Supporting Information

Discovery and implications of C_2 and C_3 perfluoroalkyl sulfonates in aqueous film forming foams (AFFF) and groundwater

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Chemicals. LC-MS grade methanol (> 99%) and GC grade 2,2,2-trifluoroethanol (> 99%) were purchased from Fisher Scientific (Fair Lawn, NJ). HPLC grade ethyl acetate (> 99%) and sodium chloride (> 99%) were purchased from Sigma Aldrich (Saint Louis, MO). B&J Brand® reagent water (HPLC grade, > 99%) and hydrochloric acid (6N) were purchased from VWR (Radnor, PA). Reagent grade ammonium acetate was acquired from Avantor Performance Materials (Center Valley, PA).

The PFS-MXA mixture of perfluoroalkyl sulfonoic acids (PFSAs) [perfluoro-1-butane sulfonate (PFBS), perfluoro-1-hexane sulfonate (PFHxS), perfluoro-1-heptane sulfonate (PFHpS), perfluoro-1-octane sulfonate (PFOS), and perfluoro-1-decane sulfonate (PFDS)] was purchased from Wellington Laboratories (Guelph, Ontario, Canada) in methanol with concentrations ranging from 1.77-1.93 µg/mL. The purity of the individual PFSAs in the PFS-MXA solution is > 98%. A mixture of mass-labeled perfluoroalkyl carboxylate and sulfonate

internal standards (MPFAC-MXA) containing perfluoro-1-hexane[$^{18}O_2$]sulfonate ([$^{18}O_2$]-PFHxS) and perfluoro-1-[1,2,3,4-$^{13}C_4$]octanesulfonate ([$^{13}C_4$]-PFOS) was purchased from Wellington Laboratories in methanol at a concentration of 2 mg/L and are between 94% and 99% isotopically pure.

Sample Extraction Method. Diluted sample (AFFF or groundwater) was added to a 15 mL polypropylene centrifuge tube (VWR) containing 0.97-1.0 g of sodium chloride. All samples for QTOF-MS and LC-MS/MS analyses were acidified with 10 µL of 6N HCl, but only samples for LC-MS/MS analysis were spiked with 1.05 ng of each isotopically-labeled internal standard. Samples for all analyses were extracted three times using 10% (v/v) 2,2,2-trifluoroethanol in ethyl acetate.

Modified LC Method. Chromatographic separations were achieved using an Agilent (Santa Clara, CA) 4.6 x 12.5 mm x 5 µm Zorbax silica guard column connected in series to a 4.6 x 12.5 mm x 5 µm Zorbax propylamine (NH_2) guard column. The silica-NH_2 guard columns were connected in series to an Agilent 4.6 x 100 mm x 3.5 µm Zorbax Eclipse Plus C18 analytical column. The aqueous mobile phase composition (A) was changed to 3% methanol in HPLC-grade water. The pre-column between the LC pumps and the 6-port valve was removed from the system, and no background PFAS contamination from the LC was observed in solvent blanks.

A shallow convex gradient was used to separate the analytes (Figure S1). The mobile phase composition was 3% MeOH in HPLC-grade water (A) and 10 mM ammonium acetate in HPLC-grade MeOH (B). Samples were loaded onto the guard columns for the first 3 minutes in 0% B. At 2.6 minutes, the needle, needle loop, and injector valve were bypassed to reduce gradient dwell time. The gradient was ramped to 25% B at 3.5 min and to 50% B at 4.5 min. At

6 min, the gradient was at 65% B and increased to 90% B at 10 min. The B mobile phase increased linearly from 10 min to 15 min (100% B) and was then held at 100% B for 4 min. The columns were allowed to reequilibrate at initial conditions for 8 min prior to the next injection. The flow rate was constant at 0.5 mL/min.

QTOF-MS Parameters. In scanning mode, quadrupole time-of-flight mass spectrometry (QTOF-MS; ABSciex, Framingham, MA) data was collected in 2200 cycles with a period cycle time of 1380 ms, a pulser frequency of 18.079 kHz, and an accumulation time of 250.0 ms. The curtain, nebulizer (gas 1), and drying (gas 2) gases were 25 psi, 50 psi, and 40 psi, respectively. The ionspray voltage was set at 4500 V with a temperature of 500°C, a collision energy of -10 V, and a declustering potential of -80 V. The starting and ending masses were 70 and 1000, respectively, for the survey scan. For the dependent (product ion) scan, the accumulation time was 180 ms, and the collision energy was -40 V. The starting and ending masses were 40 and 1000, respectively. Data were collected simultaneously from survey and product ion scanning modes. The instrument was calibrated with the APCI Negative Calibration Solution (ABSciex) before each sample was injected.

Error about the Mass Defect (CF_2 scale). The error about the mass defect (CF_2 scale) was determined using the uncertainty about the accurate mass (up to 5 ppm error).[1] From Eq 1 (main text), multiplying the error in the accurate mass by a constant did not change the magnitude of the error. Therefore, the mass (CF_2 scale) also had maximum error of 5 ppm. Similarly, in Eq 2 (main text), subtracting a constant from the mass (CF_2 scale) resulted in no change in the magnitude of the error. Thus, the mass defect (CF_2 scale) had a maximum error of 5 ppm.

Quantification by LC-MS/MS. To quantify PFEtS and PFPrS in all AFFF and groundwater samples, chromatographic separations were performed on an Agilent 1100 series HPLC (Santa Clara, CA) that was modified to inject 900 µL. The retention times of each PFSA are listed in Table S2. Details of the modified Backe *et al*[2] method are outlined above.

Analytes were detected using a TQ Detector (Waters Corporation, Milford, MA) triple quadrupole mass spectrometer that was controlled with MassLynx 4.1 (Waters Corporation) and interfaced with an electrospray ionization source. The MS/MS was operated in negative mode and multiple reaction monitoring (MRM). The precursor and product ions for both PFEtS and PFPrS observed by QTOF-MS were added to the LC-MS/MS method. The precursor ion for PFEtS was m/z 199 with daughter ions of m/z 80 and 99, while the precursor ion for PFPrS was m/z 249 with daughter ions of m/z 80 and 99. Capillary and cone voltages were not optimized for PFEtS and PFPrS and were instead based on the optimization of PFBS[2] since no commercial analytical standards were available. Parameters for the remaining PFSAs were used as previously described.[2]

Quantification of the PFEtS and PFPrS was performed using the analytical standard for PFBS, the internal standard [$^{18}O_2$]-PFHxS, and 1/*x* weighted calibration curve. Calibration standards were made in a blank extract, which consisted of reagent water taken through the extraction process. Solvent blanks consisted of 1 mL of blank extract, 500 µL of methanol, and 1.05 ng of each internal standard. Assuming equal molar response to PFBS, whole method limit of detection (LOD) for PFEtS and PFPrS were estimated to be 0.80 and 2.7 ng/L.[2] Whole method limit of quantification (LOQ) for PFEtS and PFPrS were estimated to be 1.0 and 3.3 ng/L, assuming equal molar response to PFBS.[2] All other PFSAs were quantified as described elsewhere.[2]

Sets of three quality control samples consisting of one solvent blank and two calibration standards were run every 8-10 AFFF and groundwater samples. Acceptable ranges for the quality control standards were ± 30% of the predicted values and below method LOD for the solvent blank. All solvent blanks were found to be below method LOD for all PFSAs.

Table S1. Concentrations of PFSAs in groundwater samples from Sites A-K.

Site	PFEtS (ng/L)	PFPrS (ng/L)	PFBS[a] (ng/L)	PFPeS[a] (ng/L)	PFHxS[a] (ng/L)	PFHpS[a] (ng/L)	PFOS[a] (ng/L)	PFNS[a] (ng/L)	PFDS[a] (ng/L)
A	34	1300	4100	8000	260,000	5900	69,000	99	<LOD[b]
B	530	12,000	24,000	14,000[c]	170,000[c]	4100[c]	65,000[c]	<LOD	26[c]
C	<LOD	19	85	49	740	37	1900	12	<LOD
D	<LOD	97	220	170	1200	18	130	<LOD	<LOD
E	31	2100	3100	1800	33,000	4700	550,000	3000	21
F	11	240	600	410	4800	180	7300	26	<LOD
G	4000	63,000	210,000[d]	220,000[d]	880,000[d]	23,000	380,000[d]	310	9.1
H	1400	17,000	43,000[c]	38,000	240,000[c]	11,000[c]	78,000[c]	<LOD	<LOD[c]
I	<LOD	62	130	160	5200	130	2500	<LOD	<LOD
J	220	2600	5800	4000	42,000	2200	92,000	580	30
K	7500	54,000	58,000[d]	70,000[d]	360,000[d]	23,000	210,000[d]	<LOD	<LOD[d]

[a]Whole method LOD and LOQ described in Backe et al.[2] [b]Concentration fell below whole method LOD. [c]Concentrations taken from Schultz et al.[3] [d]Concentrations taken from Backe et al.[2]

Table S2. Retention times of PFSAs.

	PFEtS	PFPrS	PFBS	PFPeS	PFHxS	PFHpS	PFOS	PFNS	PFDS
Retention time (min)	8.38	9.24	9.99	10.67	11.32	11.94	12.31	12.72	13.15

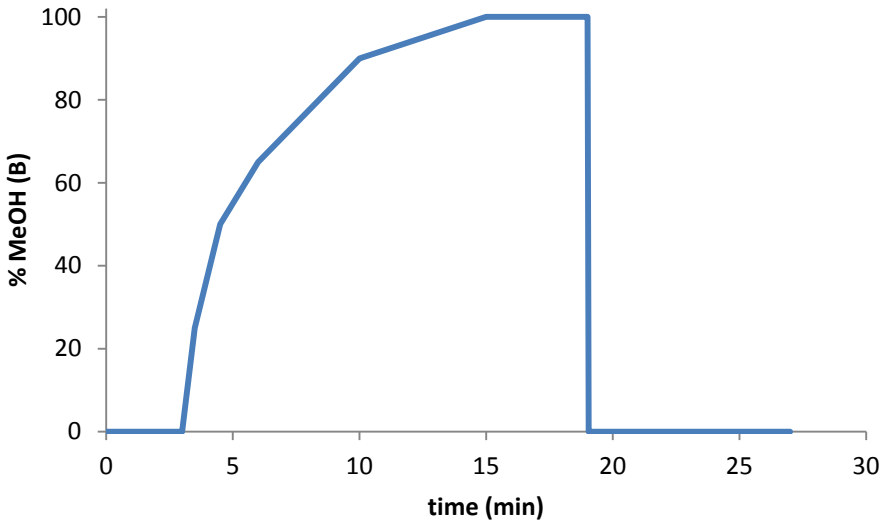

Figure S1. The HPLC gradient conditions used to separate and elute analytes. Flow rate is 0.5 mL/min.

References

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