRMs 1957 and 1958 (Organic Contaminants in Non-fortified and Fortified Human Serum, respectively), previously certified for PFC concentrations, were analyzed concurrently as control materials. The methods used in this study combine previously developed sample preparation methods with two different liquid chromatography (LC) stationary phases in unique ways to compare them robustly side-by-side. The methods offer independence from each other which is an important part of the certification process[8]. This abstract details the analytical approaches used to measure PFCs and provides the reference values assigned to SRM 1950.

Material and methods:
For all sets of SRM 1950 extracted (6 replicates per set), the calibrants, three procedural blanks, three replicates of SRM 1957, and three replicates of SRM 1958 were processed in the same manner as the samples. Figure 1 shows a flow diagram for the methods used in this study.

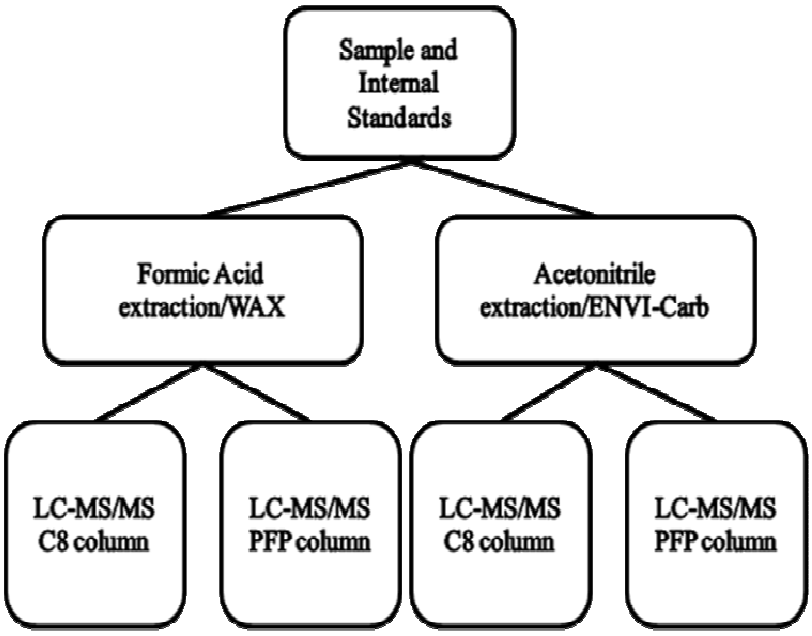

Figure 1. Methods used for the analysis of PFCs in the current study (see text for details).

Extraction/cleanup methods

Method 1: Formic Acid-WAX
A calibrant mixture of 15 PFCs, water (approximately 1 g, used as a procedural blank), and 1g aliquots of the three SRMs were weighed and gravimetrically spiked with the internal standard (IS) mixture (consisting of 7 mass labeled PFCs). Samples were diluted with 3 mL of 50% formic acid (by volume) and sonicated. Solid phase extraction (SPE) was carried out using Oasis weak anion exchange (WAX) SPE columns (Waters, Milford, MA) with the NIST method described in Keller et al.[9]. After the extracts were concentrated in volume, they were spiked with $^{13}C_2$-PFOA, vortexed, and transferred to autosampler vials. The extracts were analyzed using the two different LC methods described below.

Method 2: Acetonitrile-ENVI-Carb
Calibrants, blanks, and the SRMs were weighed into pre-cleaned glass centrifuge tubes and gravimetrically spiked with the IS mixture. Three mL of acetonitrile were added to the samples, and the samples were sonicated for 10 min. The samples were centrifuged and the supernatant was transferred to a clean tube. The acetonitrile supernatant was solvent exchanged with methanol (3 mL final volume). SPE was carried out using Supelco Supelclean ENVI-Carb SPE columns (3 mL, 250 mg 120 – 400 mesh; Bellefonte, PA) on the RapidTrace workstation (Caliper Life Sciences, Hopkinton, MA). The extracts were concentrated to approximately 0.5 mL, spiked with $^{13}C_2$-PFOA, vortexed, and transferred to autosampler vials. The extracts were analyzed using the two different LC methods described below.

LC-MS/MS methods
Extracts were analyzed using an Agilent 1100 HPLC (Santa Clara, CA) interfaced to an API 4000 negative electrospray ionization tandem mass spectrometer (Applied Biosystems, Foster City, CA).

Method 1: Agilent Zorbex Eclipse Plus C8 Column
Samples (5 µL) were injected onto an Agilent Zorbex Eclipse Plus C8 column (100 mm x 2.1 mm x 3.5 µm; Santa Clara, CA). The solvent gradient (flow rate 0.3 mL/min) expressed as volume fractions started at 50% methanol and 50% 20 mmol/L ammonium acetate in water, increased to 75% methanol by 10 min, held for 5 min, and increased to 95% methanol by 18 min, held for 2 min, before reverting back to original conditions at 20.5 min with a 14.5 min hold time. The MS/MS method included the optimization parameters from compound infusions, and at least two transitions per compound were monitored during analysis.

Method 2: Phenomenex Kinetex PFP Column
Five µL of sample were injected onto a Phenomenex Kinetex PFP column (50 mm x 3.0 mm x 2.6 µm; Torrance, CA). The solvent gradient (flow rate 0.3 mL/min) expressed as volume fraction started at 40% methanol and 60% 20 mmol/L ammonium acetate in water, increased to 65% methanol by 5 min, held for 5 min, and increased to 95% methanol by 12 min, held for 3 min, before reverting back to original conditions at 15.5 min with a 14.5 min hold time.

Results and discussion:
The methods achieved agreement with RSDs among methods below 10% for all measurable PFCs and agreement with the reference values assigned for SRMs 1957 and 1958 Certificates of Analysis (www.nist.gov/srm/) (Table 1). The two separation technique provided different selectivity for the PFCs. While the C8 stationary phase has been used previously for measurements of PFCs, to the author's knowledge, this is the first time a PFP phase has been utilized in PFC analysis. An added advantage of using the PFP column was the separation of interferences known to coelute with PFCs that have resulted in overestimation of some compounds[10,11]. The PFP column was able to separate the bile acid, taurodeoxycholic acid (TDCA), from PFOS in a short runtime (30 min).

Table 1. Average (standard deviation) of mass fractions of perfluorinated compounds (ng/g wet mass) measured in human serum SRMs 1957 and 1958 using four different methods.

	Reference Value ± Uncertainty	WAX-C8[9]	WAX-PFP	ENVI-Carb-C8	ENVI-Carb-PFP
			SRM 1957		
PFHpA	0.305 ± 0.051	0.256 (0.086)	0.298 (0.049)	0.317 (0.014)	0.323 (0.014)
PFOA	5.00 ± 0.44	4.60 (0.31)	4.56 (0.43)	4.44 (0.22)	4.38 (0.19)
PFNA	0.878 ± 0.076	0.701 (0.037)	0.690 (0.053)	0.840 (0.046)	0.824 (0.038)
PFDA	0.39 ± 0.12	0.336 (0.024)	0.335 (0.027)	0.340 (0.007)	0.338 (0.005)
PFUnA	0.172 ± 0.036	0.185 (0.011)	0.183 (0.010)	0.184 (0.010)	0.177 (0.004)
PFHxS	4.00 ± 0.83	3.91 (0.15)	4.05 (0.15)	3.68 (0.12)	3.71 (0.06)
PFOS	21.1 ± 1.3	21.3 (1.0)	20.6 (0.9)	20.9 (0.7)	19.7 (0.9)
			SRM 1958		
PFHpA		0.139 (0.010)	<0.161	0.280 (0.013)	0.281 (0.019)
PFOA	4.11 ± 0.17	3.86 (0.48)	3.77 (0.40)	3.88 (0.17)	3.84 (0.13)
PFNA	0.66 ± 0.13	0.605 (0.029)	0.613 (0.016)	0.681 (0.042)	0.677 (0.023)
PFDA		0.316 (0.103)	0.323 (0.116)	0.275 (0.012)	0.275 (0.008)
PFUnA		0.156 (0.013)	0.158 (0.015)	0.192 (0.012)	0.193 (0.015)
PFHxS	2.66 ± 0.07	2.70 (0.06)	2.66 (0.06)	2.84 (0.11)	2.88 (0.14)
PFOS	16.6 ± 0.9	16.0 (1.1)	16.2 (0.4)	16.3 (0.6)	16.4 (0.7)

Mass fractions are totals, inclusive of branched and linear isomers.
Reference values and uncertainty were previously reported and taken from the Certificates of Analysis.

Mass fractions of PFC analytes determined in SRM 1950 using the four methods are reported in Table 2. The results for PFOA, PFNA, PFDA, PFUnA, PFHxS, and PFOS were in good agreement (RSD < 10%) among methods; therefore, reference values for PFCs were assigned to SRM 1950 based on these measurements (Table 2). PFOS was detected at the highest level in SRM 1950, followed by PFOA and PFHxS. These three compounds comprised 93% of the total PFCs measured in SRM 1950. This pattern is similar to the pattern seen in other human SRMs[9]. Although the patterns among SRMs 1950, 1957, and 1958 are similar, the mass fractions for certain PFCs differ.

Table 2. Mass fraction of perfluorinated compounds (ng/g wet mass) measured in human plasma SRM 1950 using four different methods and newly calculated reference values.

	Formic Acid-WAX						**Acetonitrile-ENVI-Carb**						**Reference values**	
	C8 Column			PFP Column			C8 Column			PFP Column				
	Mean	SD	RSD (%)	Mean	SD	RSD (%)	Mean	SD	RSD (%)	Mean	SD	RSD (%)	Values	Uncertainty
PFBA	<0.288			<0.552			<3.04			<1.48				
PFPeA	<0.517			<0.679			<2.06			<3.33				
PFHxA	<0.696			<0.693			<0.648			<0.643				
PFHpA	<0.137			<0.171			0.282	0.008	3	0.280	0.010	3		
PFOA	3.39	0.48	14	3.42	0.52	15	3.17	0.18	6	3.19	0.19	6	3.21	0.06
PFNA	0.680	0.047	7	0.679	0.045	7	0.730	0.042	6	0.725	0.038	5	0.705	0.028
PFDA	0.374	0.109	29	0.374	0.108	29	0.316	0.016	5	0.312	0.010	3	0.315	0.006
PFUnA	0.177	0.036	20	0.179	0.036	20	0.184	0.010	5	0.181	0.012	7	0.182	0.003
PFDoA	<0.104			<0.105			<0.109			<0.0998				
PFTriA	<0.0708			<0.270			<0.182			<0.0686				
PFTA	<0.555			<0.937			<0.141			<0.716				
PFBS	<0.147			<0.147			<0.141			<0.141				
PFHxS	3.24	0.22	7	3.32	0.29	9	3.22	0.13	4	3.15	0.09	3	3.19	0.08
PFOS	10.4	0.4	4	10.5	0.4	4	10.2	0.4	4	10.5	0.4	4	10.4	0.1
PFOSA	<0.115			<0.295			<0.143			<0.110				

Mass fractions are totals, inclusive of branched and linear isomers. SD = standard deviation; RSD = relative standard deviation. Values shown as "<" a specified number describe the actual reporting limit.

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References:
1. Kannan K, Corsolini S, Falandysz J, Fillmann G, Kumar KS, Loganathan BG, Mohd MA, Olivero J, Van Wouwe N, Yang JH, Aldous KM. (2004) *Environ Sci Technol.* 38:4489-4495
2. Dallaire R, Ayotte P, Pereg D, Dery S, Dumas P, Langlois E, Dewailly E. (2009) *Environ Sci Technol*. 43:5130-5136
3. Ericson I, Domingo JL, Nadal M, Bigas E, Llebaria X, van Bavel B, Lindstrom G. (2009) *Arch Environ Contam Toxicol.* 57:631-638
4. Kato K, Wanigatunga AA, Needham LL, Calafat AM. (2009) *Anal Chim Acta*. 656:51-55
5. Liu J, Li J, Luan Y, Zhao Y, Wu Y. (2009) *Environ Sci Technol*. 43:4044-4048
6. Toms LM, Calafat AM, Kato K, Thompson J, Harden F, Hobson P, Sjodin A, Mueller JF. (2009) *Environ Sci Technol.* 43:4194-4199
7. Martin JW, Kannan K, Berger U, De Voogt P, Field J, Franklin J, Giesy JP, Harner T, Muir DCG, Scott B, Kaiser M, Jarnberg U, Jones KC, Mabury SA, Schroeder H, Simcik M, Sottani C, Van Bavel B, Karrman A, Lindstrom G, Van Leeuwen S. (2004) *Environ Sci Technol*. 38:248A-255A
8. May W, Parris R, Beck C, Fassett J, Greenberg R, Guenther F, Kramer G, Wise S. (2000) NIST Special Publication 260-136:available at http://ts.nist.gov/MeasurementServices/ReferenceMaterials/PUBLICATIONS.cfm
9. Keller JM, Calafat AM, Kato K, Ellefson ME, Reagen WK, Strynar M, O'Connell S, Butt CM, Mabury SA, Small J, Muir DC, Leigh SD, Schantz MM. (2010) *Anal Bioanal Chem.* 397:439-451
10. Hansen KJ, Clemen LA, Ellefson ME, Johnson HO (2001) *Environ Sci Technol.* 35:766-770
11. Benskin JP, Bataineh M, Martin JW (2007) *Anal Chem.* 79:6455-6464