



NIST Special Publication 260
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Characterization of Reference Materials 8690 to 8693

*Per- and Polyfluoroalkyl Substances (PFAS) in Four
Formulations of Aqueous Film-Forming Foams (AFFFs)*

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Abstract

The National Institute of Standards and Technology (NIST) Reference Materials (RMs) 8690 Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous Film-Forming Foams (AFFF) Formulation I, RM 8691 Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous Film-Forming Foams (AFFF) Formulation II, RM 8692 Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous Film-Forming Foams (AFFF) Formulation III, and RM 8693 Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous Film-Forming Foams (AFFF) Formulation IV deliver non-certified values of the identity and quantity of per- and polyfluoroalkyl substances (PFAS) in four formulations prepared from commercial aqueous film-forming foam (AFFF) products. These RMs are intended for use as quality control materials for PFAS measurements; they are not meant for use in establishing the metrological traceability of measurement results to the International System of Units (SI). Each unit of these RMs consists of one ampoule containing approximately 1.2 mL of an AFFF solution diluted 10:1 (volume fraction) in methanol and modified by addition of PFAS analytical standards. This publication documents the production, analytical methods, and computations involved in characterizing these products.

Keywords

aqueous film-forming foam (AFFF);
high-resolution mass spectrometry (HRMS);
liquid chromatography (LC);
non-targeted analysis (NTA);
per- and polyfluoroalkyl substances (PFAS);
tandem mass spectrometry (MS/MS).

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1. Introduction

Per- and polyfluoroalkyl substances (PFAS) are a class of organic compounds with a carbon chain that is partially or fully fluorinated. The National Institute of Standards and Technology (NIST) currently provides measurements of PFAS in Standard Reference Material[®] (SRM[®]) 1936 Great Lakes Sediment [1], SRM 1946 Lake Superior Fish Tissue [2], SRM 1950 Metabolites in Frozen Human Plasma [3], SRM 1957 Organic Contaminants in Non-Fortified Human Serum (Freeze-Dried) [4], SRM 1958 Organic Contaminants in Fortified Human Serum (Freeze-Dried) [5], SRM 2585 Organic Contaminants in House Dust [6], SRM 2586 Trace Elements in Soil Containing Lead From Paint (Nominal Mass Fraction of 500 mg/kg Lead) [7], and SRM 2781 Domestic Sludge [8]. The non-certified reference values delivered by these materials are useful for the development and validation of methods; however, the concentrations of PFAS in these materials are significantly lower compared to the concentrations in some source materials impacting U.S. Department of Defense (DoD) sites, such as aqueous film forming foams (AFFFs).

AFFFs containing PFAS have been in use since the 1960s for fire control in military/civilian aviation crash and jet fuel spill events [9]. Historically, AFFFs have been routinely applied at fire training areas during training events with little or no containment, resulting in groundwater and drinking water contamination [10,11,12]. The DoD community has identified a need for developing tools for improving the reproducibility of PFAS measurements and facilitating the development and validation of analytical methods for PFAS in complex matrices. A SERDP (Strategic Environmental Research and Development Program) project was funded in US fiscal year 2019 to develop PFAS reference materials (RMs) in Aqueous Film-Forming Foam (AFFF) matrices, Project code ER18-1664. SERDP is the Department of Defense's environmental and resilience science and technology program. As part of this project NIST has developed four AFFF RMs containing PFAS that differ from those typical of leachate from consumer products [13,14].

This publication documents the production and characterization of the four new reference materials: RM 8690 Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous Film-Forming Foams (AFFF) Formulation I, RM 8691 Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous Film-Forming Foams (AFFF) Formulation II, RM 8692 Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous Film-Forming Foams (AFFF) Formulation III, and RM 8693 Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous Film-Forming Foams (AFFF) Formulation IV. These RMs are intended for use as quality control materials for PFAS measurements; they are not meant for establishing metrological traceability to the International System of Units (SI).

1.1. Materials

Four legacy commercial AFFF products were each used in the preparation of one of the four AFFF RMs. OmniSolv LC-MS Grade methanol (Millipore Sigma, St. Louis, MO) was used in the preparation of all four RMs. A 100 µg/mL perfluorooctanoic acid (PFOA) in methanol standard (Millipore Sigma, St. Louis, MO) was used in the preparation of RM 8691.

1.2. Preparation

A preliminary interlaboratory assessment of the AFFFs used to prepare these RMs [15] indicated that the native AFFF needed to be diluted by approximately a factor of ten, volume to volume, to make material handling easier and reduce foaming.

For RMs 8690, 8692, and 8693, a 2 L high density polyethylene (HDPE) bottle was weighed and 200 mL of AFFF was gravimetrically added to the bottle via a polypropylene graduated cylinder followed by 1.6 L of gravimetrically added methanol. The mass-fraction of the constituents in the RM solutions are approximate 8.8-fold smaller than in the AFFF. A polytetrafluoroethylene (PTFE) coated stir bar was added to each bottle and all solutions were stirred via stir-plate for at least 10 min.

The same procedure was used for RM 8691 except for the addition of 0.387 g of the 100 µg/mL PFOA in methanol standard prior to the addition of the 1.6 L methanol. Given the 1591.45 g total mass of RM 8691 solution and the 0.791 g/mL density of methanol at 20 °C, the resulting solution was spiked with nominally 0.031 µg/g PFOA.

There was white precipitate at the bottom of all bottles after mixing. Based on the product safety data sheets for the AFFFs, this was an inorganic salt insoluble in methanol, such as magnesium sulfate. The bottles were stored in the dark at room temperature for a week and then were filtered through Whatman ashless 40 filters (GE Healthcare Life Sciences, Marlborough, MA). The filtered solutions were stored in 2 L HDPE bottles at room temperature until bottling.

All consumables used in the production of RMs 8690 to 8693 were rinsed with LC-MS grade methanol and allowed to dry overnight prior to use.

1.3. Packaging

The RM solutions were dispensed into amber glass ampoules by the NIST Office of Reference Materials (ORM). Each ampoule contains approximately 1.2 mL of solution. The filling order was tracked. The ampoules are stored in boxes at ORM at room temperature.

2. Identification of PFAS

2.1. Interlaboratory Study of Undiluted AFFFs

Aliquots of the four commercial AFFFs used to produce RMs 8690, 8691, 8692, and 8693 were distributed as samples in an interlaboratory study [15]. Participants were asked to provide data for any of 33 targeted PFAS they could measure using their in-house analytical methods. Table 1 lists the 28 PFAS for which at least one quantitative value was reported by at least one of the six active participants.

Table 1. PFAS Detected in the AFFF Products Used to Produce RMs 8690 to 8693.

PFAS Measurands ^a	Code	Formula	In RMs ^b
Perfluorobutanoic acid	PFBA	C ₄ HF ₇ O ₂	8690/1/2/3
Perfluoropentanoic acid	PFPeA	C ₅ HF ₉ O ₂	8690/1/2/3
Perfluorohexanoic acid	PFHxA	C ₆ HF ₁₁ O ₂	8690/1/2/3
Perfluoroheptanoic acid	PFHpA	C ₇ HF ₁₃ O ₂	8690/1/2/3
Perfluorooctanoic acid	PFOA	C ₈ HF ₁₅ O ₂	8690/1
Perfluorononanoic acid	PFNA	C ₉ HF ₁₇ O ₂	8690/1
Perfluorodecanoic acid	PFDA	C ₁₀ HF ₁₉ O ₂	8690/1/2
Perfluoroundecanoic acid	PFUnA	C ₁₁ HF ₂₁ O ₂	8690/1/2
Perfluorododecanoic acid	PFDoA	C ₁₂ HF ₂₃ O ₂	8691
Perfluorotridecanoic acid	PFTriA	C ₁₃ HF ₂₅ O ₂	8691
Perfluorotetradecanoic acid	PFTA	C ₁₄ HF ₂₇ O ₂	8691/3
Perfluorobutanesulfonic acid	PFBS	C ₄ HF ₉ O ₃ S	8690/1/2/3
Perfluoropentanesulfonic acid	PFPeS	C ₅ HF ₁₁ O ₃ S	8690/1/2
Perfluorohexanesulfonic acid	PFHxS	C ₆ HF ₁₃ O ₃ S	8690/1/2
Perfluoroheptanesulfonic acid	PFHpS	C ₇ HF ₁₅ O ₃ S	8690/1/3
Perfluorooctanesulfonic acid	PFOS	C ₈ HF ₁₇ O ₃ S	8690/1/2/3
Perfluorononanesulfonic acid	PFNS	C ₉ HF ₁₉ O ₃ S	8690/2
Perfluorodecanesulfonic acid	PFDS	C ₁₀ HF ₂₁ O ₃ S	8690
Perfluorododecanesulfonic acid	PFDoS	C ₁₂ HF ₂₅ O ₃ S	8690
Perfluorooctanesulfonamide	FOSA	C ₈ H ₂ F ₁₇ NO ₂ S	8690
<i>N</i> -methylperfluorooctanesulfonamide	<i>N</i> -MeFOSA	C ₉ H ₄ F ₁₇ NO ₂ S	8690
<i>N</i> -methylperfluorooctanesulfonamidoacetic acid	<i>N</i> -MeFOSAA	C ₁₁ H ₆ F ₁₇ NO ₄ S	8690
<i>N</i> -methylperfluorooctanesulfonamidoethanol	<i>N</i> -MeFOSE	C ₁₁ H ₄ F ₂₁ NO ₃ S	8690/3
4:2 Fluorotelomer sulfonic acid	4:2 FTS	C ₆ H ₅ F ₉ O ₃ S	8690/1/2/3
6:2 Fluorotelomer sulfonic acid	6:2 FTS	C ₈ H ₅ F ₁₃ O ₃ S	8690/1/2/3
8:2 Fluorotelomer sulfonic acid	8:2 FTS	C ₁₀ H ₅ F ₁₇ O ₃ S	8690/1/2/3
4,8-Dioxa-3H-perfluorononanoic acid	ADONA	C ₇ H ₂ F ₁₂ O ₄	8690
Hexafluoropropylene oxide dimer acid	HFPO-DA	C ₆ HF ₁₁ O ₃	8690

a Includes linear and branched isomers.

b In the legacy AFFF used to produce the RMs, for example ‘8690/2’ indicates the compound was present in RM 8690 and RM 8692.

Structures and structural identifiers for these PFAS are listed in [Appendix B](#).

2.2. Initial Screening of AFFF stock materials by Non-Targeted Analysis using High-Resolution Mass Spectrometry

Before the RM solutions were ampouled, a quick-screening method was developed using non-targeted analysis (NTA) and suspect screening analysis tools to determine the primary constituents of the AFFF stock materials.

2.2.1. Materials

All solvents used were Fisher Optima LCMS Grade (Thermo Fisher Scientific, Inc., Waltham, MA). NIST RM 8446 Perfluorinated Carboxylic Acids and Perfluorooctane Sulfonamide in Methanol [16] and RM 8447 Perfluorinated Sulfonic Acids in Methanol [17] were used as method controls.

2.2.2. Sample Preparation

Aliquots of the AFFF stock materials used for generating RMs 8690, 8691, 8692, 8693 were gravimetrically diluted in methanol with dilution factors of approximately 300-fold. Equal masses of the perfluoroalkyl carboxylic acid (PFCA) component of RM 8446 and RM 8447 were combined and gravimetrically diluted in methanol to provide a method control with known PFAS. The dilution factors for the RM 8446 and 8447 source materials were approximately 30-fold. All prepared samples were stored at room temperature.

All vials and spatulas used for sample preparation were rinsed with methanol and allowed to air-dry prior to use.

2.2.3. Instrumental Method

An Ultimate 3000 liquid chromatograph (Thermo Fisher Scientific, Inc.) with an Agilent RRHD Eclipse Plus C18 column with dimensions 2.1 mm inner diameter, 50 mm length, and 1.8 μm particle diameter (Agilent Technologies, Santa Clara, CA) was used for chromatographic separation. The injection volume for all samples was 1 μL . The mobile phase consisted of 0.1 % (by volume) formic acid in water (A) and 0.1 % (by volume) formic acid in acetonitrile (B). The flow rate was 0.3 mL/min and the mobile phase gradient program included starting conditions of 5 % B for 0.5 min, followed by a nonlinear gradient (curve setting 3) increasing to 95 % B over 10.5 min, and held at 95 % B for 4.5 min before returning to starting conditions. The column was equilibrated with starting mobile phase conditions for 7.5 min before each run. The column temperature was maintained at 20 °C.

A Thermo Q-Exactive Quadrupole-Orbitrap Hybrid Mass Spectrometer (MS) (Thermo Fisher Scientific, Inc.) was used for mass spectrometric detection. Table 2 lists the electrospray ionization source settings used in all MS experiments.

Table 2. Electrospray Source Settings.

Source Setting	Polarity		Units
	Positive	Negative	
Spray Voltage	3000	-2500	V
Capillary Temperature	350	350	°C
Probe Heater Temperature	300	300	°C
Sheath Gas	35	35	μL/min
Auxiliary Gas	10	10	μL/min

For NTA, a first-stage MS (MS1) screening method was created with sequential positive/negative polarity switching. The MS1 experiment settings were the same for both polarities. The resolution was 70,000 with an automatic gain control (AGC) target at 3×10^6 , maximum injection time (IT) of 200 ms, and a scan range from 150 mass-to-charge (m/z) to 2000 m/z .

For suspect screening analysis, an inclusion list was created (described below) for each individual sample and a data-dependent second-stage MS (DD-MS2) experiment was performed after each MS1 survey scan. The scan sequence went as follows: negative MS1, negative DD-MS2, positive MS1, positive DD-MS2. The MS1 experiment settings were the same as the non-targeted analysis method. The DD-MS2 settings included resolution of 35,000 with an AGC target at 1×10^5 , maximum IT of 50 ms, a maximum number of most abundant ions (topN) of 5, an isolation window of 4 m/z , normalized collision energy of 15, 30, and 45, an intensity threshold of 1.6×10^5 with dynamic exclusion of 5.0 s, and “if idle select others” off so that the DD-MS2 experiment would not pick ions outside of the inclusion list.

The instrument was calibrated in both polarities using the manufacturer’s calibration solution within 24 hours of sample analysis.

2.2.4. Non-Targeted Data Analysis

After instrumental analysis, the MS1 data was converted into mzML using Proteowizard msconvert version 3.0.19127 (<http://proteowizard.sourceforge.net/>) with the vendor’s peak-picking algorithm and threshold peak filter of absolute intensities over 1. Using in-house developed R-scripts, m/z values with intensities over 1×10^6 were extracted, and adducts and isotopes including $[M+Na]^+$, $^{18}O_1$, $^{34}S_1$, $^{13}C_1$, and 2H_1 were removed from the mass list. Chromatographic quality of the mass features was checked with minimum peak width of 0.1 min, maximum peak width of 5 min, peak width determination at 10 % peak height, peak slope limit of 0.001, and peak slope determination width of 25 points. Mass features were compared to a PFAS list developed from literature compilations [11,12,14,18]. All compounds included in the compound list were used to create DD-MS2 inclusion lists.

The MS1 + DD-MS2 data was converted into mzML using the above settings. Using in-house developed R-scripts, the inclusion list was used to extract MS2 data and determine the identity of the mass features. The inclusion list start and end times were expanded by 0.5 min to include the entire peak with background. For data extraction the maximum allowed mass difference was 10 ppm, the minimum mass error match window was 0.0025 Da, and the

isolation width was 0.5 Da. The extracted parent ions were compared to the PFAS list and the fragment ions were compared to a fragment ion list prepared from [11,19]. Fragment ions with absolute intensities above 5 % of total ion intensity were annotated. Library matching was not possible for all compounds.

Using the PFAS list described above, inclusion lists were developed for each individual AFFF stock material. The rest of this section will refer to the RM number that used the individual AFFF stock material. RMs 8690, 8691, 8692, and 8693 had a total of 14, 3, 4, and 8 potential PFAS, respectively. Some PFAS are most likely isomers of the same compound and therefore do not represent separate identifications. Structural isomers such as branched and linear chain perfluorooctanesulfonic acid (PFOS) are difficult to identify separately without individual standards for all isomers. Based on the MS1 alone, no compounds could be identified beyond matching compound masses. DD-MS2 information is required for increased confidence in identification.

2.2.5. Suspect Screening

All the expected PFAS in the method control solution were identified using the NTA workflow, including a variety of annotated fragments for each compound. This indicates that the workflow was within control and identified the correct compounds. Additional compounds may be present in the source RMs as impurities or produced by in-source fragmentation. An example of an annotated PFAS identification is given in Fig. 1. Table 3 lists all the PFAS identified in the method control solution and the level of confidence with which the identification was made [20].

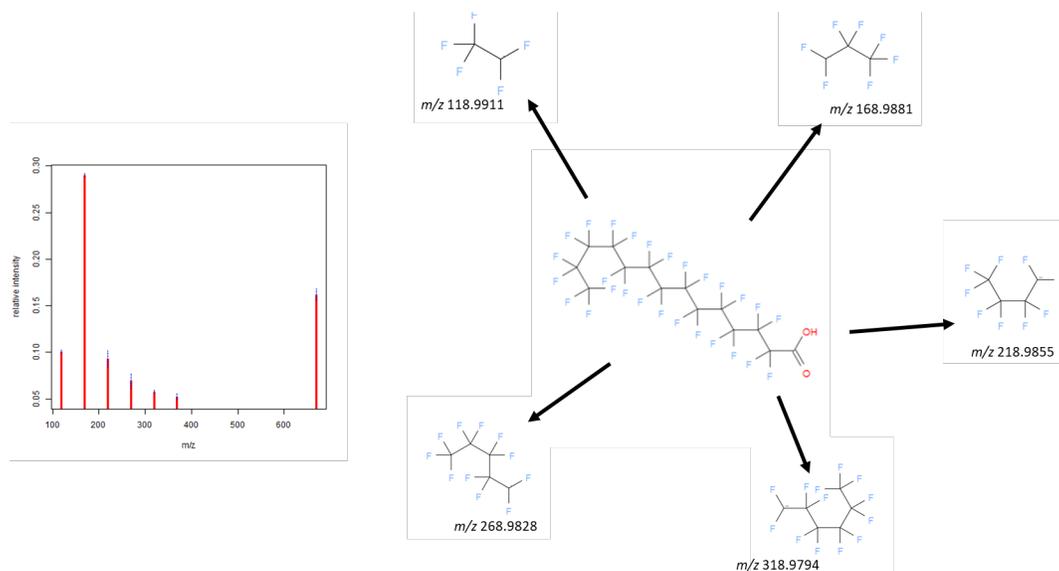


Fig. 1. Example of Fragment Annotation for Perfluorododecanoic acid.

The consensus mass spectrum is shown on the left with error bars representing standard deviation of the relative intensity and m/z ratio. The individual fragments surround the parent structure on the right.

Table 3. PFAS Identified in Method Control Solution Prepared from RM 8446 and RM 8447.

Observed <i>m/z</i>	Ion	Neutral Mass ^a , Da	Formula	Bias, ^b ppm	Identified Compound	Level ^c
298.9421	M-H	299.9494	C ₄ HF ₉ O ₃ S	-2.95	Perfluorobutanesulfonic acid	1
312.9727	M-H	313.9799	C ₆ HF ₁₁ O ₂	-0.50	Perfluorohexanoic acid	1
362.9694	M-H	363.9767	C ₇ HF ₁₃ O ₂	-0.55	Perfluoroheptanoic acid	1
398.9353	M-H	399.9425	C ₆ HF ₁₃ O ₃ S	-3.33	Perfluorohexanesulfonic acid	1
412.9661	M-H	413.9734	C ₈ HF ₁₅ O ₂	-0.72	Perfluorooctanoic acid	1
462.9630	M-H	463.9702	C ₉ HF ₁₇ O ₂	-0.61	Perfluorononanoic acid	1
498.9295	M-H	499.9368	C ₈ HF ₁₇ O ₃ S	-1.48	Perfluorooctanesulfonic acid	1
498.9301	M-H	499.9373	C ₈ HF ₁₇ O ₃ S	-0.32	Perfluorooctanesulfonic acid	1
498.9306	M-H	499.9379	C ₈ HF ₁₇ O ₃ S	0.78	Perfluorooctanesulfonic acid	1
512.9594	M-H	513.9667	C ₁₀ HF ₁₉ O ₂	-1.21	Perfluorodecanoic acid	1
562.9563	M-H	563.9636	C ₁₁ HF ₂₁ O ₂	-0.97	Perfluoroundecanoic acid	1
612.9532	M-H	613.9605	C ₁₂ HF ₂₃ O ₂	-0.66	Perfluorododecanoic acid	1
662.9507	M-H	663.9580	C ₁₃ HF ₂₅ O ₂	0.35	Perfluorotridecanoic acid	1
712.9473	M-H	713.9545	C ₁₄ HF ₂₇ O ₂	0.00	Perfluorotetradecanoic acid	1
318.9790	M-H	319.9863	C ₆ HF ₁₃	-2.37	1H-Perfluorohexane	2b
346.9745	M-H	347.9818	C ₇ HF ₁₃ O	-0.45	1,1,2,2,3,3,4,4,5,5-Decafluoro-1-((trifluorovinyl)oxy)pentane	2b
368.9758	M-H	369.9831	C ₇ HF ₁₅	-2.06	1H-Perfluoroheptane	2b
396.9713	M-H	397.9786	C ₇ H ₃ F ₁₃ O ₄	-9.50	Fluorinated triethylene glycol monomethyl ether	2b
418.9731	M-H	419.9804	C ₈ HF ₁₇	-0.76	1H-Perfluorooctane	2b
430.9568	M-H	431.9640	C ₈ F ₁₆ O ₂	-0.57	Perfluoro(2-propoxypropyl vinyl ether)	2b
518.9666	M-H	519.9739	C ₁₀ HF ₂₁	-0.77	1H-Perfluorodecane	2b
726.9473	M-H	727.9545	C ₁₄ H ₅ F ₂₅ O ₃ S	-2.03	1H,1H,2H,2H-Perfluoroalkane-1-sulfonic acid	2b

- a) Calculated from the observed *m/z* and the molar mass of the ion postulated as producing the neutral parent. Here, all neutralizing ions are ¹H⁺.
- b) Difference between the molar mass of the proposed neutral compound, *m*_{neutral}, and the calculated mass of compounds with the proposed elemental formula, *m*_{formula}, expressed as “ppm”: $10^6 \times (m_{\text{neutral}} - m_{\text{formula}}) / m_{\text{formula}}$.
- c) Identification confidence level [20]: 1) confirmed structure by reference standard, 2b) probable structure established by MS1, MS2 evidence.

Fourteen PFAS were identified in RM 8690, including the perfluoroalkyl sulfonic acids PFOS, PFHxS, and PFBS verified using retention time matching against RM 8447. Due to low concentrations of the perfluoroalkyl sulfonic acids, MS2 fragments were not produced using the DD-MS2 experiment. Two quaternary amine sulfonamide PFAS were identified with annotated fragments, an example is shown in Fig. 2.

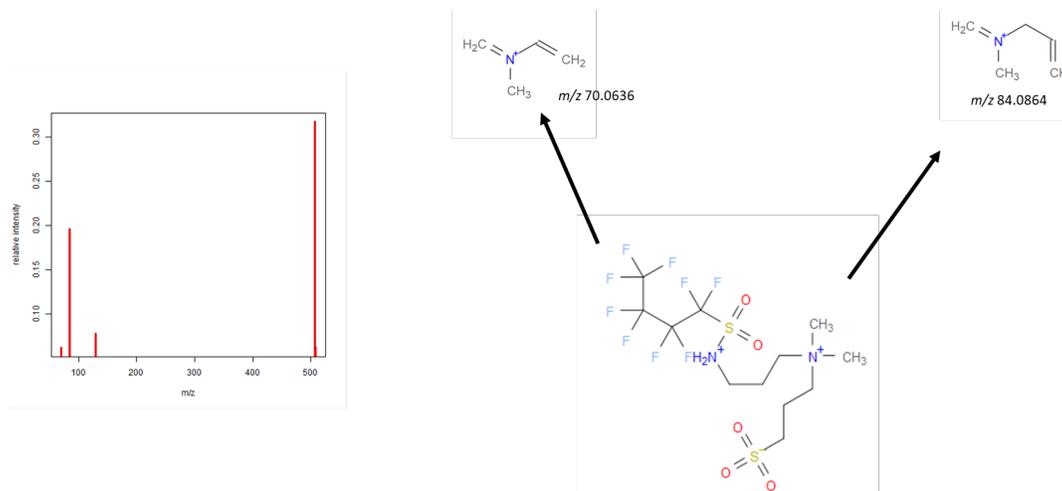


Fig. 2. Example of Fragment Annotation for an Amine Sulfonamide PFAS.

The consensus mass spectrum is shown on the left with error bars representing standard deviation of the relative intensity and m/z ratio. The individual fragments surround the parent structure on the right. The compound is identified as *N,N*-dimethyl-3-((perfluorobutyl)sulfonamido)-*N*-(3-sulfopropyl)propan-1-aminium.

The compounds identified in RMs 8690, 8691, 8692, and 8693 stock materials are the same as the PFAS identified in AFFFs produced using electrofluorination or fluorotelomerization [14]. A compound detected having the nominal m/z 586 with the sulfonate fragment ion as the only annotated fragment is shown in Fig. 3.

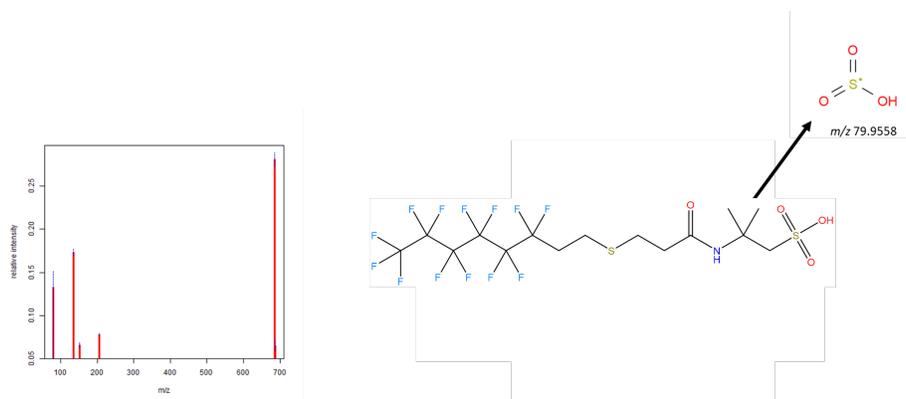


Fig. 3. Example of Fragment Annotation for a Sulfonic Acid PFAS.

The consensus mass spectrum is shown on the left with error bars representing standard deviation of the relative intensity and m/z ratio. The sulfonate fragment and the parent structure are on the right. The compound is identified as 2-methyl-2-(3-((1H,1H,2H,2H-perfluoro-1-octyl)thio)propanamido)propane-1-sulfonic acid (6:2 Fluorotelomer thioether amido sulfonic acid).

All PFAS identified in RMs 8690, 8691, 8692, or 8693 through in this screening study are listed in Table 4. Code names, structures, and structural identifiers are listed in [Appendix B](#).

Table 4. PFAS Identified in RM 8690, 8691, 8692, and/or 8693 By the Initial Screen.

Observed m/z	Ion	Neutral Mass ^a , Da	Formula	Bias, ^b ppm	Tentatively Identified Compound	In Stock Materials
248.9460	M-H	249.9532	C ₃ HF ₇ O ₃ S	-0.92	Perfluoropropanesulfonic acid	8690
298.9430	M-H	299.9502	C ₄ HF ₉ O ₃ S	-0.10	Perfluorobutanesulfonic acid	8690
348.9393	M-H	349.9466	C ₅ HF ₁₁ O ₃ S	-1.32	Perfluoropentanesulfonic acid	8690
383.0467	M-H	384.0540	C ₉ H ₁₃ F ₉ N ₂ O ₂ S	-3.65	Perfluorobutane sulfonamido amine	8690/2/3
383.0692	M-H	384.0764	C ₁₄ H ₁₃ F ₉ O ₂	-1.95	4-[3-(Perfluorobutyl)-1-propyloxy]benzyl alcohol	8690
398.9363	M-H	399.9436	C ₆ HF ₁₃ O ₃ S	-0.81	Perfluorohexanesulfonic acid	8690
399.0412	M-H	400.0484	C ₉ H ₁₃ F ₉ N ₂ O ₃ S	-4.69	Perfluorobutane sulfonamido amine oxide	8690
426.9676	M-H	427.9749	C ₈ H ₃ F ₁₃ O ₃ S	-0.73	6:2 Fluorotelomer sulfonic acid	8693
457.0699	M+	457.0699	C ₁₁ H ₂₀ O ₅ S ₂ N ₂ F ₇	0.47	<i>N,N</i> -dimethyl-3-((perfluoropropyl)sulfonamido)- <i>N</i> -(3-sulfopropyl)propan-1-aminium	8690
483.0416	M-H	484.0489	C ₁₁ H ₁₃ F ₁₃ N ₂ O ₂ S	-0.28	Perfluorohexane sulfonamido amine	8690
496.0809	M+H	495.0737	C ₁₈ H ₁₄ F ₉ NO ₅	1.67	1-[[[[4-(2-(Perfluorobutyl)-1-ethyl)phenyl]methoxy]carbonyl]oxy]-2,5-pyrrolidinedione	8692/3
498.9303	M-H	499.9375	C ₈ HF ₁₇ O ₃ S	0.11	Perfluorooctanesulfonic acid	8690
507.0650	M+	507.0650	C ₁₂ H ₂₀ O ₅ S ₂ N ₂ F ₉	-2.91	<i>N,N</i> -dimethyl-3-((perfluorobutyl)sulfonamido)- <i>N</i> -(3-sulfopropyl)propan-1-aminium	8690
527.0671	M-H	528.0744	C ₁₃ H ₁₇ F ₁₃ N ₂ O ₃ S	-1.54	<i>N,N</i> -Dimethyl-3-((perfluorohexyl)ethylsulfonyl)aminopropanamine <i>N</i> -oxide	8693
551.0456	M+H	550.0383	C ₁₂ H ₁₃ F ₁₅ N ₂ O ₃ S	-4.41	Perfluoroheptane sulfonamido amine oxide	8693
555.0627	M-H	556.0700	C ₁₄ H ₁₇ F ₁₃ N ₂ O ₄ S	-0.21	<i>N</i> -[3-(Dimethylamino)propyl]- <i>N</i> -[(tridecafluorohexyl)sulfonyl]-beta-alanine	8690
569.0780	M-H	570.0853	C ₁₅ H ₁₉ F ₁₃ N ₂ O ₄ S	-0.90	6:2 Fluorotelomer sulfonamide betaine	8693
586.0403	M-H	587.0476	C ₁₅ H ₁₈ F ₁₃ NO ₄ S ₂	1.01	6:2 Fluorotelomer thioether amido sulfonic acid	8690/1/2/3
602.0346	M-H	603.0419	C ₁₅ H ₁₈ F ₁₃ NO ₅ S ₂	0.00	6:2 Fluorotelomer sulfinyl amido sulfonic acid	869/2/3
686.0349	M-H	687.0421	C ₁₇ H ₁₈ F ₁₇ NO ₄ S ₂	2.25	8:2 Fluorotelomer thioether amido sulfonic acid	8691

- a) Calculated from the observed m/z and the molar mass of the ion postulated as producing the neutral parent. Here, all neutralizing ions are $^1\text{H}^+$.
- b) Difference between the molar mass of the proposed neutral compound, m_{neutral} , and the calculated mass of compounds with the proposed elemental formula, m_{formula} , expressed as “ppm”: $10^6 \times (m_{\text{neutral}} - m_{\text{formula}})/m_{\text{formula}}$.

2.3. Secondary Non-Targeted Analysis of Reference Materials using High-Resolution Mass Spectrometry

Based on the non-targeted analysis of the stock materials (section 2.2), RMs 8690, 8691, 8692, and 8693 may contain constituents other than those included in typical (or even expanded) targeted analytical methods. Relative to targeted chemical analyses, liquid-chromatography high resolution mass spectrometry (LC-HRMS) analyses provide an opportunity to detect unknown or unanticipated chemicals in complex samples [11,12,14,21].

2.3.1. Materials

Solvents used in sample preparation were Alfa Aesar LC-MS Grade (Thermo Fisher Scientific, Waltham, MA). Solvents used for LC-HRMS analysis were Fisher Optima LCMS Grade (Thermo Fisher Scientific, Inc., Waltham, MA). Deionized (DI) water was produced by an in-house Milli-Q system. All glassware used for sample preparation were rinsed with methanol and allowed to air-dry prior to use. AFFFs were subsamples of the original AFFFs used to create the RMs.

2.3.2. Sample Preparation

The RMs were gravimetrically diluted in methanol in 15 mL polypropylene tubes (VWR, Radnor, PA) to give final AFFF dilutions of approximately 1:5000. After dilution, 1 mL aliquots were transferred to methanol-rinsed autosampler vials.

2.3.3. HRMS Instrumental Method

Chromatographic separation was performed with a Vanquish ultra-performance liquid chromatograph (UPLC; Thermo Fisher Scientific, Inc., Waltham, MA), using a Waters Acquity UPLC HSS T3 column (2.1 mm inner diameter, 100 mm length, 1.8 μ m particle diameter; Waters Corporation, Milford, MA) and Waters Acquity UPLC HSS T3 VanGuard Pre-column (2.1 mm inner diameter, 5 mm length, 1.8 μ m particle diameter; Waters Corporation, Milford, MA). Mass spectrometric detection was performed with a Thermo Q-Exactive Quadrupole-Orbitrap Hybrid Mass Spectrometer (Thermo Fisher Scientific Inc., Waltham, MA). Table 5 lists the MS detector; Table 6 the UPLC, and Table 7 the Orbitrap parameter settings that were used.

Table 5. Mass Spectrometer Detector Settings.

Source Parameter	ESI Positive (ESI+)	ESI Negative (ESI-)
Spray Voltage (kV)	3	-2.5
Capillary Temperature (°C)	350	350
Sheath Gas Flow Rate	35	35
Aux Gas Flow Rate	10	10
Sweep Gas Flow Rate	2	2
Aux Gas Heater Temperature (°C)	300	300
S-Lens RF Level	50	50

Table 6. Ultra-Performance Liquid Chromatograph Parameter Settings.

Parameter	Setting
Column temperature	45 °C
Eluent A	DI water + 0.1 % formic acid (v/v)
Eluent B	Methanol + 0.1 % formic acid (v/v)
Eluent flow rate	0.3 mL/min
Injection volume	5 µL
Autosampler temperature	10 °C
Gradient	5% B at 0-0.5 min, 50% B at 1.5 min, 100% B 10-15 min, 5% B at 15.5-20 min

Table 7. Orbitrap Parameter Settings.

	Orbitrap Parameter	Setting (MS1)	Setting (ddMS2)	
Overall Method	Source type	ESI	ESI	
	Use lock masses	Best	Best	
	Chromatographic peak width (FWHM)	3 s	3 s	
	Runtime	0 to 20 min	0 to 20 min	
Full MS	Polarity	Positive / Negative	Positive / Negative	
	In-source CID	0.0 eV	0.0 eV	
	Default charge state	1	1	
	Microscans	1	1	
	Resolution	70,000	70,000	
	AGC target	1×10 ⁶	1×10 ⁶	
	Maximum IT	100 ms	100 ms	
	Number of scan ranges	1	1	
	Scan range	100 to 1200 m/z	100 to 1200 m/z	
	Spectrum data type	Centroid	Centroid	
	Data Dependent-MS ²	Microscans		1
		Resolution		17,500
AGC target			1×10 ⁵	
Maximum IT			50 ms	
Loop count			5	
MSX count			1	
TopN			5	
Isolation window			1.5 m/z	
Isolation offset			0.0 m/z	
Scan range			50 to 1200 m/z	
Fixed first mass			50 m/z	
NCE			Stepped 15, 30, 45 eV	
Spectrum data type			Centroid	
Data Dependency	Minimum AGC target		5×10 ³	
	Intensity threshold		1×10 ⁵	
	Multiple charge states		all	
	Exclude isotopes		on	
	Dynamic exclusion time		0.3 s	

The instrument was calibrated in both polarities (positive and negative) using the manufacturer's calibration solution on the first day of sample analysis. A methanol blank was injected before and after samples to check for column carry-over: none was observed. Triplicate injections of each of the 1:5000 dilutions were analyzed by LC-HRMS.

2.3.4. HRMS Data Extraction

Non-targeted HRMS MS1 data were extracted in Compound Discoverer version 3.1. The workflow steps and associated settings are provided in Table 8.

Table 8. Workflow steps and settings for HRMS data extraction in Compound Discoverer.

Step 1: Input files

Step 2: Select Spectra

Workflow Step	Parameter	Setting (ESI+)	Setting (ESI-)
General Settings	Precursor Selection	Use MS(n-1)	Use MS(n-1)
	Use Isotope Pattern in Precursor Reevaluation	True	True
	Provide Profile Spectra	Automatic	Automatic
	Store Chromatograms	False	False
Spectrum Properties Filter	Lower RT Limit	0.5	0.5
	Upper RT Limit	20	20
	Min Precursor Mass	100 Da	100 Da
	Max Precursor Mass	1200 Da	1200 Da
	Total Intensity Threshold	500,000	100,000
	Minimum Peak Count	1	1
Scan Event Filters	Mass Analyzer	Is FTMS	Is FTMS
	MS Order	Any	Any
	Activation Type	Any	Any
	Min Collision Energy	0	0
	Max Collision Energy	1000	1000
	Scan Type	Any	Any
	Polarity Mode	Is +	Is -
Peak Filters	Signal/Noise Threshold (Fourier Transform only)	3	3
Replacements for Unrecognized Properties	Unrecognized Charge Replacements	1	1
	Unrecognized Mass Analyzer Replacements	FTMS	FTMS
	Unrecognized MS Order Replacements	MS1	MS1
	Unrecognized Activation Type Replacements	HCD	HCD
	Unrecognized Polarity Replacements	+	-
	Unrecognized MS1 Resolution@200 Replacements	70,000	70,000
	Unrecognized MS2 Resolution@200 Replacements	17,500	17,500

Step 3: Align Retention Times

Workflow Step	Parameter	Setting (ESI+)	Setting (ESI-)
General Settings	Alignment Model	Adaptive Curve	Adaptive Curve
	Alignment Fallback	Linear Model	Linear Model
	Maximum Shift [min]	0.6	0.6
	Shift Reference File	True	True
	Mass Tolerance	7 ppm	7 ppm
	Remove Outlier	True	True

Step 4: Detect Compounds

Workflow Step	Parameter	Setting (ESI+)	Setting (ESI-)
General Settings	Mass Tolerance [ppm]	7 ppm	7 ppm
	Intensity Tolerance [%]	30	30
	S/N Threshold	3	3
	Min. Peak Intensity	500,000	100,000
	Ions	[M+H] ⁺ +1	[M-H] ⁻ -1
	Base Ions	[M+H] ⁺ +1	[M-H] ⁻ -1
	Min Element Counts	C H	C H
	Max Element Counts	C ₉₀ H ₁₉₀ Br ₃ Cl ₈ F ₅₀ N ₁₀ O ₁₈ P ₃ S ₅	C ₉₀ H ₁₉₀ Br ₃ Cl ₈ F ₅₀ N ₁₀ O ₁₈ P ₃ S ₅
Peak Detection	Filter Peaks	True	True
	Max Peak Width [min]	0.8	0.8
	Remove Singlets	False	False
	Min # Scans per Peak	3	3
	Min # Isotopes	1	1

Step 5: Group Compounds

Workflow Step	Parameter	Setting (ESI+)	Setting (ESI-)
Compound Consolidation	Mass Tolerance	10 ppm	10 ppm
	RT Tolerance [min]	0.3	0.3
Fragment Data Selection	Preferred Ions	[M+H] ⁺ +1	[M-H] ⁻ -1

After initial data extraction, features were retained that: 1) were present in all three analytical replicates, 2) had peak area greater than three-fold that of the methanol blanks, and 3) had peak area $> 1 \times 10^5$ (ESI-) or $\geq 5 \times 10^5$ (ESI+). To prioritize features for MS/MS data acquisition, features were screened against an in-house PFAS database (n = 4041 compounds). Additionally, CF₂-based homologous series screening was applied to prioritize additional potential PFAS.

2.3.5. Analysis

Table 9 lists the PFAS identified in this LC-HRMS study. The identifications align with many reported in the initial screening, however some of the previously identified PFAS either were not observed or were assigned a different identity. Table 10 and Table 11 provide the chemical standards and internal standards used in PFAS identification.

Code names, structures, and structural identifiers are listed in [Appendix B](#).

Table 9. PFAS Identified in LC-HRMS Analysis.

Observed <i>m/z</i>	Ion	Neutral Mass ^a , Da	Formula	Bias, ^b ppm	Tentatively Identified Compound	In RMs
486.0461	M-H-	487.0534	C ₁₃ H ₁₈ F ₉ NO ₄ S ₂	-0.04	4:2 Fluorotelomer thioether amido sulfonic acid	8691
586.0397	M-H-	587.0470	C ₁₅ H ₁₈ F ₁₃ NO ₄ S ₂	0.02	6:2 Fluorotelomer thioether amido sulfonic acid	8690/1/2/3
686.0333	M-H-	687.0406	C ₁₇ H ₁₈ F ₁₇ NO ₄ S ₂	0.51	8:2 Fluorotelomer thioether amido sulfonic acid	8691
786.0269	M-H-	787.0342	C ₁₉ H ₁₈ F ₂₁ NO ₄ S ₂	0.47	10:2 Fluorotelomer thioether amido sulfonic acid	8691
886.0205	M-H-	887.0278	C ₂₁ H ₁₈ F ₂₅ NO ₄ S ₂	0.03	12:2 Fluorotelomer thioether amido sulfonic acid	8691
602.0346	M-H-	603.0419	C ₁₅ H ₁₈ F ₁₃ NO ₅ S ₂	0.20	6:2 Fluorotelomer sulfinyl amido sulfonic acid	8690/1/2/3
618.0295	M-H-	619.0368	C ₁₅ H ₁₈ F ₁₃ NO ₆ S ₂	0.23	6:2 Fluorotelomer sulfonyl amido sulfonic acid	8691
452.0349	M+H+	451.0276	C ₁₁ H ₁₀ F ₁₃ NOS	1.51	3-[(3,3,4,4,5,5,6,6,7,7,8,8,8-Tridecafluorooctyl)sulfanyl]propanamide	8691/2/3
552.0285	M+H+	551.0212	C ₁₃ H ₁₀ F ₁₇ NOS	1.39	3-[(3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-Heptadecafluorodecyl)sulfanyl]propanamide	8691
652.0221	M+H+	651.0148	C ₁₅ H ₁₀ F ₂₁ NOS	2.18	3-[(3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,12-Henicosafluorododecyl)sulfanyl]propanamide	8691
752.0157	M+H+	751.0084	C ₁₇ H ₁₀ F ₂₅ NOS	2.23	3-[(3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,13,13,14,14,14-Pentacosafuorotetradecyl)sulfanyl]propanamide	8691
471.0995	M+H+	470.0922	C ₁₃ H ₁₉ F ₉ N ₂ O ₄ S	2.21	4:2 Fluorotelomer sulfonamide betaine	8693
571.0931	M+H+	570.0858	C ₁₅ H ₁₉ F ₁₃ N ₂ O ₄ S	0.00	6:2 Fluorotelomer sulfonamide betaine	8690/3
285.0691	M+H+	284.0618	C ₇ H ₁₃ F ₅ N ₂ O ₂ S	3.54	Perfluoroethane sulfonamido amine	8690
335.0659	M+H+	334.0586	C ₈ H ₁₃ F ₇ N ₂ O ₂ S	2.91	Perfluoropropane sulfonamido amine	8690
385.0627	M+H+	384.0554	C ₉ H ₁₃ F ₉ N ₂ O ₂ S	2.48	Perfluorobutane sulfonamido amine	8690
435.0595	M+H+	434.0522	C ₁₀ H ₁₃ F ₁₁ N ₂ O ₂ S	3.63	Perfluoropentane sulfonamido amine	8690
485.0563	M+H+	484.0490	C ₁₁ H ₁₃ F ₁₃ N ₂ O ₂ S	0.00	Perfluorohexane sulfonamido amine	8690
535.0531	M+H+	534.0458	C ₁₂ H ₁₃ F ₁₅ N ₂ O ₂ S	4.41	Perfluoroheptane sulfonamido amine	8690
585.0499	M+H+	584.0426	C ₁₃ H ₁₃ F ₁₇ N ₂ O ₂ S	3.45	Perfluorooctane sulfonamido amine	8690
357.0902	M+H+	356.0829	C ₁₀ H ₁₇ F ₅ N ₂ O ₄ S	3.03	<i>N</i> -[3-(Dimethylamino)propyl]- <i>N</i> -[(pentafluoroethyl)sulfonyl]-beta-alanine	8690
407.087	M+H+	406.0797	C ₁₁ H ₁₇ F ₇ N ₂ O ₄ S	3.36	<i>N</i> -[3-(Dimethylamino)propyl]- <i>N</i> -[(heptafluoropropyl)sulfonyl]-beta-alanine	8690
457.0838	M+H+	456.0765	C ₁₂ H ₁₇ F ₉ N ₂ O ₄ S	3.39	<i>N</i> -[3-(Dimethylamino)propyl]- <i>N</i> -[(nonafluorobutyl)sulfonyl]-beta-alanine	8690
507.0806	M+H+	506.0733	C ₁₃ H ₁₇ F ₁₁ N ₂ O ₄ S	3.65	<i>N</i> -[3-(Dimethylamino)propyl]- <i>N</i> -[(undecafluoropentyl)sulfonyl]-beta-alanine	8690
557.0774	M+H+	556.0701	C ₁₄ H ₁₇ F ₁₃ N ₂ O ₄ S	3.78	<i>N</i> -[3-(Dimethylamino)propyl]- <i>N</i> -[(tridecafluorohexyl)sulfonyl]-beta-alanine	8690
607.0742	M+H+	606.0669	C ₁₅ H ₁₇ F ₁₅ N ₂ O ₄ S	2.44	<i>N</i> -[3-(Dimethylamino)propyl]- <i>N</i> -[(pentadecafluoroheptyl)sulfonyl]-beta-alanine	8690
657.071	M+H+	656.0637	C ₁₆ H ₁₇ F ₁₇ N ₂ O ₄ S	2.68	<i>N</i> -[3-(Dimethylamino)propyl]- <i>N</i> -[(heptadecafluorooctyl)sulfonyl]-beta-alanine	8690
527.0679	M-H-	528.0752	C ₁₃ H ₁₇ F ₁₃ N ₂ O ₃ S	-0.29	<i>N,N</i> -Dimethyl-3-((perfluorohexyl)ethylsulfonyl)aminopropanamine- <i>N</i> -oxide	8693

- a) Calculated from the observed *m/z* and the molar mass of the ion postulated as producing the neutral parent.
- b) Difference between the molar mass of the proposed neutral compound, m_{neutral} , and the calculated mass of compounds with the proposed elemental formula, m_{formula} , expressed as “ppm”: $10^6 \times (m_{\text{neutral}} - m_{\text{formula}})/m_{\text{formula}}$.

Table 10. Calibrants used in identification of PFAS

Acronym	Analyte	CAS Number	Diagnostic Ions, m/z ^a		
			M ₁	M ₂	M ₃
PFBA	Perfluoro-n-butanoic acid	375-22-4	213	169	
PFPeA	Perfluoro-n-pentanoic acid	2706-90-3	263	219	119
PFHxA	Perfluoro-n-hexanoic acid	307-24-4	313	269	119
PFHpA	Perfluoro-n-heptanoic acid	375-85-9	363	319	169
PFOA	Perfluoro-n-octanoic acid	335-67-1	413	369	169
PFNA	Perfluoro-n-nonanoic acid	375-95-1	463	419	219
PFDA	Perfluoro-n-decanoic acid	335-76-2	513	469	219
PFUnA	Perfluoro-n-undecanoic acid	2058-94-8	563	519	269
PFDoA	Perfluoro-n-dodecanoic acid	307-55-1	613	569	269
PFTTrDA	Perfluoro-n-tridecanoic acid	72629-94-8	663	619	269
PFTeDA	Perfluoro-n-tetradecanoic acid	376-06-7	713	669	369
HFPO-DA	2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-propanoic acid	13252-13-6	329	285	169
ADONA	2,2,3-Trifluoro-3-(1,1,2,2,3,3-hexafluoro-3-(trifluoromethoxy)propoxy)propanoic acid	919005-14-4	377	251	
FBSA	Perfluoro-1-butanefulfonamide	30334-69-1	298	78	169
FHxSA	Perfluoro-1-hexanesulfonamide	41997-13-1	398	78	169
FOSA	Perfluoro-1-octanesulfonamide	754-91-6	498	78	169
N-MeFOSAA	<i>N</i> -methylperfluoro-1-octanesulfonamidoacetic acid	2355-31-9	570	419	512
N-EtFOSAA	<i>N</i> -ethylperfluoro-1-octanesulfonamidoacetic acid	2991-50-6	584	419	526
PFPrS	Perfluoro-1-propanesulfonate	423-41-6	248.9	80	98.9
PFBS	Perfluoro-1-butanefulfonate	375-73-5	299	80	99
PFPeS	Perfluoro-1-pentanesulfonate	2706-91-4	349	80	99
PFHxS	Perfluoro-1-hexanesulfonate	355-46-4	399	80	99
PFHpS	Perfluoro-1-heptanesulfonate	375-92-8	449	80	99
PFOS	Perfluoro-1-octanesulfonate	1763-23-1	499	80	99
PFNS	Perfluoro-1-nonanesulfonate	68259-12-1	549	80	99
PFDS	Perfluoro-1-decanesulfonate	335-77-3	599	80	99
4:2 FTS	1H,1H,2H,2H-perfluoro-1-hexanesulfonate	757124-72-4	327	307	81
6:2 FTS	1H,1H,2H,2H,-perfluoro-1-octanesulfonate	27619-97-2	427	407	81
8:2 FTS	1H,1H,2H,2H,-perfluoro-1-decanesulfonate	39108-34-4	527	507	81
9Cl-PF3ONS	9-chlorohexadecafluoro-3-oxanonane-1-sulfonate	756426-58-1	531	351	353
11Cl-PF3OUdS	11-chloroeicosafluoro-3-oxaundecane-1-sulfonate	763051-92-9	631	451	
N-TAmP-FHxSA	<i>N</i> -[3-(perfluoro-1-hexanesulfonamido)propan-1-yl]- <i>N,N,N</i> -trimethylammonium	38850-51-0	499.1	60.1	86.1
N-AP-FHxSA	<i>N</i> -(3-dimethylaminopropan-1-yl)perfluoro-1-hexanesulfonamide	50598-28-2	485.4	85.1	70.1
6:2 FTAB	<i>N</i> -(carboxymethyl)- <i>N,N</i> -dimethyl- <i>N</i> -[3-(1H,1H,2H,2H-perfluoro-1-octanesulfonamido)propan-1-yl]ammonium	34455-29-3	569.1	446.1	223.1
			571	104	58

^a Values in regular font indicate ions for negative mode electrospray ionization; values in bold font indicate ions for positive mode electrospray ionization

Table 11. Isotopically labeled commercially available standards used as internal standards

Acronym	Analyte	Diagnostic Ions, <i>m/z</i>	
		<i>M</i> ₁	<i>M</i> ₂
¹³ C ₃ -PFBA	Perfluoro- <i>n</i> -[¹³ C ₃]-butanoic acid	216	172
¹³ C ₄ -PFBA	Perfluoro- <i>n</i> -[¹³ C ₄]-butanoic acid	217	172
¹³ C ₅ -PFPeA	Perfluoro- <i>n</i> -[¹³ C ₅]-pentanoic acid	268	223
¹³ C ₂ -PFHxA	Perfluoro- <i>n</i> -[1,2,3,4,6- ¹³ C ₂]-hexanoic acid	315	270
¹³ C ₅ -PFHxA	Perfluoro- <i>n</i> -[1,2,3,4,6- ¹³ C ₅]-hexanoic acid	318	273
¹³ C ₄ -PFHpA	Perfluoro- <i>n</i> -[1,2,3,4- ¹³ C ₄]-heptanoic acid	367	322
¹³ C ₈ -PFOA	Perfluoro- <i>n</i> -[¹³ C ₈]-octanoic acid	421	376
¹³ C ₂ -PFOA	Perfluoro- <i>n</i> -[¹³ C ₂]-octanoic acid	415	370
¹³ C ₉ -PFNA	Perfluoro- <i>n</i> -[¹³ C ₉]-nonanoic acid	472	427
¹³ C ₂ -PFDA	Perfluoro- <i>n</i> -[1,2,3,4,5,6- ¹³ C ₂]-decanoic acid	515	470
¹³ C ₆ -PFDA	Perfluoro- <i>n</i> -[1,2,3,4,5,6- ¹³ C ₆]-decanoic acid	519	474
¹³ C ₂ -PFUnA	Perfluoro- <i>n</i> -[1,2,3,4,5,6- ¹³ C ₂]-undecanoic acid	565	520
¹³ C ₇ -PFUnA	Perfluoro- <i>n</i> -[1,2,3,4,5,6- ¹³ C ₇]-undecanoic acid	570	525
¹³ C ₂ -PFDoA	Perfluoro- <i>n</i> -[1,2- ¹³ C ₂]-dodecanoic acid	615	570
¹³ C ₂ -PFTeDA	Perfluoro- <i>n</i> -[1,2- ¹³ C ₂]-tetradecanoic acid	715	670
¹³ C ₃ -HFPO-DA	2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-[¹³ C ₃]-propanoic acid	267	169
¹³ C ₈ -FOSA	Perfluoro- <i>n</i> -[¹³ C ₈]-octanesulfonamide	506	78
<i>d</i> ₃ - <i>N</i> -MeFOSAA	<i>N</i> -methyl- <i>d</i> ₃ -perfluoro-1-octanesulfonamidoacetic acid	573	419
<i>d</i> ₅ - <i>N</i> -EtFOSAA	<i>N</i> -ethyl- <i>d</i> ₅ -perfluoro-1-octanesulfonamidoacetic acid	589	419
¹³ C ₃ -PFBS	Perfluoro- <i>n</i> -[2,3,4- ¹³ C ₃]-butanesulfonate	302	99
¹³ C ₃ -PFHxS	Perfluoro- <i>n</i> -[1,2,3- ¹³ C ₃]-hexanesulfonate	402	99
¹⁸ O ₂ -PFHxS	Perfluoro- <i>n</i> -hexane [¹⁸ O ₂]-sulfonate	403	84
¹³ C ₄ -PFOS	Perfluoro- <i>n</i> -[¹³ C ₄]-octanesulfonate	503	80
¹³ C ₈ -PFOS	Perfluoro- <i>n</i> -[¹³ C ₈]-octanesulfonate	507	99
¹³ C ₂ -4:2 FTS	1H,1H,2H,2H-perfluoro- <i>n</i> -[1,2- ¹³ C ₂]-hexanesulfonate	329	81
¹³ C ₂ -6:2 FTS	1H,1H,2H,2H,-perfluoro- <i>n</i> -[1,2- ¹³ C ₂]-octanesulfonate	429	81
¹³ C ₂ -8:2 FTS	1H,1H,2H,2H,-perfluoro- <i>n</i> -[1,2- ¹³ C ₂]-decanesulfonate	529	81
Prometon- <i>d</i> ₃	6-Methoxy- <i>N</i> ² , <i>N</i> ⁴ -di(propan-2-yl)-1,3,5-triazine-2,4-diamine- <i>d</i> ₃	229	
Diphenyl- <i>d</i> ₁₀ -urea	<i>N,N'</i> -Diphenylurea- <i>d</i> ₁₀	223	
Metolachlor- <i>d</i> ₆	(<i>RS</i>)-2-Chloro- <i>N</i> -(2-ethyl-6-methyl-phenyl)- <i>N</i> -(1-methoxypropan-2-yl)acetamide- <i>d</i> ₆	290	
Diocetyl phthalate- <i>d</i> ₄	Bis(2-ethylhexyl) benzene-1,2-dicarboxylate- <i>d</i> ₄	395	

2.4. Suspect Screening by a Commercial Laboratory

One ampoule of each RM was shipped to Eurofins Environment Testing Australia Pty Ltd. (Murarrie, Australia) for suspect screening of PFAS.

Aliquots of the RMs were diluted 1:100 with methanol before analysis using liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QToF-MS). Data was acquired in both positive and negative mode, using both data dependent and data independent acquisition. The mass range scanned was from 50 *m/z* to 1700 *m/z* and MS2 scans were performed. The data acquired was screened against Eurofins' in-house per- and polyfluoroalkyl substances spectral library created from 76 authentic standards and the PubChem [22], ChemSpider [23], and the NORMAN Suspect Exchange List [24] public chemical databases.

Table 12 lists the PFAS identified by the commercial laboratory and the RM(s) in which each compound was identified.

Structures and structural identifiers are listed in [Appendix B](#).

Table 12. PFAS Identified by a Commercial Laboratory in RM 8690, 8691, 8692, and/or 8693.

Identified PFAS	Formula	In RMs ^a
Perfluoropropane sulfonic acid	C ₃ HF ₇ O ₃ S	8690
Perfluorobutane sulfonic acid	C ₄ HF ₉ O ₃ S	8690
Perfluoropentane sulfonic acid	C ₅ HF ₁₁ O ₃ S	8690
Perfluorohexane sulfonic acid	C ₆ HF ₁₃ O ₃ S	8690
Perfluoroheptane sulfonic acid	C ₇ HF ₁₅ O ₃ S	8690
Perfluorooctane sulfonic acid	C ₈ HF ₁₇ O ₃ S	8690
Perfluorohexane sulfonamido amine	C ₁₁ H ₁₃ F ₁₃ N ₂ O ₂ S	8690
4:2 Fluorotelomer thioether amido sulfonic acid	C ₁₃ H ₁₈ F ₉ NO ₄ S ₂	8691
6:2 Fluorotelomer sulfonate	C ₈ H ₅ F ₁₃ O ₃ S	8693
6:2 Fluorotelomer thioether propanoic acid	C ₁₁ H ₉ F ₁₃ O ₂ S	8692
6:2 Fluorotelomer sulfonamide amine	C ₁₃ H ₁₇ F ₁₃ N ₂ O ₂ S	8693
6:2 Fluorotelomer thioether hydroxyammonium	C ₁₄ H ₁₉ F ₁₃ NOS	8692/3
6:2 Fluorotelomer sulfoxide hydroxyammonium	C ₁₄ H ₁₉ F ₁₃ NO ₂ S	8692/3
6:2 Fluorotelomer thioether amido sulfonic acid	C ₁₅ H ₁₈ F ₁₃ NO ₄ S ₂	8691/2/3
6:2 Fluorotelomer sulfinyl amido sulfonic acid	C ₁₅ H ₁₈ F ₁₃ NO ₅ S ₂	8691/2/3
6:2 Fluorotelomer sulfonamide betaine	C ₁₅ H ₁₉ F ₁₃ N ₂ O ₄ S	8693
8:2 Fluorotelomer thioether amido sulfonic acid	C ₁₇ H ₁₈ F ₁₇ NO ₄ S ₂	8691
10:2 Fluorotelomer thioether amido sulfonic acid	C ₁₉ H ₁₈ F ₂₁ NO ₄ S ₂	8691
3-[(3,3,4,4,5,5,6,6,7,7,8,8,8-Tridecafluorooctyl)sulfanyl]propanamide	C ₁₁ H ₁₀ F ₁₃ NOS	8691/2/3
3-[(3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,10-Heptafluorodecyl)sulfanyl]propanamide	C ₁₃ H ₁₀ F ₁₇ NOS	8691
3-[(3,3,4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,12-Henicosafluorododecyl)sulfanyl]propanamide	C ₁₅ H ₁₀ F ₂₁ NOS	8691
<i>N,N</i> -Dimethyl-3-((perfluorohexyl)ethylsulfonyl)aminopropanamine- <i>N</i> -oxide	C ₁₃ H ₁₇ F ₁₃ N ₂ O ₃ S	8693

^a In the legacy AFFF used to produce the RMs, for example '8690/2' indicates the compound was present in RM 8690 and RM 8692.

2.5. Targeted Analysis by a Commercial Laboratory

All but three of the 28 PFAS that were detected above the reporting limit in at least one of the targeted analysis studies discussed in Section 3 were identified in the initial interlaboratory study (Section 2.1) and/or by NTA (Sections 2.2, 2.3, and 2.4). Table 13 lists the three PFAS identified and semi-quantified or quantified during targeted analysis that were not identified in the previous sections.

Structures and structural identifiers are listed in Appendix B.

Table 13. Additional PFAS Identified by a Commercial Laboratory in RMs 8690 to 8693.

Identified PFAS	Formula	In RMs
Perfluorobutanesulfonamide	C ₄ H ₂ F ₉ NO ₂ S	8690/1/2/3
Perfluorohexanesulfonamide	C ₆ H ₂ F ₁₃ NO ₂ S	8690/1/2/3
<i>N</i> -[3-(Perfluoro-1-hexanesulfonamido)propan-1-yl]- <i>N,N,N</i> -trimethylammonium	C ₁₂ H ₁₆ F ₁₃ N ₂ O ₂ S	8690/3

2.6. Summary List of Identified PFAS in RMs 8690, 8691, 8692, and/or 8693

Table 14 lists the 70 PFAS that have been thus far identified in RMs 8690, 8691, 8692, and/or 8693. The PFAS are listed roughly in order of increasing structural complexity. The compounds are here identified by a consensus chemical name, code name (often a modified acronym), and molecular formula. These three identifiers can be used to query the structural descriptors provided in Appendix B.

Table 14 also identifies the studies in which the PFAS were identified. Those that were quantitatively reported by at least one participant in the initial ILS (Section 2.1) are coded as being in set “A”. Those reported in the initial NTA screening study are coded as being in set “B” (Section 2.2). Those reported in the secondary NTA study are coded as being reported in set “C” (Section 2.3). Those reported by the commercial laboratory are coded as being reported in set “D” (Section 2.4). Those that are quantitatively reported in at least one NIST targeted analysis are coded as being reported in set “Z” (Section 3).

Note: Appendix B provides the Simplified Molecular-Input Line-Entry System (SMILES) [25] and International Chemical Identifier (InChI) [26,27] molecular structure line-text descriptors along with a 2D diagram of the compound. When known, the unique Distributed Structure-Searchable Toxicity (DSSTox) Structural Identifier (DTXSID) [28,29] and Chemical Abstract Service Registry Number (CAS) [30] are also provided.

Table 14. Summary of PFAS Identified in RM 8690, 8691, 8692, and/or 8693.

Identified PFAS	Code	Formula	In Sets ^a	In RMs ^b
Perfluorobutanoic acid	PFBA	C ₄ HF ₇ O ₂	DZ	8690/1/2/3
Perfluoropentanoic acid	PFPeA	C ₅ HF ₉ O ₂	DZ	8690/1/2/3
Perfluorohexanoic acid	PFHxA	C ₆ HF ₁₁ O ₂	DZ	8690/1/2/3
Perfluoroheptanoic acid	PFHpA	C ₇ HF ₁₃ O ₂	DZ	8690/1/2/3
Perfluorooctanoic acid	PFOA	C ₈ HF ₁₅ O ₂	DZ	8690/1
Perfluorononanoic acid	PFNA	C ₉ HF ₁₇ O ₂	Z	8690/1
Perfluorodecanoic acid	PFDA	C ₁₀ HF ₁₉ O ₂	DZ	8690/1/2
Perfluoroundecanoic acid	PFUnA	C ₁₁ HF ₂₁ O ₂	Z	8690/1/2
Perfluorododecanoic acid	PFDoA	C ₁₂ HF ₂₃ O ₂	Z	8691
Perfluorotridecanoic acid	PFTriA	C ₁₃ HF ₂₅ O ₂	Z	8691
Perfluorotetradecanoic acid	PFTA	C ₁₄ HF ₂₇ O ₂	Z	8691/3
2,3,3,3-Tetrafluoro-2-(heptafluoropropoxy)propanoic acid	HFPO-DA	C ₆ HF ₁₁ O ₃	Z	8690
4,8-Dioxa-3H-perfluorononanoic acid	ADONA	C ₇ H ₂ F ₁₂ O ₄	Z	8690
Perfluoropropanesulfonic acid	PFPrS	C ₃ HF ₇ O ₃ S	ACD	8690/1/2/3
Perfluorobutanesulfonic acid	PFBS	C ₄ HF ₉ O ₃ S	ACDZ	8690/1/2/3
Perfluoropentanesulfonic acid	PFPeS	C ₅ HF ₁₁ O ₃ S	ACDZ	8690/1/2
Perfluorohexanesulfonic acid	PFHxS	C ₆ HF ₁₃ O ₃ S	ACDZ	8690/1/2
Perfluoroheptanesulfonic acid	PFHpS	C ₇ HF ₁₅ O ₃ S	CDZ	8690/1/3
Perfluorooctanesulfonic acid	PFOS	C ₈ HF ₁₇ O ₃ S	ACDZ	8690/1/2/3
Perfluorononanesulfonic acid	PFNS	C ₉ HF ₁₉ O ₃ S	Z	8690/2
Perfluorodecanesulfonic acid	PFDS	C ₁₀ HF ₂₁ O ₃ S	DZ	8690
Perfluorododecanesulfonic acid	PFDoS	C ₁₂ HF ₂₅ O ₃ S	Z	8690
Perfluorobutanesulfonamide	PFBSA	C ₄ H ₂ F ₉ NO ₂ S	D	8690/1/2/3
Perfluorohexanesulfonamide	PFHxSA	C ₆ H ₂ F ₁₃ NO ₂ S	D	8690/1/2/3
Perfluorooctanesulfonamide	PFOSA	C ₈ H ₂ F ₁₇ NO ₂ S	Z	8690
<i>N</i> -methyl perfluorooctanesulfonamide	<i>N</i> -MeFOSA	C ₉ H ₄ F ₁₇ NO ₂ S	Z	8690
Perfluoroethane sulfonamido amine	<i>N</i> -AP-FESA	C ₇ H ₁₃ F ₅ N ₂ O ₂ S	BD	8690
Perfluoropropane sulfonamido amine	<i>N</i> -AP-FPrSA	C ₈ H ₁₃ F ₇ N ₂ O ₂ S	BD	8690
Perfluorobutane sulfonamido amine	<i>N</i> -AP-FBSA	C ₉ H ₁₃ F ₉ N ₂ O ₂ S	ABD	8690/2/3
Perfluorobutane sulfonamido amine oxide	<i>N</i> -OxAmP-FBSA	C ₉ H ₁₃ F ₉ N ₂ O ₃ S	A	8690
Perfluoropentane sulfonamido amine	<i>N</i> -AP-FPeSA	C ₁₀ H ₁₃ F ₁₁ N ₂ O ₂ S	BD	8690
Perfluorohexane sulfonamido amine	<i>N</i> -AP-FHxSA	C ₁₁ H ₁₃ F ₁₃ N ₂ O ₂ S	ABCD	8690/2
<i>N</i> -[3-(perfluoro-1-hexanesulfonamido)propan-1-yl]- <i>N,N,N</i> -trimethylammonium	<i>N</i> -TAmP-FHxSA	C ₁₂ H ₁₆ F ₁₃ N ₂ O ₂ S	D	8690/3
Perfluoroheptane sulfonamido amine	<i>N</i> -AP-FHpSA	C ₁₂ H ₁₃ F ₁₅ N ₂ O ₂ S	BD	8690
Perfluoroheptane sulfonamido amine oxide	<i>N</i> -OxAmP-FHpSA	C ₁₂ H ₁₃ F ₁₅ N ₂ O ₃ S	A	8693
Perfluorooctane sulfonamido amine	<i>N</i> -AP-FOSA	C ₁₃ H ₁₃ F ₁₇ N ₂ O ₂ S	BD	8690
<i>N,N</i> -dimethyl- <i>N</i> -(3-perfluoroalkylsulfonamidopropan-1-yl)- <i>N</i> -(-sulfopropan-1-yl)ammonium	<i>N</i> -SPAmP-FPrSA	C ₁₁ H ₂₀ F ₇ N ₂ O ₅ S ₂	A	8690
<i>N,N</i> -dimethyl- <i>N</i> -(3-perfluoroalkylsulfonamidobutan-1-yl)- <i>N</i> -(-sulfopropan-1-yl)ammonium	<i>N</i> -SPAmP-FBSA	C ₁₂ H ₂₀ F ₉ N ₂ O ₅ S ₂	A	8690
<i>N</i> -methylperfluorooctanesulfonamidoethanol	<i>N</i> -MeFOSE	C ₁₁ H ₄ F ₂₁ NO ₃ S	Z	8690/3
<i>N</i> -methylperfluorooctanesulfonamidoacetic acid	<i>N</i> -MeFOSAA	C ₁₁ H ₆ F ₁₇ NO ₄ S	Z	8690
<i>N</i> -[3-(dimethylamino)propyl]- <i>N</i> -[(pentafluoroethyl)sulfonyl]-beta-alanine	PFASAC n=2	C ₁₀ H ₁₇ F ₅ N ₂ O ₄ S	BD	8690

Identified PFAS	Code	Formula	In Sets ^a	In RMs ^b
<i>N</i> -[3-(dimethylamino)propyl]- <i>N</i> -[(heptafluoropropyl)sulfonyl]-beta-alanine	PFASAC n=3	C ₁₁ H ₁₇ F ₇ N ₂ O ₄ S	BD	8690
<i>N</i> -[3-(dimethylamino)propyl]- <i>N</i> -[(nonafluorobutyl)sulfonyl]-beta-alanine	PFASAC n=4	C ₁₂ H ₁₇ F ₉ N ₂ O ₄ S	BD	8690
<i>N</i> -[3-(dimethylamino)propyl]- <i>N</i> -[(undecafluoropentyl)sulfonyl]-beta-alanine	PFASAC n=5	C ₁₃ H ₁₇ F ₁₁ N ₂ O ₄ S	BD	8690
<i>N</i> -[3-(dimethylamino)propyl]- <i>N</i> -[(tridecafluorohexyl)sulfonyl]-beta-alanine	PFASAC n=6	C ₁₄ H ₁₇ F ₁₃ N ₂ O ₄ S	ABD	8690
<i>N</i> -[3-(dimethylamino)propyl]- <i>N</i> -[(pentadecafluoroheptyl)sulfonyl]-beta-alanine	PFASAC n=7	C ₁₅ H ₁₇ F ₁₅ N ₂ O ₄ S	BD	8690
<i>N</i> -[3-(dimethylamino)propyl]- <i>N</i> -[(heptadecafluoroocetyl)sulfonyl]-beta-alanine	PFASAC n=8	C ₁₆ H ₁₇ F ₁₇ N ₂ O ₄ S	BD	8690
4:2 Fluorotelomer sulfonic acid	4:2 FTS	C ₆ H ₅ F ₉ O ₃ S	DZ	8690/1/2/3
6:2 Fluorotelomer sulfonic acid	6:2 FTS	C ₈ H ₅ F ₁₃ O ₃ S	ACDZ	8690/1/2/3
8:2 Fluorotelomer sulfonic acid	8:2 FTS	C ₁₀ H ₅ F ₁₇ O ₃ S	DZ	8690/1/2/3
6:2 Fluorotelomer thioether propanoic acid	6:2 FtTP	C ₁₁ H ₉ F ₁₃ O ₂ S	C	8692
6:2 Fluorotelomer thioether propanamide	6:2 FtTPAm	C ₁₁ H ₁₀ F ₁₃ NOS	BCD	8691/2/3
8:2 Fluorotelomer thioether propanamide	8:2 FtTPAm	C ₁₃ H ₁₀ F ₁₇ NOS	BCD	8691
10:2 Fluorotelomer thioether propanamide	10:2 FtTPAm	C ₁₅ H ₁₀ F ₂₁ NOS	BCD	8691
12:2 Fluorotelomer thioether propanamide	12:2 FtTPAm	C ₁₇ H ₁₀ F ₂₅ NOS	B	8691
6:2 Fluorotelomer thioether hydroxyammonium	6:2 FTSHA	C ₁₄ H ₁₉ F ₁₃ NOS	C	8692/3
6:2 Fluorotelomer sulfoxide hydroxyammonium	6:2 FTSHA Sulfoxide	C ₁₄ H ₁₉ F ₁₃ NO ₂ S	C	8692/3
4:2 Fluorotelomer thioether amido sulfonic acid	4:2 FTtAoS	C ₁₃ H ₁₈ F ₉ NO ₄ S ₂	BCD	8691
6:2 Fluorotelomer thioether amido sulfonic acid	6:2 FTtAoS	C ₁₅ H ₁₈ F ₁₃ NO ₄ S ₂	ABCD	8690/1/2/3
6:2 Fluorotelomer sulfinyl amido sulfonic acid	6:2 FtSiAoS	C ₁₅ H ₁₈ F ₁₃ NO ₅ S ₂	ABCD	8690/1/2/3
6:2 Fluorotelomer sulfonyl amido sulfonic acid	6:2 FtSoAoS	C ₁₅ H ₁₈ F ₁₃ NO ₆ S ₂	B	8691
8:2 Fluorotelomer thioether amido sulfonic acid	8:2 FTtAoS	C ₁₇ H ₁₈ F ₁₇ NO ₄ S ₂	ABCD	8691
10:2 Fluorotelomer thioether amido sulfonic acid	10:2 FTtAoS	C ₁₉ H ₁₈ F ₂₁ NO ₄ S ₂	BCD	8691
12:2 Fluorotelomer thioether amido sulfonic acid	12:2 FTtAoS	C ₂₁ H ₁₈ F ₂₅ NO ₄ S ₂	B	8691
4:2 Fluorotelomer sulfonamide betaine	4:2 FTAB	C ₁₃ H ₁₉ F ₉ N ₂ O ₄ S	BD	8690/3
6:2 Fluorotelomer sulfonamide betaine	6:2 FTAB	C ₁₅ H ₁₉ F ₁₃ N ₂ O ₄ S	ABCD	8690/1/2/3
6:2 Fluorotelomer sulfonamide amine	6:2 FTAA	C ₁₃ H ₁₇ F ₁₃ N ₂ O ₂ S	C	8693
<i>N,N</i> -dimethyl-3-((perfluorohexyl)ethylsulfonyl)aminopropanamine oxide	6:2 FTAA-Ox	C ₁₃ H ₁₇ F ₁₃ N ₂ O ₃ S	ABCD	8693
1-[[[4-(2-(Perfluorobutyl)-1-ethyl)phenyl)methoxy]carbonyl]oxy]-2,5-pyrrolidinedione	not assigned	C ₁₈ H ₁₄ F ₉ NO ₅	A	8692/3
4-[3-(Perfluorobutyl)-1-propyloxy]benzyl alcohol	not assigned	C ₁₄ H ₁₃ F ₉ O ₂	A	8690

a) Compound identified in: A) interlaboratory study (Section 2.1), B) initial screening non-targeted analysis (Section 2.2); C) high-resolution mass spectrometric non-targeted analysis (Section 2.3), D) commercial laboratory screening (Section 2.4); Z) one or more of the NIST targeted analyses (Section 3).

3. Targeted Quantitative Measurements by NIST

The studies discussed in this section provided quantitative results based on targeted analysis performed by NIST using calibrants purchased from Wellington Laboratories (Guelph, Ontario). The reported results are metrologically traceable to the calibrants used but not to the International System of Units (SI). Table 15 lists the PFAS calibrants used.

Table 15. Calibration Materials Used in Targeted Analyses.

Code Name ^a	Product ^b	µg/mL ^c	Code Name ^a	Product ^b	µg/mL ^c
PFBA	PFAC30PAR	1	PFNS	PFAC30PAR	1
PFPeA	PFAC30PAR	1	PFDS	PFAC30PAR	1
PFHxA	PFAC30PAR	1	PFECHS	PFECHS	50
PFHpA	PFAC30PAR	1	PFEESA	PFAC-MXG	2
PFOA	PFAC30PAR	1	9Cl-PF3ONS	PFAC30PAR	1
PFNA	PFAC30PAR	1	11Cl-PF3OUdS	PFAC30PAR	1
PFDA	PFAC30PAR	1	PFBSA	PFAC30PAR	1
PFUnA	PFAC30PAR	1	PFHxSA	PFAC30PAR	1
PFDoA	PFAC30PAR	1	PFOSA	PFAC30PAR	1
PFTriA	PFAC30PAR	1	N-MeFBSA	N-MeFBSA-M	50
PFTA	PFAC30PAR	1	N-MeFOSA	N-MeFOSA-M	50
HFPO-DA	PFAC30PAR	1	N-EtFOSA	N-EtFOSA-M	50
ADONA	PFAC30PAR	1	N-AP-FHxSA	N-AP-FHxSA	50
PF4OPeA	PFAC-MXG	2	N-TAmP-FHxSA	N-TAmP-FHxSA	50
PF5OHxA	PFAC-MXG	2	N-MeFOSAA	PFAC30PAR	1
3,6-OPFHpA	PFAC-MXG	2	N-EtFOSAA	PFAC30PAR	1
PFPrS	L-PFPRS	50	5:3 FTB	5:3FTB	50
PFBS	PFAC30PAR	1	5:1:2 FTB	5:1:2FTB	50
PFPeS	PFAC30PAR	1	4:2 FTS	PFAC30PAR	1
PFHxS	PFAC30PAR	1	6:2 FTS	PFAC30PAR	1
PFHpS	PFAC30PAR	1	8:2 FTS	PFAC30PAR	1
PFOS	PFAC30PAR	1	6:2 FTAB	N-CMAmP-6:2FOSA	50

- a) Calibrants are supplied as the linear isomer or specified ratio of isomers dissolved in methanol.
b) Wellington product descriptor. Different lots of some of the products have been used.
c) Concentration in µg/mL of solution. All products are supplied as 1.2 mL solution in a sealed glass ampoule.

Table 16 lists the isotopically-labeled internal standards for compounds detected in ESI positive (ESI+) mode. Table 17 lists the isotopically-labeled PFAS standards, all purchased from Wellington Labs, used as internal standards for compounds detected in ESI negative (ESI-) mode.

Table 16. Isotopically-Labeled Internal Standards for Positive Mode Electrospray Ionization.

Code Name	Chemical Name	Product ^a	mg ^b
Prometon- <i>d</i> ₃	6-Methoxy- <i>d</i> ₃ -2- <i>N</i> ,4- <i>N</i> -di(propan-2-yl)-1,3,5-triazine-2,4-diamine	CDN Isotopes D-6802	10
Diphenyl- <i>d</i> ₁₀ -urea	1,3-Diphenyl- <i>d</i> ₁₀ -urea	CDN Isotopes D-7664	50
Metolachlor- <i>d</i> ₆	(±)-2-chloro- <i>N</i> -(2-ethyl-6-methylphenyl)- <i>N</i> -(1,1,1,2,3,3-hexadeuterio-3-methoxypropan-2-yl)acetamide	CDN Isotopes D-5647	50
Diocetyl phthalate- <i>d</i> ₄	Diocetyl 3,4,5,6-tetradeuteriobenzene-1,2-dicarboxylate	Sigma Aldrich 488887	100

- a) CDN Isotopes: C/D/N ISOTOPES Inc., Pointe-Claire, Quebec, Canada.
Sigma Aldrich: now a brand of Merck KGaA, Darmstadt, Germany.
b) The isotopically-labeled standards were supplied as neat compounds.

Table 17. Isotopically-Labeled PFAS Used as Internal Standards.

Code Name ^a	Chemical Name ^a	Product ^b	µg/mL ^c
¹³ C ₃ -PFBA	Perfluoro-n-[2,3,4- ¹³ C ₃]butanoic acid	MPFAC-C-IS	2
¹³ C ₄ -PFBA	Perfluoro-n-[¹³ C ₄]butanoic acid	MPFAC-24ES	1
¹³ C ₅ -PFPeA	Perfluoro-n-[¹³ C ₅]pentanoic acid	MPFAC-24ES	1
¹³ C ₂ -PFHxA	Perfluoro-n-[1,2- ¹³ C ₂]hexanoic acid	MPFHxA	50
¹³ C ₅ -PFHxA	Perfluoro-n-[1,2,3,4,6- ¹³ C ₅]hexanoic acid	MPFAC-24ES	1
¹³ C ₄ -PFHpA	Perfluoro-n-[1,2,3,4- ¹³ C ₄]heptanoic acid	MPFAC-24ES	1
¹³ C ₂ -PFOA	Perfluoro-n-[1,2- ¹³ C ₂]octanoic acid	MPFAC-C-IS	2
¹³ C ₈ -PFOA	Perfluoro-n-[¹³ C ₈]octanoic acid	MPFAC-24ES	1
¹³ C ₉ -PFNA	Perfluoro-n-[¹³ C ₉]nonanoic acid	MPFAC-24ES	1
¹³ C ₂ -PFDA	Perfluoro-n-[1,2- ¹³ C ₂]decanoic acid	MPFAC-C-IS	2
¹³ C ₆ -PFDA	Perfluoro-n-[1,2,3,4,5,6- ¹³ C ₆]decanoic acid	MPFAC-24ES	1
¹³ C ₂ -PFUnA	Perfluoro-n-[1,2- ¹³ C ₂]undecanoic acid	MPFUdA	50
¹³ C ₇ -PFUnA	Perfluoro-n-[1,2,3,4,5,6- ¹³ C ₇]undecanoic acid	MPFAC-24ES	1
¹³ C ₂ -PFDoA	Perfluoro-n-[1,2- ¹³ C ₂]dodecanoic acid	MPFAC-24ES	1
¹³ C ₂ -PFTeDA	Perfluoro-n-[1,2- ¹³ C ₂]tetradecanoic acid	MPFAC-24ES	1
¹³ C ₃ -HFPO-DA	2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-[¹³ C ₃]-propanoic acid	M3HFPO-DA	50
¹³ C ₃ -PFBS	Perfluoro-1-[2,3,4- ¹³ C ₃]butanesulfonic acid	MPFAC-24ES	1
¹³ C ₃ -PFHxS	Perfluoro-1-[1,2,3- ¹³ C ₃]hexanesulfonic acid	MPFAC-24ES	1
¹⁸ O ₂ -PFHxS	Perfluoro-1-hexane(¹⁸ O ₂)sulfonic acid	MPFHxS	50
¹³ C ₄ -PFOS	Perfluoro-1-[1,2,3,4- ¹³ C ₄]octanesulfonic acid	MPFAC-C-IS	2
¹³ C ₈ -PFOS	Perfluoro-1-[¹³ C ₈]octanesulfonic acid	MPFAC-24ES	1
¹³ C ₈ -FOSA	Perfluoro-n-[¹³ C ₈]octanesulfonamide	MPFAC-24ES	1
<i>d</i> ₃ -N-MeFOSA	<i>N</i> -methyl- <i>d</i> ₃ -perfluoro-1-octanesulfonamide	<i>d</i> -N-MeFOSA-M	50
<i>d</i> ₅ -N-EtFOSA	<i>N</i> -ethyl- <i>d</i> ₅ -perfluoro-1-octanesulfonamide	<i>d</i> -N-EtFOSA-M	50
<i>d</i> ₃ -N-MeFOSAA	<i>N</i> -methyl- <i>d</i> ₃ -perfluoro-1-octanesulfonamidoacetic acid	MPFAC-24ES	1
<i>d</i> ₅ -N-EtFOSAA	<i>N</i> -ethyl- <i>d</i> ₅ -perfluoro-1-octanesulfonamidoacetic acid	MPFAC-24ES	1
¹³ C ₂ -4:2 FTS	1H,1H,2H,2H-perfluoro-n-[1,2- ¹³ C ₂]hexanesulfonic acid	MPFAC-24ES	1
¹³ C ₂ -6:2 FTS	1H,1H,2H,2H-perfluoro-n-[1,2- ¹³ C ₂]octanesulfonic acid	MPFAC-24ES	1
¹³ C ₂ -8:2 FTS	1H,1H,2H,2H-perfluoro-n-[1,2- ¹³ C ₂]decanesulfonic acid	MPFAC-24ES	1

- a) Isotopically-labeled standards were supplied in methanol.
b) Wellington product descriptor. Multiple lots of some of the products were used.
c) Concentration in µg/mL of solution. All products were supplied as 1.2 mL solutions in sealed glass ampoules.

All standards were stored at -20 °C until analysis.

3.1. Between-Ampoule Homogeneity Assessment

The between-ampoule homogeneity of the four RMs was assessed with LC-MS/MS measurements of the first, last, and eight intermediate ampoules of each RM.

3.1.1. Materials

Table 18 lists the 10 ampoules of each of the four RMs that were used, by their ampoule-fill order.

Table 18. Ampoules Used for the Between-Ampoule Homogeneity Assessment.

RM 8690	RM 8691	RM 8692	RM 8693
1	1	1	1
79	49	282	226
142	195	354	387
291	404	494	488
317	683	637	648
471	766	715	863
675	805	791	1003
855	1088	1035	1115
1005	1138	1126	1183
1148	1288	1347	1260

Methanol (Alfa Aesar) was used for the preparation and/or dilution of the calibration solutions, internal standard solutions (IS solutions), and samples. Table 19 lists the composition of the two IS solutions prepared from the compounds listed in Table 16 and Table 17.

Table 19. Internal Standard Solutions.

Internal Standard IS1		Internal Standard IS2	
Compound	Concentration, $\mu\text{g/g}$	Compound	Concentration, $\mu\text{g/g}$
$^{13}\text{C}_4$ -PFBA	0.0183	$^{13}\text{C}_3$ -PFBA	0.00673
$^{13}\text{C}_5$ -PFPeA	0.0183	$^{13}\text{C}_2$ -PFOA	0.00673
$^{13}\text{C}_5$ -PFHxA	0.0183	$^{13}\text{C}_2$ -PFHxA	0.00755
$^{13}\text{C}_4$ -PFHpA	0.0183	$^{13}\text{C}_2$ -PFDA	0.00673
$^{13}\text{C}_8$ -PFOA	0.0183	$^{13}\text{C}_2$ -PFUnA	0.00779
$^{13}\text{C}_9$ -PFNA	0.0183	$^{18}\text{O}_2$ -PFHxS	0.00787
$^{13}\text{C}_6$ -PFDA	0.0183	$^{13}\text{C}_4$ -PFOS	0.00645
$^{13}\text{C}_7$ -PFUnA	0.0175	Prometon- d_3	0.0158
$^{13}\text{C}_2$ -PFDoA	0.0179	Diphenyl- d_{10} -urea	0.0151
$^{13}\text{C}_2$ -PFTA	0.0183	Metolachlor- d_6	0.169
$^{13}\text{C}_3$ -PFBS	0.0183	Diocetyl phthalate- d_4	0.0385
$^{13}\text{C}_3$ -PFHxS	0.0183		
$^{13}\text{C}_8$ -PFOS	0.0183		
$^{13}\text{C}_8$ -FOSA	0.0179		
d_3 -NMeFOSAA	0.0177		
d_5 -NEtFOSAA	0.0178		
$^{13}\text{C}_2$ -4:2 FTS	0.0183		
$^{13}\text{C}_2$ -6:2 FTS	0.0183		
$^{13}\text{C}_2$ -8:2 FTS	0.0174		
$^{13}\text{C}_3$ -HFPO-DA	0.0183		

Table 20 lists the composition of three working standards (WS) prepared from the PFAS calibrants listed in Table 15; it also identifies the isotopically-labeled compound used as an internal standard for each PFAS measurand (Paired IS).

Table 20. Calibration Solution Concentrations and Internal Standard Pairings.

Calibrant	Concentration, µg/g			Paired IS
	WS1	WS1.5	WS2	
PFBA	0.115	0.0124	0.00113	¹³ C ₄ -PFBA
PFPeA	0.115	0.0124	0.00113	¹³ C ₅ -PFPeA
PFHxA	0.115	0.0124	0.00113	¹³ C ₅ -PFHxA
PFHpA	0.115	0.0124	0.00113	¹³ C ₄ -PFHpA
PFOA	0.115	0.0124	0.00113	¹³ C ₈ -PFOA
PFNA	0.115	0.0124	0.00113	¹³ C ₉ -PFNA
PFDA	0.115	0.0124	0.00113	¹³ C ₆ -PFDA
PFUnA	0.115	0.0124	0.00113	¹³ C ₇ -PFUnA
PFDoA	0.115	0.0124	0.00113	¹³ C ₂ -PFDoA
PFTriA	0.115	0.0124	0.00113	¹³ C ₂ -PFTDA
PFTA	0.115	0.0124	0.00113	¹³ C ₂ -PFTDA
HFPO-DA	0.115	0.0124	0.00113	¹³ C ₃ -HFPO-DA
ADONA	0.110	0.0119	0.00109	¹³ C ₈ -PFOA
PFBSA	0.115	0.0124	0.00113	¹³ C ₄ -PFHpA
PFHxSA	0.115	0.0124	0.00113	¹³ C ₈ -PFOS
PFOSA	0.115	0.0124	0.00113	¹³ C ₈ -PFOSA
N-MeFOSAA	0.117	0.0127	0.00115	<i>d</i> ₃ -MeFOSAA
N-EtFOSAA	0.117	0.0127	0.00115	<i>d</i> ₅ -EtFOSAA
PFPrS	0.083	0.0090	0.00081	¹³ C ₄ -PFBA
PFBS	0.103	0.0112	0.00102	¹³ C ₄ -PFBA
PFPeS	0.110	0.0119	0.00108	¹³ C ₃ -PFHxS
PFHxS	0.106	0.0115	0.00105	¹³ C ₃ -PFHxS
PFHpS	0.111	0.0120	0.00109	¹³ C ₈ -PFOS
PFOS	0.108	0.0117	0.00107	¹³ C ₈ -PFOS
PFNS	0.112	0.0122	0.00110	¹³ C ₈ -PFOS
PFDS	0.113	0.0122	0.00111	¹³ C ₈ -PFOS
4:2 FTS	0.109	0.0118	0.00107	¹³ C ₂ -4:2 FTS
6:2 FTS	0.111	0.0120	0.00109	¹³ C ₂ -6:2 FTS
8:2 FTS	0.112	0.0121	0.00110	¹³ C ₂ -8:2 FTS
9Cl-PF3ONS	0.109	0.0118	0.00107	¹³ C ₈ -PFOS
11Cl-PF3OU _n DS	0.110	0.0119	0.00108	¹³ C ₈ -PFOS
N-AP-FHxSA	0.108	0.0117	0.00106	¹³ C ₂ -6:2 FTS
N-TAmP-FHxSA	0.104	0.0113	0.00102	¹³ C ₂ -6:2 FTS
6:2 FTAB	0.108	0.0117	0.00106	¹³ C ₂ -6:2 FTS

All standards were stored at -20 °C until analysis.

3.1.2. Sample Preparation

Based on the preliminary assessment, ampoules for RM 8690 and RM 8693 were gravimetrically diluted with 200 µL RM from each ampoule into 9.8 mL methanol to create Dilution 1. Dilution 2 was prepared from 400 µL of Dilution 1 and 600 µL of methanol. A known mass of approximately 800 µL of Dilution 2 was added to precleaned autosampler vials. A known mass of approximately 200 µL of each of the two IS solutions were added to the autosampler vials. While the exact dilution factors varied slightly between samples, the average combined dilution is approximately 180-fold.

Based on the preliminary assessment, ampoules for RM 8691 and 8692 did not need significant dilution. A known mass of approximately 200 μL of RM from each ampoule was added to precleaned autosampler vials. Then, a known mass of approximately 200 μL of each of the two IS solution and an additional 400 μL of methanol were added to the autosampler vials. While the exact dilution factors varied slightly between samples, the average is approximately five-fold.

Methanol blanks spiked with both IS mixtures were prepared in triplicate from known masses of approximately 800 μL methanol and 200 μL of each IS solution.

3.1.3. Instrumental Method

Chromatographic separation was performed using an Agilent Infinity II liquid chromatograph (Agilent, Santa Clara, CA). The autosampler tray temperature was set to the ambient air temperature, roughly 25 °C. The mobile phases consisted of 10 mM ammonium acetate in laboratory deionized water (A) and 10 mM ammonium acetate in HPLC grade methanol (B) with a flow rate of 0.350 mL/min and initial composition of 100 % A, 0 % B. The initial mobile phase was equilibrated at this composition for at least 15 min prior to injection. After the 10 μL injection, the mobile phase was changed to 65 % A, 35 % B over 3 min, then changed to 0% A and 100% B over the next 22 minutes and held at this composition for 7 min before returning to initial conditions over the next 13 min. A retention column was placed before the pumps (Eclipse Plus C18, 4.6 mm ID, 50 mm, 5 μm particle size). The chromatographic column set up was a Zorbax Diol (4.6 mm ID, 12.5 mm, 6 μM particle size) attached to an Agilent InfinityLab Poroshell 120 EC-C18 column (4.6 mm ID, 100 mm, 2.7 μM particle size). The column temperature was maintained at ambient air temperature for the entire run. Mass spectrometric detection was performed using an AB Sciex API 5500+ Triple Quadrupole Mass Spectrometer (AB Sciex, Framingham, MA).

Fig. 4 displays the chromatographic separation of the PFAS calibrants in the WS.

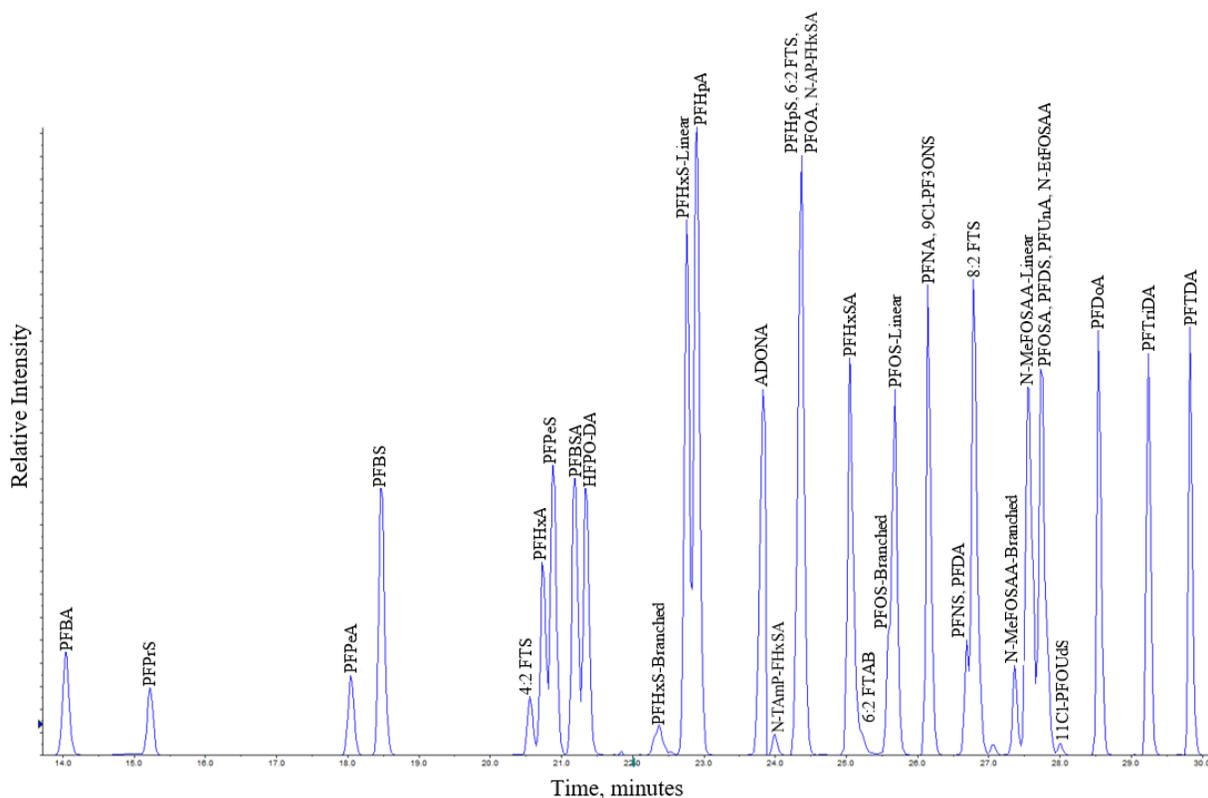


Fig. 4. Exemplar Chromatographic Separation of the PFAS Calibrants

3.1.4. Quantification

Linear calibration models for each of the 34 PFAS measurands listed in Table 20 were estimated using the five calibrant solutions described in Table 21. The intercepts of the models were not forced through zero. All calibration curves had Pearson correlation $R^2 \geq 0.99$.

Table 21. Calibrants for Homogeneity Assessment.

Calibrant	WS1 g	WS1.5 g	WS2 g	IS1 g	IS2 g	Total g	PFBA ^a µg/g
Cal A	0.23578			0.15853	0.15983	0.55414	0.04893
Cal B		0.82242		0.16013	0.16043	1.14298	0.00892
Cal C		0.14705		0.16125	0.15999	0.46829	0.00389
Cal D			0.82311	0.15937	0.15869	1.14117	0.00082
Cal E			0.07843	0.16037	0.16044	0.39924	0.00022

a PFBA is a representative measurand.

Measurands were quantified using the relative response ratio to its paired IS (Table 20).

Reporting limits (RLs) were determined as the maximum of the blank plus three times the standard deviation (SD) of the measurand in the blanks or the lowest detectable concentration among the calibrants. Of the 34 measurands assessed, 17 had measured values above their RL in at least one of the RMs. Table 22, Table 23, Table 24, and Table 25 report the measured values for RMs 8690, 8691, 8692, and 8693.

Table 26 lists the means, SDs, and relative SDs expressed as a coefficient of variation (CV) for all measurands. The SDs are displayed as a function of their means in Fig. 5. While the relationship is somewhat variable for concentrations of less than about 0.02 $\mu\text{g/g}$, it is reasonably well modelled as a constant CV of 6 %. Since only one result was obtained for each ampoule, this variability is a composite of method repeatability and material heterogeneity.

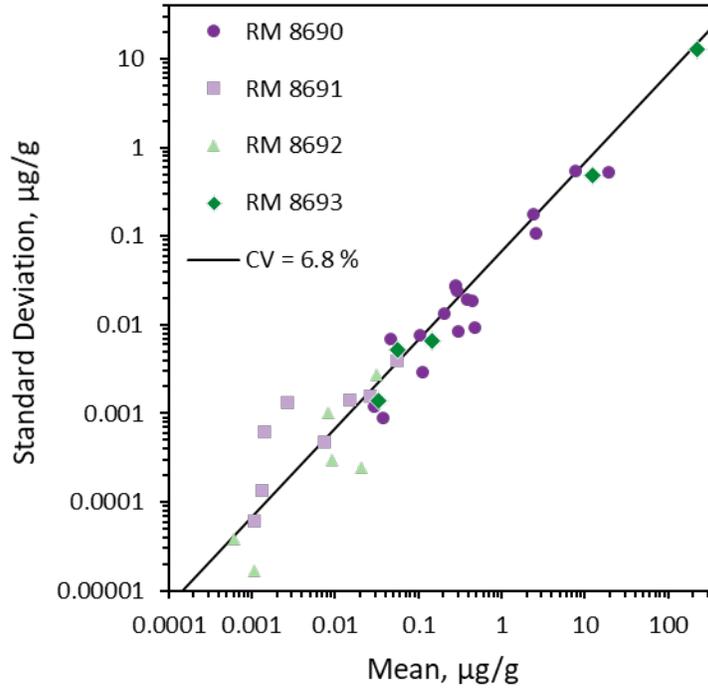


Fig. 5. Between-Ampoule Homogeneity Standard Deviation as a Function of Mean Value.

Table 22. RM 8690: Between-Ampoule Homogeneity Study Results, µg/g.

Compound	Ampoule Number based on Fill Order									
	1	79	142	291	317	471	675	855	1005	1148
PFBA	0.109	0.113	0.116	0.108	0.110	0.114	0.107	0.112	0.111	0.114
PFHxA	0.286	0.291	0.235	0.307	0.285	0.281	0.308	0.241	0.254	0.308
PFHpA	0.107	0.106	0.086	0.104	0.105	0.106	0.106	0.117	0.105	0.103
PFOA	0.300	0.317	0.298	0.250	0.302	0.246	0.296	0.251	0.300	0.259
PFTDA			0.077							
PFBSA	0.0380	0.0381	0.0384	0.0398	0.0382	0.0362	0.0374	0.0380	0.0380	0.0377
PFHxSA	0.0294	0.0319	0.0280	0.0304	0.0300	0.0283	0.0296	0.0284	0.0302	0.0289
PFPrS	0.316	0.302	0.296	0.309	0.308	0.294	0.316	0.315	0.298	0.307
PFBS	0.475	0.459	0.477	0.480	0.485	0.467	0.493	0.484	0.476	0.477
PFPeS	0.404	0.372	0.367	0.400	0.402	0.363	0.367	0.379	0.370	0.416
PFHxS	2.67	2.85	2.53	2.56	2.63	2.63	2.55	2.65	2.72	2.47
PFHpS	0.449	0.465	0.436	0.434	0.453	0.434	0.434	0.461	0.492	0.445
PFOS	18.5	20.0	18.7	19.2	19.7	19.1	19.6	19.5	20.0	19.4
PFDS	0.0467	0.0515	0.0488	0.0475	0.0548	0.0513	0.0316	0.0448	0.0511	0.0385
6:2 FTS	0.212	0.214	0.193	0.202	0.223	0.189	0.213	0.179	0.209	0.205
N-AP-FHxSA	8.18	8.62	7.18	8.33	7.86	7.41	7.26	7.02	7.60	8.23
N-TAmP-FHxSA	0.302	0.335	0.298	0.286	0.305	0.262	0.280	0.251	0.313	0.303
6:2 FTAB	2.50	2.62	2.29	2.58	2.44	2.20	2.39	2.05	2.43	2.45

Table 23. RM 8691: Between-Ampoule Homogeneity Study Results, µg/g.

Compound	Ampoule Number based on Fill Order									
	1	49	195	404	683	766	805	1088	1138	1288
PFBA	0.00754	0.00750	0.00766	0.00733	0.00750	0.00733	0.00748	0.00722	0.00769	0.00760
PFHxA	0.0151	0.0155	0.0158	0.0149	0.0149	0.0156	0.0158	0.0145	0.0154	0.0149
PFHpA	0.00137	0.00134	0.00135	0.00129	0.00135	0.00134	0.00133	0.00127	0.00124	0.00136
PFOA	0.0259	0.0266	0.0269	0.0258	0.0253	0.0260	0.0261	0.0254	0.0259	0.0263
PFDA	0.00141	0.00157	0.00161	0.00155	0.00154	0.00132	0.00174	0.00138	0.00111	0.00119
PFBSA	0.00109	0.00109	0.00108	0.00106	0.00107	0.00106	0.00107	0.00107	0.00110	0.00112
6:2 FTS	0.0533	0.0547	0.0566	0.0537	0.0558	0.0563	0.0572	0.0553	0.0553	0.0562
6:2 FTAB	0.00247	0.00264	0.00285	0.00335	0.00232	0.00286	0.00224	0.00219	0.00334	0.00290

Table 24. RM 8692: Between-Ampoule Homogeneity Study Results, µg/g.

Compound	Ampoule Number based on Fill Order									
	1	282	354	494	637	715	791	1035	1126	1347
PFBA	PFBA	0.0206	0.0208	0.0209	0.0201	0.0207	0.0206	0.0205	0.0204	0.0205
PFHxA	PFHxA	0.00912	0.00910	0.00951	0.00914	0.00937	0.00937	0.00855	0.00932	0.00926
PFHpA	PFHpA	0.000629	0.000664	0.000603	0.000583	0.000577	0.000611	0.000653	0.000664	0.000640
PFBSA	PFBSA	0.00111	0.00109	0.00106	0.00107	0.00106	0.00107	0.00107	0.00107	0.00107
6:2 FTS	6:2 FTS	0.0358	0.0303	0.0357	0.0302	0.0293	0.0299	0.0316	0.0299	0.0312
6:2 FTAB	6:2 FTAB	0.00950	0.00789	0.00988	0.00709	0.00709	0.00917	0.00839	0.00880	0.00790

Table 25. RM 8693: Between-Ampoule Homogeneity Study Results, µg/g.

Compound	Ampoule Number based on Fill Order									
	1	226	387	488	648	863	1003	1115	1183	1260
PFBA	0.0551	0.0596	0.0554	0.0519	0.0704	0.0553	0.0577	0.0530	0.0567	0.0553
PFHxA	0.149	0.152	0.147	0.139	0.136	0.142	0.151	0.154	0.155	0.142
PFBSA	0.0326	0.0335	0.0338	0.0342	0.0369	0.0330	0.0337	0.0324	0.0332	0.0319
6:2 FTS	12.9	12.4	12.3	11.6	13.1	12.3	12.14	13.1	12.0	12.4
6:2 FTAB	227	222	222	193	228	221	212	243	214	220

Table 26. Summary of Between-Ampoule Homogeneity Results for RMs 8690 to 8693.

	RM 8690			RM 8691			RM 8692			RM 8693		
	Mean µg/g	SD µg/g	CV %									
PFBA	0.111	0.003	2.6	0.00749	0.00048	6.4	0.0206	0.0002	1.2	0.0570	0.0052	9.1
PFHxA	0.280	0.027	9.8	0.0152	0.0014	9.2	0.00915	0.00030	3.3	0.147	0.007	4.5
PFHpA	0.105	0.008	7.2	0.00132	0.00014	10.2	0.000618	0.000038	6.2			
PFOA	0.282	0.027	9.6	0.0260	0.0016	6.0						
PFDA				0.00144	0.00062	43.1						
PFPrS	0.306	0.008	2.7									
PFBS	0.477	0.010	2.0									
PFPeS	0.384	0.019	5.1									
PFHxS	2.63	0.11	4.1									
PFHpS	0.450	0.019	4.1									
PFOS	19.4	0.5	2.7									
PFDS	0.0467	0.0069	14.9									
PFBSA	0.0380	0.0009	2.3	0.00108	0.00006	5.6	0.00107	0.00002	1.6	0.0335	0.0014	4.1
PFHxSA	0.0295	0.0012	4.0									
6:2 FTS	0.204	0.013	6.6	0.0554	0.0040	7.1	0.0320	0.0028	8.7	12.4	0.5	4.0
N-AP-FHxSA	7.77	0.55	7.1									
N-TAmP-FHxSA	0.294	0.025	8.4									
6:2 FTAB	2.40	0.17	7.3	0.0027	0.0013	48.9	0.0083	0.0010	12.1	220	13	5.9

3.1.5. Between-Ampoule Homogeneity Assessment

The PFAS homogeneity for each RM was investigated through comparisons of the measurand concentrations as functions of the sample ampoule filling order, shown in Fig. 6, Fig. 7, Fig. 8, and Fig. 9. There are no apparent systematic trends based on ampoule filling order.

While there are peaks and troughs in the traces of some measurands, the deviations are not coordinated across measurands within a given RM. This pattern suggests measurand-specific measurement variability rather than ampoule-specific heterogeneity.

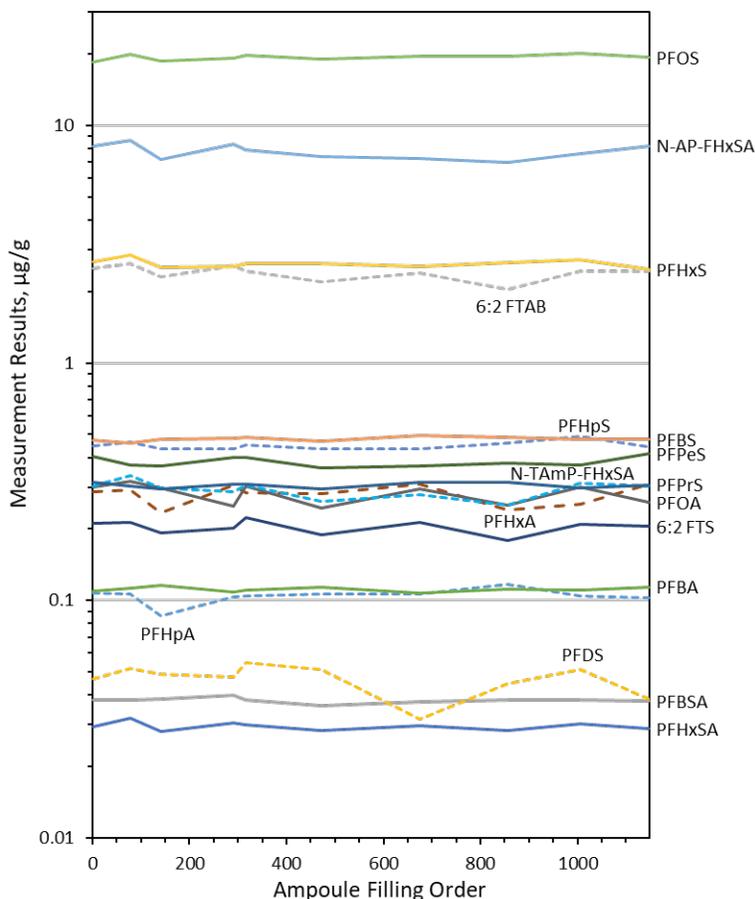


Fig. 6. RM 8690 Measurand Results as a Function of Production Sequence.

Each line connects the results for one measurand across ten ampoules. Results are displayed on a logarithmic axis to accommodate the concentration range spanned by the measurements.

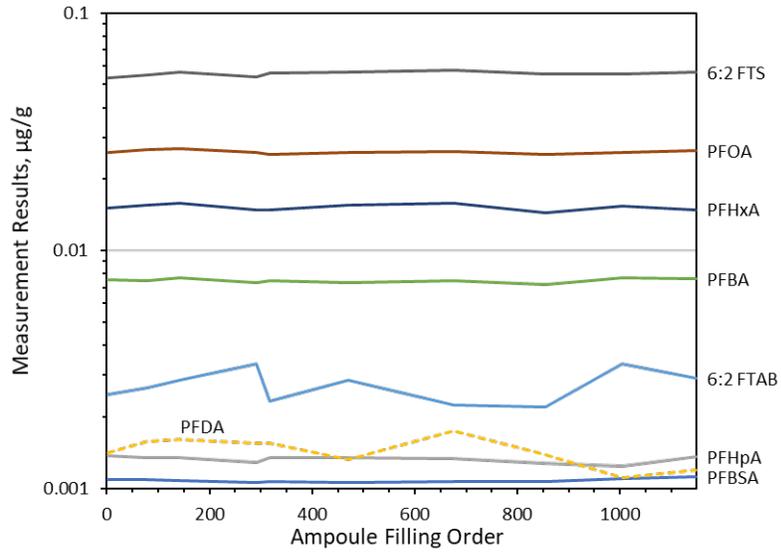


Fig. 7. RM 8691 Measurand Results as a Function of Production Sequence

Each line connects the results for one measurand across ten ampoules. Results are displayed on a logarithmic axis to accommodate the concentration range spanned by the measurements.

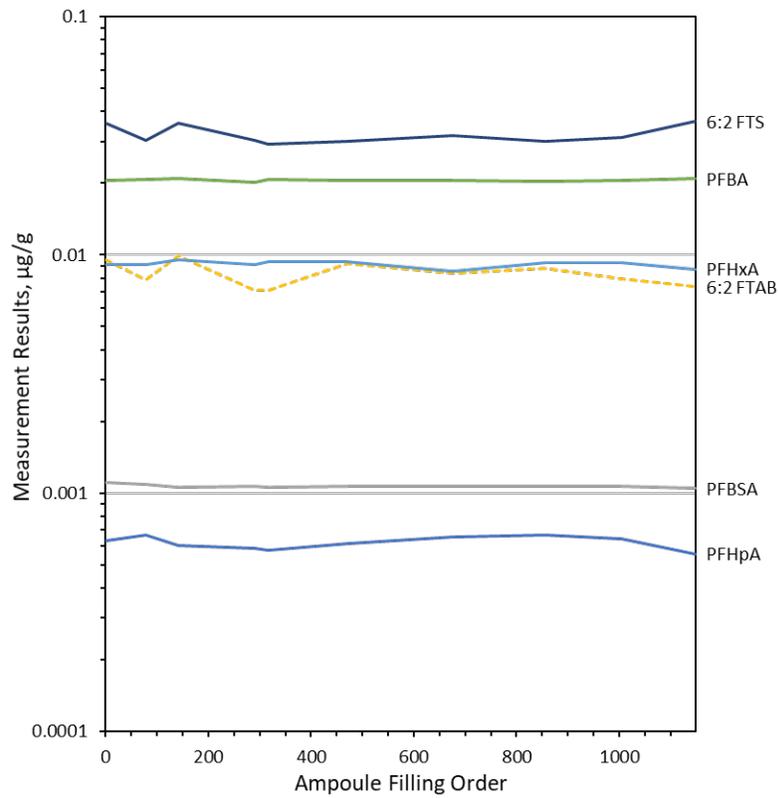


Fig. 8. RM 8692 Measurand Results as a Function of Production Sequence

Each line connects the results for one measurand across ten ampoules. Results are displayed on a logarithmic axis to accommodate the concentration range spanned by the measurements.

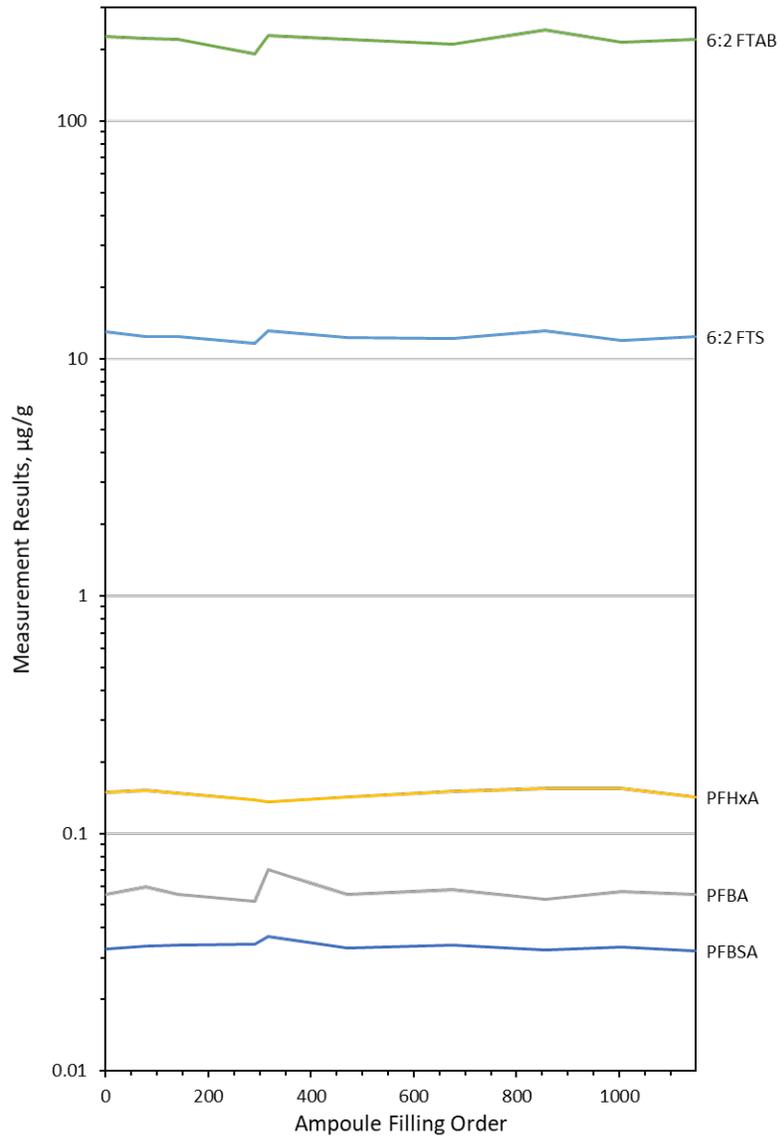


Fig. 9. RM 8693 Measurand Results as a Function of Production Sequence

Each line connects the results for one measurand across ten ampoules. Results are displayed on a logarithmic axis to accommodate the concentration range spanned by the measurements.

3.2. Within-Ampoule Homogeneity Assessment

The within-ampoule homogeneity of the four RMs was assessed with LC-MS/MS measurements of three aliquots from three ampoules of each RM. This study also addresses whether PFAS in methanol solution vertically stratify within an ampoule.

3.2.1. Materials

Table 27 lists the three ampoules of each of the four RMs that were used.

Table 27. Ampoules Used for the Within-Ampoule Homogeneity Assessment.

RM 8690	RM 8691	RM 8692	RM 8693
79	195	354	387
317	766	715	863
675	1138	1126	1183

All other materials (including methanol, calibrants, and internal standards) are as described in Section 3.1.1. All standards were stored at -20 °C until analysis

3.2.2. Sample Preparation

To evaluate whether vertical stratification was observed within an ampoule, three aliquots were removed from each of the three selected ampoules for each RM. Ampoules were opened, transferred into 1.5 mL amber autosampler vials (VWR, Radnor, PA), capped, and allowed to rest at room temperature for at least 24 hours. Then three, 200 µL aliquots, were taken from the 1.5 mL vial, with no shaking, and diluted based on concentrations observed in 3.1.4. The aliquots, 200 µL each, were removed in sequence with an effort to take the aliquots from the top, middle, and bottom of the 1.5 mL vial.

For RMs 8691 and 8692, a five-fold dilution was performed where 200 µL aliquots were added to 200 µL of each IS solution and an additional 400 µL methanol directly to an autosampler vial (ASV). For RMs 8690 and 8693, an initial 50-fold dilution was prepared using 200 µL RM in 9800 µL methanol (D1). Then, a 125-fold dilution was prepared by adding 400 µL of D1 into an additional 600 µL methanol (D2). Then, an 800 µL aliquot of D2 was transferred into an autosampler vial with 200 µL of each IS solution, yielding final dilution levels of approximately 175-fold (g/g; exact mass known).

3.2.3. Instrumental Method

The method was as described in Section 3.1.3.

3.2.4. Quantification

Linear calibration models for each of the 34 PFAS measurands listed in Table 20 were estimated using the five calibrant solutions described in Table 21. The intercepts of the models were not forced through zero. All calibration curves had Pearson correlation $R^2 \geq 0.99$. Table 28,

Table 29, Table 30, and Table 31 provide the measurement results for RMs 8690, 8691, 8692, and 8693. Table 32 lists the means, SDs, and CVs for all measurands.

Table 28. RM 8690: Within-Ampoule Homogeneity Study Results, µg/g.

PFAS	Ampoule 79			Ampoule 317			Ampoule 675		
	Top	Middle	Bottom	Top	Middle	Bottom	Top	Middle	Bottom
PFBA	0.0893	0.0919	0.1020	0.0851	0.0862	0.0874	0.1060	0.1090	0.1030
PFHxA	0.302	0.314	0.278	0.330	0.297	0.307	0.353	0.324	0.295
PFHpA	0.0515	0.0673	0.0603	0.0430	0.0421	0.0650	0.0323	0.0794	0.0624
PFOA	0.295	0.284	0.286	0.273	0.298	0.288	0.270	0.291	0.264
PFBSA	0.0304	0.0290	0.0294	0.0317	0.0309	0.0300	0.0306	0.0305	0.0315
PFHxSA	0.0459	0.0438	0.0468	0.0462	0.0459	0.0453	0.0460	0.0452	0.0462
PFPrS	0.314	0.294	0.318	0.304	0.301	0.308	0.333	0.304	0.298
PFBS	0.475	0.407	0.470	0.427	0.446	0.443	0.487	0.446	0.452
PFPeS	0.367	0.393	0.327	0.374	0.358	0.352	0.380	0.387	0.299
PFHxS	2.38	2.75	2.15	2.59	2.62	2.72	2.75	2.59	2.13
PFHpS	0.296	0.313	0.316	0.322	0.335	0.336	0.333	0.332	0.289
PFOA	18.3	17.3	19.5	18.8	19.0	20.4	20.2	19.3	16.9
6:2 FTS	0.201	0.176	0.194	0.192	0.168	0.162	0.181	0.185	0.231
N-AP-FHxSA	9.55	8.79	9.22	9.30	8.58	8.00	9.46	7.91	9.53
N-TAmP-FHxSA	0.357	0.306	0.311	0.317	0.291	0.279	0.331	0.256	0.354
6:2 FTAB	3.31	3.04	3.06	3.22	2.86	2.50	3.04	2.56	3.41

Table 29. RM 8691: Within-Ampoule Homogeneity Study Results, µg/g.

PFAS	Ampoule 195			Ampoule 766			Ampoule 1138		
	Top	Middle	Bottom	Top	Middle	Bottom	Top	Middle	Bottom
PFBA	0.00809	0.00726	0.00780	0.00704	0.00738	0.00731	0.00680	0.00719	0.00707
PFHxA	0.0181	0.0139	0.0143	0.0143	0.0152	0.0152	0.0140	0.0173	0.0162
PFOA	0.0262	0.0258	0.0256	0.0248	0.0256	0.0289	0.0241	0.0276	0.0256
PFBS	0.000854	0.000800	0.000797	0.000798	0.000795	0.000803	0.000797	0.000853	0.000788
PFHxSA	0.000824	0.000776	0.000774	0.000779	0.000773	0.000782	0.000773	0.000832	0.000764
PFPrS	0.00125	0.00119	0.00117	0.00123	0.00093	0.00115	0.00121	0.00100	0.00110
8:2 FTS	0.00333	0.00265	0.00349	0.00262	0.00428	0.00279	0.00214	0.00434	0.00332
6:2 FTS	0.0604	0.0525	0.0576	0.0560	0.0574	0.0557	0.0561	0.0546	0.0591
6:2 FTAB	0.00204	0.00152	0.00203	0.00187	0.00218	0.00195	0.00138	0.00163	0.00191

Table 30. RM 8692: Within-Ampoule Homogeneity Study Results, µg/g.

PFAS	Ampoule 354			Ampoule 715			Ampoule 1126		
	Top	Middle	Bottom	Top	Middle	Bottom	Top	Middle	Bottom
PFBA	0.0204	0.0207	0.0203	0.0207	0.0204	0.0206	0.0199	0.0210	0.0210
PFHxA	0.01010	0.01030	0.00929	0.00960	0.01040	0.01020	0.00919	0.00939	0.00880
PFBSA	0.000821	0.000826	0.000799	0.000798	0.000816	0.000832	0.000821	0.000808	0.000821
PFHxSA	0.000788	0.000792	0.000767	0.000761	0.000783	0.000801	0.000787	0.000775	0.000786
PFPrS	0.000977	0.000980	0.000940	0.000942	0.000983	0.000977	0.000964	0.000958	0.000979
6:2 FTS	0.0321	0.0360	0.0357	0.0362	0.0370	0.0366	0.0389	0.0375	0.0388
N-AP-FHxSA			0.00246	0.00111	0.00122		0.00297	0.00174	0.00138
6:2 FTAB	0.0183	0.0199	0.0198	0.0226	0.0212	0.0210	0.0205	0.0218	0.0195

Table 31. RM 8693: Within-Ampoule Homogeneity Study Results, $\mu\text{g/g}$.

PFAS	Ampoule 387			Ampoule 863			Ampoule 1183		
	Top	Middle	Bottom	Top	Middle	Bottom	Top	Middle	Bottom
PFBA	0.0466	0.0369	0.0456	0.0434	0.0400	0.0420	0.0442	0.0463	0.0462
PFHxA	0.169	0.158	0.184	0.177	0.189	0.177	0.155	0.176	0.184
PFBSA	0.0249	0.0249	0.0247	0.0249	0.0262	0.0247	0.0227	0.0246	0.0253
PFHxSA	0.0237	0.0239	0.0237	0.0239	0.0252	0.0238	0.0218	0.0236	0.0243
PFPrS	0.0291	0.0292	0.0290	0.0291	0.0307	0.0290	0.0266	0.0289	0.0297
6:2 FTS	14.2	14.2	13.9	14.1	13.9	16.9	14.2	13.7	15.2
6:2 FTAB	269	269	269	261	260	307	260	253	281

Table 32. Summary of Within-Ampoule Homogeneity Results for RMs 8690 to 8693, $\mu\text{g/g}$.

PFAS	RM 8690			RM 8691			RM 8692			RM 8693		
	Mean $\mu\text{g/g}$	SD $\mu\text{g/g}$	CV %									
PFBA	0.0955	0.0094	9.8	0.00733	0.00040	5.4	0.02056	0.00035	1.7	0.0435	0.0033	7.6
PFHxA	0.311	0.022	7.1	0.0154	0.0015	9.8	0.00970	0.00057	5.9	0.174	0.012	6.7
PFHpA	0.0559	0.0148	27									
PFOA	0.2832	0.0117	4.1	0.0260	0.0014	5.5						
PFBSA	0.03044	0.00089	2.9	0.000809	0.000025	3.1	0.000816	0.000012	1.4	0.02477	0.00092	3.7
PFHxSA	0.04570	0.00086	1.9	0.000786	0.000024	3.1	0.000782	0.000013	1.6	0.02377	0.00089	3.7
PFPrS	0.3082	0.0119	3.9	0.00114	0.00011	9.5	0.000967	0.000017	1.7	0.0290	0.0011	3.7
PFBS	0.450	0.025	5.5									
PFPeS	0.360	0.030	8.4									
PFHxS	2.520	0.244	9.7									
PFHpS	0.3191	0.0172	5.4									
PFOS	18.87	1.20	6.3									
6:2 FTS	0.188	0.020	10.9	0.0566	0.0024	4.2	0.0365	0.0020	5.5	14.47	1.00	6.9
8:2 FTS				0.00322	0.00075	23						
N-AP-FHxSA	8.93	0.64	7.2				0.00181	0.00075	41			
N-TAmP-FHxSA	0.311	0.033	10.7									
6:2 FTAB	3.00	0.31	10.5	0.00183	0.00027	15	0.0205	0.0013	6.3	270	16	5.9

The SDs are displayed as a function of their means in Fig. 10. While the relationship is again somewhat variable for concentrations of less than about $0.02 \mu\text{g/g}$, it is reasonably well modelled as a constant CV of 5.9 %. This result was similar to that for the between-ampoule results, which suggests that the within-ampoule sampling location is not a significant source of variability. Since only one result was obtained for each ampoule, this variability is a composite of method repeatability and material heterogeneity.

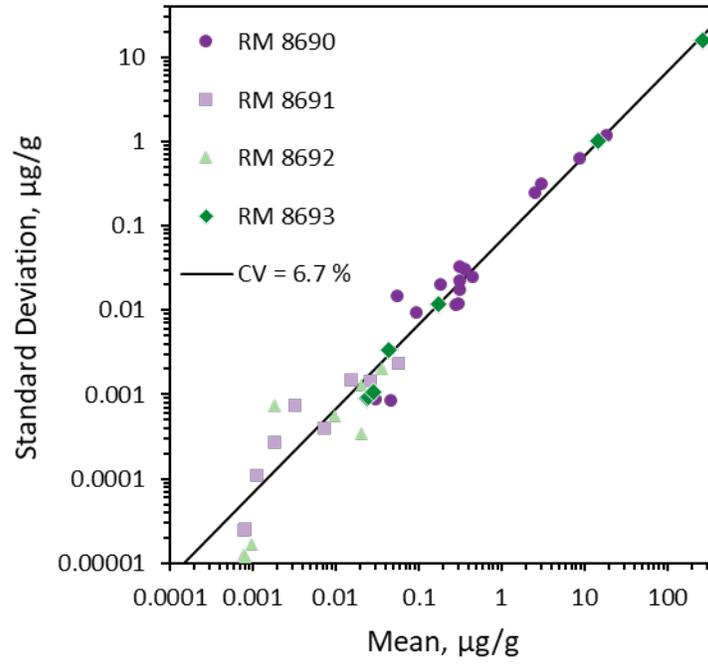


Fig. 10. Within-Ampoule Homogeneity Standard Deviation as a Function of Mean Value.

3.2.5. Within-Ampoule Homogeneity Assessment

The PFAS homogeneity for each RM was investigated through comparisons of the measured concentrations as functions of the sample ampoule filling order, shown in Fig. 11, Fig. 12, Fig. 13, and Fig. 14. As can be expected, the relative differences tend to be larger for lower concentration measurands. There are no trends that are consistent across sampling location.

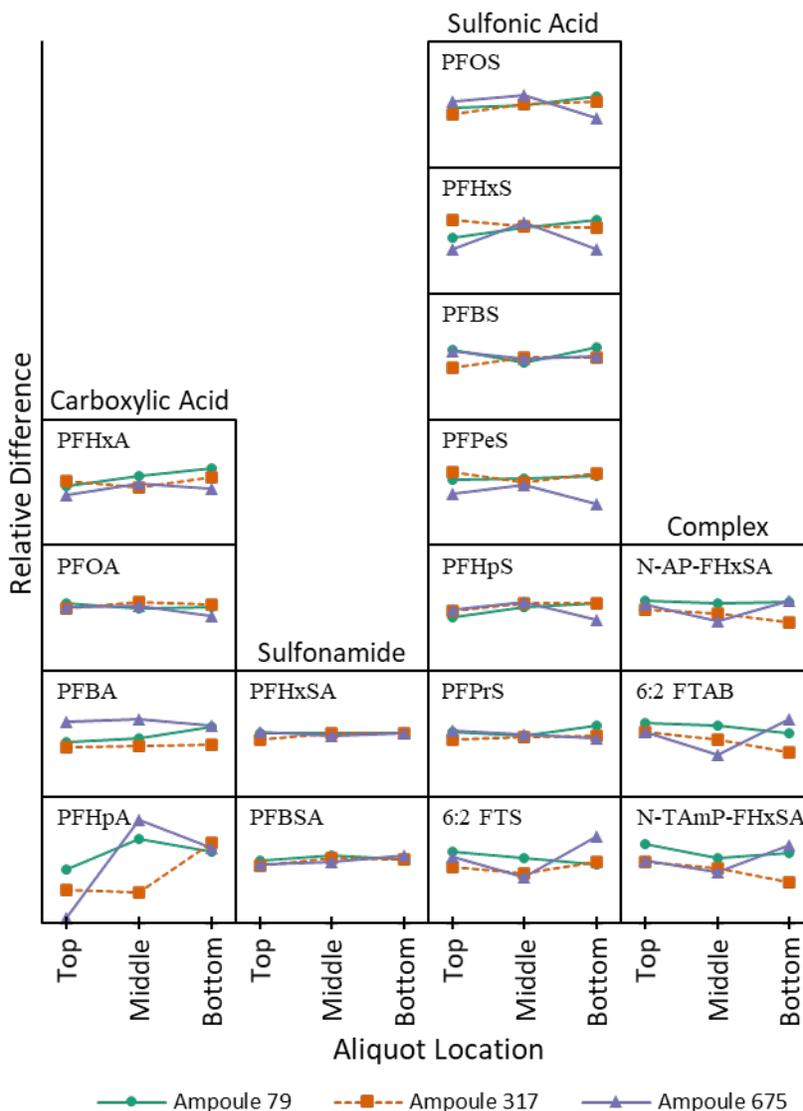


Fig. 11. RM 8690 Measurand Results as a Function of Within-Ampoule Aliquot Location.

Each rectangle provides the measurement results for three sampling locations (top, middle, and bottom) for three ampoules. The results are displayed as the base₁₀ logarithm of the ratio between the individual result and the mean of all results for the measurand, centered within the rectangle and scaled so that the most divergent of all results (N-AP-FHxSA in RM 8692) are just contained within the rectangle. The measurands in the first three columns share structure: carboxylic acid, sulfonamide, and sulfonic acid. The measurands in the fourth column are complex sulfonamides. Measurands within each column are sorted, bottom to top, by increasing concentration.

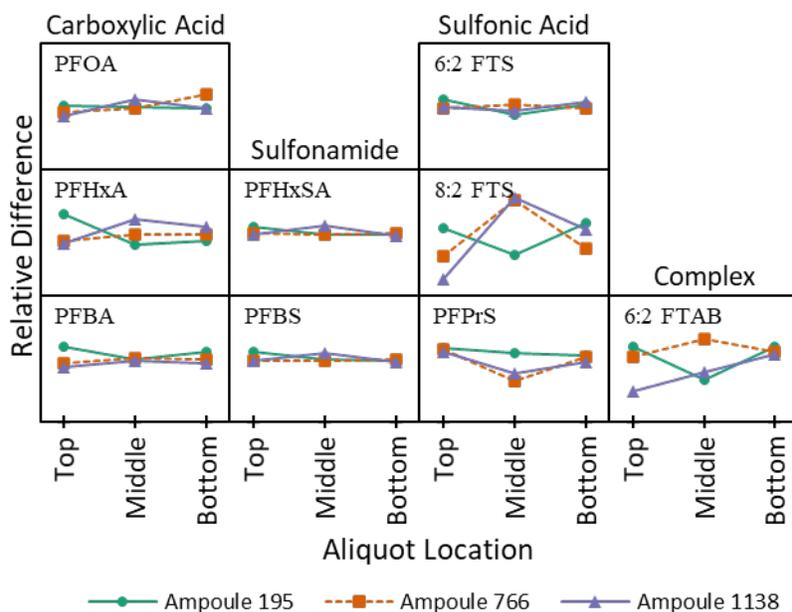


Fig. 12. RM 8691 Measurand Results as a Function of Within-Ampoule Aliquot Location.

Each rectangle provides the measurement results for three sampling locations (top, middle, and bottom) for three ampoules. The results are displayed as the base₁₀ logarithm of the ratio between the individual result and the mean of all results for the measurand, centered within the rectangle and scaled so that the most divergent of all results (N-AP-FHxSA in RM 8692) are just contained within the rectangle. The measurands in the first three columns share structure: carboxylic acid, sulfonamide, and sulfonic acid. Measurands within each column are sorted, bottom to top, by increasing concentration.

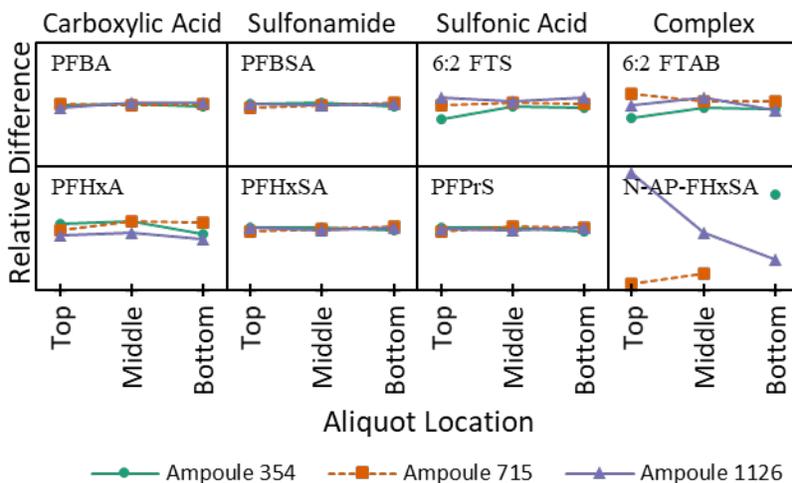


Fig. 13. RM 8692 Measurand Results as a Function of Within-Ampoule Aliquot Location.

Each rectangle provides the measurement results for three sampling locations (top, middle, and bottom) for three ampoules. The results are displayed as the base₁₀ logarithm of the ratio between the individual result and the mean of all results for the measurand, centered within the rectangle and scaled so that the most

divergent of all results (N-AP-FHxSA in this RM) are just contained within the rectangle. The measurands in the first three columns share structure: carboxylic acid, sulfonamide, and sulfonic acid. The measurands in the fourth column are complex sulfonamides. Measurands within each column are sorted, bottom to top, by increasing concentration.

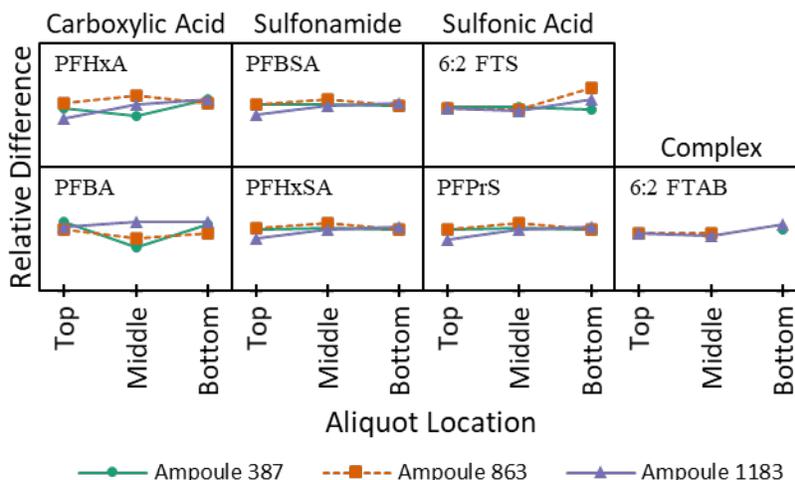


Fig. 14. RM 8693 Measurand Results as a Function of Within-Ampoule Aliquot Location.

Each rectangle provides the measurement results for three sampling locations (top, middle, and bottom) for three ampoules. The results are displayed as the base₁₀ logarithm of the ratio between the individual result and the mean of all results for the measurand, centered within the rectangle and scaled so that the most divergent of all results (N-AP-FHxSA in RM 8692) are just contained within the rectangle. The measurands in the first three columns share structure: carboxylic acid, sulfonamide, and sulfonic acid. Measurands within each column are sorted, bottom to top, by increasing concentration.

3.3. Confirmatory Method: High-Resolution Mass Spectrometry

An independent quantitative assessment of RM 8690, 8691, 8692, and 8693 was conducted using an LC-HRMS method distinct from that used for homogeneity assessments.

3.3.1. Materials

Table 33 lists the single ampoules of each of the four RMs that were used. All ampoules had been used in the homogeneity assessment studies.

Table 33. Ampoules Used in the LC-HRMS Confirmatory Study.

RM 8690	RM 8690	RM 8692	RM 8693
317	683	715	1183

Alfa Aesar LC-MS Grade methanol (Thermo Fisher Scientific, Waltham, MA) was used during sample preparation and analysis. The same two IS solutions described in Table 19 were used. Table 34 lists the composition of three working standards (WS) prepared from the PFAS calibrants listed in Table 15; it also identifies the IS pair(s) used for each PFAS measurand. For

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positive electrospray ionization compounds, four paired IS were used for semi-quantitative measurements.

All standards were stored at -20 °C until analysis.

Table 34. Calibration Solutions Used in the LC-HRMS Confirmatory Study.

Calibrant	Concentration, µg/g			Paired IS	ESI ^a
	WS1	WS1.5	WS2		
PFBA	0.115	0.0124	0.00113	¹³ C ₄ -PFBA	-
PFPeA	0.115	0.0124	0.00113	¹³ C ₅ -PFPeA	-
PFHxA	0.115	0.0124	0.00113	¹³ C ₅ -PFHxA	-
PFHpA	0.115	0.0124	0.00113	¹³ C ₄ -PFHpA	-
PFOA	0.115	0.0124	0.00113	¹³ C ₈ -PFOA	-
PFNA	0.115	0.0124	0.00113	¹³ C ₉ -PFNA	-
PFDA	0.115	0.0124	0.00113	¹³ C ₆ -PFDA	-
PFUnA	0.115	0.0124	0.00113	¹³ C ₇ -PFUnA	-
PFDoA	0.115	0.0124	0.00113	¹³ C ₂ -PFDoA	-
PFTriA	0.115	0.0124	0.00113	¹³ C ₂ -PFTriA	-
PFTA	0.115	0.0124	0.00113	¹³ C ₂ -PFTA	-
PFBSA	0.115	0.0124	0.00113	¹³ C ₄ -PFHpA	-
PFHxSA	0.115	0.0124	0.00113	¹³ C ₈ -PFOS	-
PFOSA	0.115	0.0124	0.00113	¹³ C ₈ -FOSA	-
HFPO-DA	0.115	0.0124	0.00113	¹³ C ₃ -HFPO-DA	-
N-MeFOSAA	0.117	0.0127	0.00120	<i>d</i> ₃ -MeFOSAA	-
N-EtFOSAA	0.117	0.0127	0.00120	<i>d</i> ₅ -EtFOSAA	-
PFPrS	0.083	0.0090	0.00081	¹³ C ₄ -PFBA	-
PFBS	0.103	0.0112	0.00102	¹³ C ₄ -PFBA	-
PFPeS	0.110	0.0119	0.00108	¹³ C ₃ -PFHxS	-
PFHxS	0.106	0.0115	0.00100	¹³ C ₃ -PFHxS	-
PFHpS	0.111	0.0120	0.00109	¹³ C ₈ -PFOS	-
PFOS	0.108	0.0117	0.00110	¹³ C ₈ -PFOS	-
PFNS	0.112	0.0122	0.00110	¹³ C ₈ -PFOS	-
PFDS	0.113	0.0122	0.00111	¹³ C ₈ -PFOS	-
4:2 FTS	0.109	0.0118	0.00107	¹³ C ₂ -4:2 FTS	-
6:2 FTS	0.111	0.0120	0.00109	¹³ C ₂ -6:2 FTS	-
8:2 FTS	0.112	0.0121	0.00110	¹³ C ₂ -8:2 FTS	-
ADONA	0.110	0.0119	0.00109	¹³ C ₈ -PFOA	-
9Cl-PF3ONS	0.109	0.0118	0.00107	¹³ C ₈ -PFOS	-
11Cl-PF3OU _n DS	0.110	0.0119	0.00108	¹³ C ₈ -PFOS	-
PF4OPeA	0.125	0.0136	0.00123	¹³ C ₅ -PFPeA	-
PF5OHxA	0.125	0.0136	0.00123	¹³ C ₅ -PFHxA	-
3,6-OPFH _p A	0.125	0.0136	0.00123	¹³ C ₅ -PFHxA	-
PFEESA	0.125	0.0136	0.00123	¹³ C ₅ -PFHxA	-
N-MeFBSA-M	0.108	0.0118	0.00107	<i>d</i> ₃ -MeFOSAA	-
N-MeFOSA-M	0.109	0.0118	0.00108	<i>d</i> ₃ -MeFOSAA	-
N-EtFOSA-M	0.107	0.0116	0.00106	<i>d</i> ₅ -EtFOSAA	-
N-AP-FHxSA	0.108	0.0117	0.00106	Prometon- <i>d</i> ₃ , Diphenyl- <i>d</i> ₁₀ -urea, Metolachlor- <i>d</i> ₆ , Dioctyl phthalate- <i>d</i> ₄	+
N-TAmP-FHxSA	0.104	0.0113	0.00102	Prometon- <i>d</i> ₃ , Diphenyl- <i>d</i> ₁₀ -urea, Metolachlor- <i>d</i> ₆ , Dioctyl phthalate- <i>d</i> ₄	+
6:2 FTAB	0.108	0.0117	0.00106	Prometon- <i>d</i> ₃ , Diphenyl- <i>d</i> ₁₀ -urea, Metolachlor- <i>d</i> ₆ , Dioctyl phthalate- <i>d</i> ₄	+

a ESI mode used to quantify the PFAS.

3.3.2. Sample Preparation

Based on preliminary analyses, each RM was diluted to five levels in methanol so that analytes of interest were within the range of the calibration curve. All samples were prepared gravimetrically. For each RM, a 50-fold dilution was prepared by diluting 200 µL RM in 9800 µL methanol (D1). Then, a 600-fold dilution (D2) and 1500-fold dilution (D3) were prepared using 200 µL of D1 in 2200 µL methanol and 200 µL of D1 in 5800 µL methanol, respectively. Then, a 3000-fold dilution was prepared using 400 µL of D2 in 1600 µL methanol. A 6000-fold dilution was made by adding 200 µL of D2 dilution in 1800 µL methanol. A 15000-fold dilution was prepared using 200 µL of D2 dilution in 4800 µL methanol.

For each of the 600-fold (D2), 1500-fold (D3), 3000-fold, 6000-fold, and 15000-fold dilutions, a 600 μL aliquot was transferred with 200 μL each of the IS solutions to an autosampler vial. Final dilutions levels (v/v) of 1000-fold, 2500-fold, 5000-fold, 10000-fold, and 25000-fold were achieved. A single sample was prepared for each RM at each dilution level.

3.3.3. Instrumental Method

The method was as described in Section 2.3.3.

A methanol blank and IS control were each re-injected in full-scan (MS1) mode every 8-12 samples. All ESI- analyses were performed first, followed by all ESI+ analyses. For each RM, triplicate injections of each dilution were performed sequentially in MS1 mode, with a single injection of the 1:1000 and 1:2500 dilution levels for MS/MS data acquisition after the respective MS1 analyses. The calibration curve (single injection per calibration level, MS1 mode) was analyzed at the beginning of the analytical batch and re-analyzed between analysis of each RM, yielding five total calibration curve analyses per ESI detection polarity. For all targeted PFAS analytes, < 0.1% carry-over was observed in methanol blanks following the highest calibration level or the least dilute sample of any RM based on comparison of analyte peak areas.

3.3.4. Quantification

Peak area data (from MS1 full scan data) were extracted in XCalibur Quan (version 4.2.47) using a 5-ppm mass error setting for $[\text{M}+\text{H}]^+$ or $[\text{M}-\text{H}]^-$ adducts in ESI+ and ESI-, respectively. Levels of 37 PFAS in the candidate RMs were calculated using the linear equation of the calibration curve, not forcing the intercept through zero. The PFAS calibration curves had good correlation coefficient (R^2) values: ≥ 0.997 for PFTriA, PFTA, N-MeFBSA, and N-MeFOSA, ≥ 0.999 for all others. Reporting limits (RLs) were calculated as the lowest detectable calibration point divided by the mass of RM in the sample: nanogram measurand per gram of RM.

Compounds were quantified using a relative response ratio to the internal standard that matched the compound based on chemical structure and/or retention time. For PFAS analyzed in ESI+, no PFAS internal standards were available; thus, concentrations were quantified with each of the four available non-PFAS ESI+ internal standards and averaged. Final concentrations are summed totals of linear and (if present) branched isomers.

None of the targeted PFAS were observed above the RL for RM 8691, The quantitative results for RMs 8690, 8692, and 8693 are presented in Table 35, Table 36, and Table 37.

The SDs for the PFAS detected in multiple dilutions and/or with multiple IS pairs are displayed as a function of their means in Fig. 15. Since only the PFAS present in concentration above about 0.1 $\mu\text{g}/\text{g}$ were detected in multiple levels, there are too few (Mean, SD) pairs for reliable analysis. However, the measurements for PFAS quantified using ESI- mode can be characterized as having a CV of about 4 %. The measurements made using ESI+ mode can be characterized as having a CV of about 9 %.

Table 35. RM 8690: HRMS Confirmatory Study Results, µg/g.

PFAS	Paired IS	ESI	Sample Dilutions					Mean	SD
			1:1000	1:2500	1:5000	1:10000	1:25000		
PFHxA	¹³ C ₅ -PFHxA	-	0.199					0.199	
PFOA	¹³ C ₈ -PFOA	-	0.267					0.267	
PFPrS	¹³ C ₃ -PFBS	-	0.288					0.288	
PFBS	¹³ C ₃ -PFBS	-	0.446					0.446	
PFPeS	¹³ C ₃ -PFHxS	-	0.411					0.411	
PFHxS	¹³ C ₃ -PFHxS	-	2.823	2.684	2.638	2.476		2.655	0.143
PFHpS	¹³ C ₈ -PFOS	-	0.308					0.308	
PFOS	¹³ C ₈ -PFOS	-	19.78	19.05	19.66	19.30	19.37	19.43	0.29
N-AP-FHxSA	prometon- <i>d</i> ₃	+	10.38	10.32	10.86			10.67	0.85
	diphenyl- <i>d</i> ₁₀ -urea	+	8.21	9.99	10.73	10.87	11.62		
	metolachlor- <i>d</i> ₆	+	10.21	10.20	10.75	10.73	11.06		
	dioctyl phthalate- <i>d</i> ₄	+	10.75	10.73	11.27	11.15	12.51		
N-TAmP-FHxSA	prometon- <i>d</i> ₃	+	0.246					0.260	0.038
	diphenyl- <i>d</i> ₁₀ -urea	+	0.226						
	metolachlor- <i>d</i> ₆	+	0.253						
	dioctyl phthalate- <i>d</i> ₄	+	0.315						
6:2 FTAB	prometon- <i>d</i> ₃	+	2.101	2.153				2.13	0.22
	diphenyl- <i>d</i> ₁₀ -urea	+	1.669	2.162					
	metolachlor- <i>d</i> ₆	+	2.077	2.157					
	dioctyl phthalate- <i>d</i> ₄	+	2.263	2.451					

Table 36. RM 8692: HRMS Confirmatory Study Results, µg/g.

PFAS	Paired IS	ESI	Sample Dilutions					Mean	SD
			1:1000	1:2500	1:5000	1:10000	1:25000		
PFOS	¹³ C ₈ -PFOS	-	0.578					0.578	

Table 37. RM 8693: HRMS Confirmatory Study Results, µg/g.

PFAS	Paired IS	ESI	Sample Dilutions					Mean	SD
			1:1000	1:2500	1:5000	1:10000	1:25000		
6:2 FTS	¹³ C ₈ -PFOS	-	15.06	14.86	14.95	15.17	15.12	15.03	0.12
PFOS	¹³ C ₈ -PFOS	-	0.982					0.982	
N-TAmP-FHxSA	prometon- <i>d</i> ₃	+	1.129	1.178	1.187			1.224	0.091
	diphenyl- <i>d</i> ₁₀ -urea	+	1.153	1.232	1.306				
	metolachlor- <i>d</i> ₆	+	1.141	1.193	1.231				
	dioctyl phthalate- <i>d</i> ₄	+	1.194	1.278	1.460				
6:2 FTAB	prometon- <i>d</i> ₃	+	248.9	272.3	282.4	300.7	293.6	275.7	17.1
	diphenyl- <i>d</i> ₁₀ -urea	+	248.1	268.9	277.7	297.3	290.0		
	metolachlor- <i>d</i> ₆	+	249.3	269.7	280.0	294.5	289.1		
	dioctyl phthalate- <i>d</i> ₄	+	249.6	260.1	274.0	287.6	281.2		

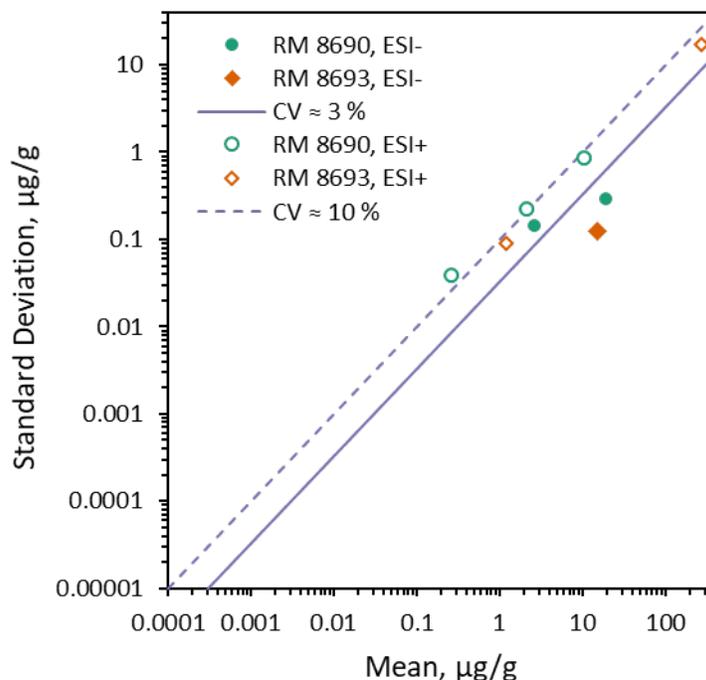


Fig. 15. LC-HRMS Confirmatory Result Standard Deviations as a Function of Mean Value.

3.4. Confirmatory Method: Triple-Quadrupole Mass Spectrometry

An independent assessment of RM 8690, 8691, 8692, and 8693 was conducted using an LC-MS/MS method.

3.4.1. Materials

The materials used in this study were the same as described in Section 3.3.1.

3.4.2. Sample Preparation

The samples evaluated in this study were those described in Section 3.3.2.

3.4.3. Instrumental Method

The method is as described in Section 3.1.3.

A methanol blank was injected between RM dilutions and observed to be less than the reporting limit for all measurands of interest. For each RM, four injections of each 1:2500 dilution were analyzed. The calibration curve was analyzed at the beginning of the analytical batch. Calibration solutions D and E (see Table 21) were injected every 10 samples as quality controls; the calculated concentrations of the calibrants in these solutions were between 70-130% of the mean value.

3.4.4. Quantification

Mass fractions of 34 PFAS in the candidate RMs were calculated using the linear equation of the calibration curve, not forcing the intercept through zero. Good R^2 values (≥ 0.99) were observed for all PFAS calibration curves. Compounds were quantified using a relative response ratio to the internal standard compound that most closely matched the compound. Where matched internal standards were not available, an IS in the same homologous series was used; e.g., $^{13}\text{C}_8$ -FOSA was used for FHxSA. When no homologue match existed, the IS with the closest matching retention time was used; e.g., $^{13}\text{C}_2$ -6:2 FTS was used for 6:2 FTAB. Final concentrations are totals including branched and linear isomers.

No carry-over was observed following the highest calibration level or the least dilute sample of any RM.

RLs were calculated as the lowest detectable calibration point divided by the mass of RM in the sample. None of the target PFAS were detected in RM 8691 or 8692. The quantitative results for RMs 8690 and 8693 are presented in Table 38 and Table 39.

The SDs for the PFAS detected in multiple dilutions are displayed as a function of their means in Fig. 16. Since only the PFAS present in concentrations above about 0.1 $\mu\text{g/g}$ were detected in multiple levels, there are too few (Mean, SD) pairs for reliable analysis. However, the measurements for PFAS quantified using either the ESI- or ESI+ modes can be characterized as having a CV of about 15 %.

Table 38. RM 8690: MS/MS Confirmatory Study Results, $\mu\text{g/g}$.

PFAS	Paired IS	ESI ^a	1:1000	1:2500 ^b	1:5000	1:10000	1:25000	Mean	SD
PFHxA	$^{13}\text{C}_5$ -PFHxA	-	0.434	0.578				0.506	0.102
PFPoS	$^{13}\text{C}_3$ -PFBS	-	0.454	0.649				0.552	0.138
PFBS	$^{13}\text{C}_3$ -PFBS	-	0.443	0.402				0.423	0.029
PFPoS	$^{13}\text{C}_3$ -PFHxS	-	0.427	0.433	0.534			0.465	0.060
PFHxS	$^{13}\text{C}_3$ -PFHxS	-	2.478	2.838	3.302	3.554		3.04	0.48
PFPoS	$^{13}\text{C}_8$ -PFOS	-	0.342	0.316				0.329	0.018
PFOS	$^{13}\text{C}_8$ -PFOS	-	19.954	19.966	19.547	19.059	21.314	19.97	0.84
N-AP-FHxSA	$^{13}\text{C}_2$ -6:2 FTS	+	11.737	11.197				11.47	0.38
N-TAmP-FHxSA	$^{13}\text{C}_2$ -6:2 FTS	+	0.500	0.549				0.525	0.035
6:2 FTAB	$^{13}\text{C}_2$ -6:2 FTS	+	3.88	2.959	3.033	1.934		2.95	0.80

a ESI mode used to quantify the PFAS.

b Average of four injections. All other results based upon single injections.

Table 39. RM 8693: MS/MS Confirmatory Study Results, $\mu\text{g/g}$.

PFAS	Paired IS	ESI ^a	1:1000	1:2500 ^b	1:5000	1:10000	1:25000	Mean	SD
6:2 FTS	$^{13}\text{C}_2$ -6:2 FTS	-	15.204	14.739	17.265	19.895	18.894	17.2	2.2
PFOS	$^{13}\text{C}_8$ -PFOS	-	0.96					0.960	
6:2 FTAB	$^{13}\text{C}_2$ -6:2 FTS	+	314.75	330.4	296.28	325.75	289.52	311.3	17.9

a ESI mode used to quantify the PFAS.

b Average of four replicate injections. All other results based upon single injections.

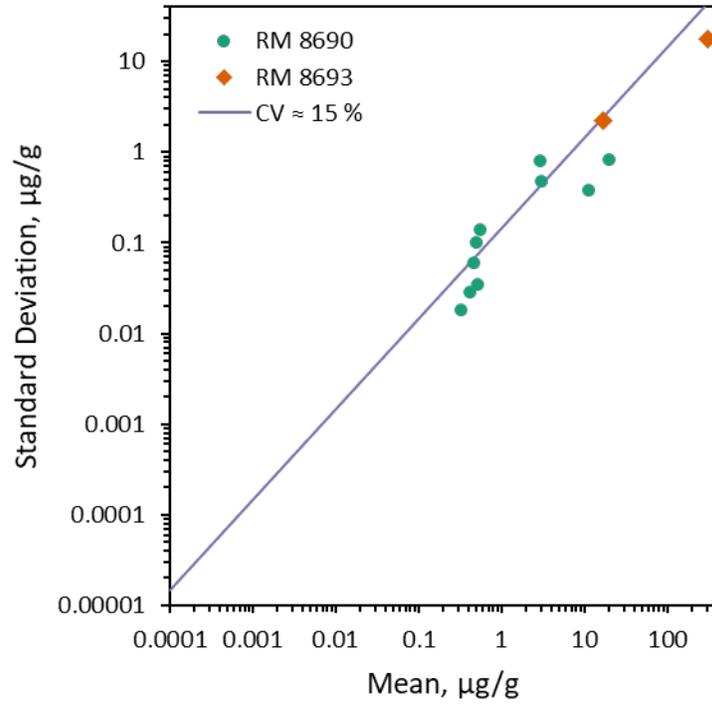


Fig. 16. LC-MS/MS Confirmatory Result Standard Deviations as a Function of Mean Value.

3.5. DoD AFFF01 Method/EPA Draft Method 1633

The U.S. Department of Defense AFFF01 analytical method [31] and U.S. Environmental Protection Agency draft method 1633 [32] for PFAS became available in 2021. These methods were used to provide an independent assessment of the PFAS in RMs 8690, 8691, 8692, and 8693. In addition, the measured values of PFAS were used to evaluate stability of the RMs.

3.5.1. Materials

One ampoule of each RM was obtained from ORM; the production sequence of these ampoules is not known. All solvents were Fisher Optima LCMS grade (Waltham, MA). Table 40 lists the target PFAS, the concentration of the analytical standards in three WSs, and the paired IS used in quantitation.

Table 40. Calibration Working Solutions Used with Method AFFF01.

Calibrant	Concentration, µg/g			Paired IS
	WS1	WS1.5	WS2	
PFBA	0.111	0.011	0.0017	¹³ C ₄ -PFBA
PFPeA	0.111	0.011	0.0017	¹³ C ₅ -PFPeA
PFHxA	0.111	0.011	0.0017	¹³ C ₅ -PFHxA
PFHpA	0.111	0.011	0.0017	¹³ C ₄ -PFHpA
PFOA	0.111	0.011	0.0017	¹³ C ₈ -PFOA
PFNA	0.111	0.011	0.0017	¹³ C ₉ -PFNA
PFDA	0.111	0.011	0.0017	¹³ C ₆ -PFDA
PFUdA	0.111	0.011	0.0017	¹³ C ₇ -PFUdA
PFDoA	0.111	0.011	0.0017	¹³ C ₂ -PFDoA
PFTriA	0.111	0.011	0.0017	¹³ C ₂ -PFTeDA
PFTA	0.111	0.011	0.0017	¹³ C ₂ -PFTeDA
PFBSA	0.111	0.011	0.0017	¹³ C ₄ -PFHpA
PFHxSA	0.111	0.011	0.0017	¹³ C ₈ -PFOS
FOSA	0.111	0.011	0.0017	¹³ C ₈ -FOSA
HFPO-DA	0.111	0.011	0.0017	¹³ C ₃ -HFPO-DA
N-MeFOSAA	0.111	0.011	0.0017	<i>d</i> ₃ -MeFOSAA
N-EtFOSAA	0.111	0.011	0.0017	<i>d</i> ₅ -EtFOSAA
PFPrS	0.098	0.010	0.0015	¹³ C ₄ -PFBA
PFBS	0.100	0.010	0.0016	¹³ C ₄ -PFBS
PFPeS	0.107	0.011	0.0017	¹³ C ₃ -PFHxS
PFHxS	0.103	0.011	0.0016	¹³ C ₃ -PFHxS
PFHpS	0.108	0.011	0.0017	¹³ C ₈ -PFOS
PFOS	0.106	0.011	0.0017	¹³ C ₈ -PFOS
PFNS	0.109	0.011	0.0017	¹³ C ₈ -PFOS
PFDS	0.109	0.011	0.0017	¹³ C ₈ -PFOS
4:2 FTS	0.106	0.011	0.0017	¹³ C ₂ -4:2 FTS
6:2 FTS	0.108	0.011	0.0017	¹³ C ₂ -6:2 FTS
8:2 FTS	0.109	0.011	0.0017	¹³ C ₂ -8:2 FTS
ADONA	0.107	0.011	0.0017	¹³ C ₈ -PFOA
9Cl-PF3ONS	0.106	0.011	0.0017	¹³ C ₈ -PFOS
11Cl-PF3OUdS	0.107	0.011	0.0017	¹³ C ₈ -PFOS
PF4OPeA	0.122	0.012	0.0019	¹³ C ₄ -PFBA
PF5OHxA	0.122	0.012	0.0019	¹³ C ₅ -PFPeA
3,6-OPFHpA	0.122	0.012	0.0019	¹³ C ₄ -PFHpA
PFEEESA	0.111	0.011	0.0017	¹³ C ₄ -PFHpA
N-MeFBSA	0.126	0.013	0.0020	<i>d</i> ₃ -N-MeFOSA
N-MeFOSA	0.102	0.010	0.0016	<i>d</i> ₃ -N-MeFOSA
N-EtFOSA	0.103	0.011	0.0016	<i>d</i> ₃ -N-EtFOSA

An eight-point calibration curve was prepared gravimetrically using the analytical standards and the internal standards. Table 41 lists the composition of the eight calibration solutions. These calibrants were not taken through extraction as per method specifications.

Table 41. Calibration Standards Used with Method AFFF01.

Calibrant	WS1 g	WS1.5 g	WS2 g	IS g	Total g	PFBA ^a µg/g
Cal A	0.49056			0.15846	0.64902	0.08390
Cal B	0.48969			0.15878	0.64847	0.08382
Cal C	0.09661			0.15628	0.25289	0.04240
Cal D		0.19809		0.15671	0.35480	0.00614
Cal E			0.39558	0.15624	0.55182	0.00122
Cal F			0.15866	0.15562	0.31428	0.00086
Cal G			0.07701	0.1586	0.23561	0.00056
Cal H			0.03857	0.15635	0.19492	0.00034

a PFBA is a representative measurand.

3.5.2. Sample Preparation

3.5.2.1. Quality Assurance Samples

The AFFF01 method requires evaluation of several quality assurance (QA) materials: a Method Blank (MB), two Laboratory Control Samples (LCS), and a Low-Level Laboratory Control Sample (LLCS). The MB was 0.02 g water. The LCSs contained approximately 2 ng/mL of each of the analytical standards and 0.02 g water. The LLCS contained approximately 0.2 ng/mL of each of the analytical standards and 0.02 g water. These QA materials were added to 60 mL HDPE bottles and processed through the same extraction method as samples.

3.5.2.2. Extractions

Extractions were completed as specified in AFFF01.

For each sample and QA material the mass of a 60 mL HDPE bottle (VWR, Radnor, PA) was determined to four decimal places, approximately 0.02 g of sample added, weighed, and 60 mL of water added and swirled to mix. All samples were left to sit overnight (at least 12 h). Then 200 µL of IS solution was added to each container, weighed, and swirled to mix. The sample pH was verified to be in the range of (6.5 ± 0.5) pH using pH paper; no adjustments were deemed necessary.

SPE WAX cartridges (Oasis WAX 6cc Cartridge 150 mg 30 µm, PN:186002493) were packed with methanol-rinsed silanized glass wool (Supelco, PN:20411) to at least half the height of each cartridge barrel. The cartridges and silanized wool were added to a vacuum manifold and washed with 15 mL of 1% methanolic ammonium hydroxide and 5 mL of 0.3 mol/L formic acid. The cartridges were not allowed to go dry. All rinses were discarded.

Sample was added at no more than 5 mL/min. The cartridge and loaded sample were rinsed with 5 mL reagent water twice and 5 mL of 1:1 methanol:0.1 mol/L formic acid at 5 mL/min. The cartridges were allowed to go dry for at least 15 sec. All rinses were discarded.

15 mL polypropylene vials were attached to the SPE manifold for collection. A 5 mL aliquot of 1% methanolic ammonium hydroxide elution solvent was added to the inside of the corresponding 60 mL sample bottles. A glass pipette was used to transfer the bottle rinse to its corresponding SPE cartridge. Elution solvent was pulled through the cartridges into the collection tubes. Sample bottles were discarded.

The extracts were removed from the SPE manifold and 50 μ L of 50 % acetic acid (in water) added to the extract and vortexed. 10 mg of black carbon was added to each sample. The samples were placed on a wrist action shaker for 5 min then centrifuged at 294 rad/s (2800 rpm) for 10 min.

A 5 mL polypropylene syringe equipped with a 25 mm, 0.2 μ m syringe filter (Pall Corporation, PN:4436T) was used to filter the entire extract into each collection tube. 1 mL of each extract was added to an autosampler vial for analysis.

3.5.3. Instrumental Method

Chromatographic separation was performed on an Agilent 1260 Infinity liquid chromatograph (Agilent, Santa Clara, CA) attached to a SCIEX API 5000+ LC-MS/MS with QJet Ion Guide mass spectrometer (AB SCIEX, Framingham, MA). An analytical hold-up column was put in place directly after the purge valve (Eclipse Plus C18, 4.6mm ID, 50 mm, 5 μ M particle size) to separate instrumental contamination. Chromatographic separation was achieved with an analytical guard cartridge, Phenomenex Kinetex Evo C18 (2.1 mm ID, sub-2 μ M particle size, PN:AJ0-9298; Torrance, CA) attached to a Waters Acquity UPLC BEH C18 column (2.1 mm ID, 50 mm, 1.7 μ m particle size, PN:186002350; Milford, MA). Injections were 10 μ L and the autosampler compartment was set to 10 $^{\circ}$ C. Mobile phase A was 2 mmol/L ammonium acetate in 95:5 water/acetonitrile; phase B was acetonitrile. Table 42 provides the gradient and flow rate profile.

Table 42. Chromatographic Gradient and Flow Rate Profile Used with Method AFFF01.

Time, min	Phase A, %	Phase B, %	Flow, μ L/min
0	98	2	350
0.2	98	2	350
4	70	30	350
7	45	55	350
9	25	75	200
18	5	95	200
18.4	98	2	350
19.8	98	2	350
20	98	2	350

Table 43 provides the SCIEX 5000+ parameter settings that were used for electrospray ionization in both ESI+ and ESI- polarities.

Table 43. Electrospray Ionization Detection Parameters Used with with Method AFFF01 for both polarities.

Source Parameter	ESI+	ESI-
Curtain Gas	20	20
Collision Gas	9	9
IonSpray Voltage	4500	-4500
Temperature	350	350
Ion Source Gas 1	15	15
Ion Source Gas 2	0	0

3.5.4. Quantification and Stability Assessment

Concentrations of PFAS in the samples were calculated using the linear calibration curve, not forcing the intercept through zero. Good R^2 values (≥ 0.99) were observed for the calibration curves for PFAS. Compounds were quantified using a relative response ratio to the paired IS. Final concentrations are summed totals of branched and linear isomers (when present).

RLs were determined as the value of the lowest calibrant detectable. PFBA values are not reported because of known background contamination; no other PFAS was detected in the MB at a concentration at or above the RL.

Table 44 lists the results for the LCS and LLLCS samples. Most (28 of 35) of the CVs for the two LCS samples are less than 10 % but CVs for both low concentration (PFPrS) and high concentration (PFDoA) measurands exceed 50 %. Measurand concentrations in the LLLCS were below the RL for about half of the targets. For the PFAS with quantitative LLLCS results, the ratios between the LCS mean and the LLLCS result range from 2 to 52; however, the median ratio of 11 is very close to the nominal 10-fold differences between the unextracted samples. Since this is a draft method, and soliciting feedback at this time, this information will be shared with the authors of the draft method.

Table 44. Results for the Quality Assurance Samples with with Method AFFF01.

PFAS	LCS		LLLCS		PFAS	LCS		LLLCS
	Mean, $\mu\text{g/g}$	CV, %	$\mu\text{g/g}$	Ratio ^a		Mean, $\mu\text{g/g}$	CV, %	
PFPeA	0.003246	3.2	0.001132	3	PFHxA	0.003238	27.9	<RL
PFHpA	0.003314	4.9	0.000308	11	PFOA	0.003166	1.2	<RL
PFTA	0.004013	6.3	0.000103	39	PFNA	0.003194	12.1	<RL
PFBSA	0.002882	5.8	0.000441	7	PFDA	0.003496	1.1	<RL
N-EtFOSAA	0.003672	10.5	0.000320	11	PFUnA	0.003704	4.5	<RL
PFBS	0.003336	5.4	0.001433	2	PFDoA	0.006037	63.9	<RL
PFPeS	0.003738	3.1	0.000118	32	PFTriA	0.004999	7.5	<RL
PFHxS	0.003329	7.6	0.000225	15	PFHxSA	0.003614	1.9	<RL
4:2 FTS	0.003824	22.9	0.000181	21	FOSA	0.003905	2.3	<RL
6:2 FTS	0.004976	50.7	0.000193	26	N-MeFOSAA	0.003278	10.9	<RL
8:2 FTS	0.003696	0.2	0.000074	50	PFPrS	0.001171	66.2	<RL
PF4OPeA	0.004286	6.0	0.000259	17	PFHpS	0.003280	8.0	<RL
PF5OHxA	0.004213	2.0	0.000605	7	PFOS	0.003215	5.9	<RL
3,6-OPFHpA	0.003882	2.6	0.001402	3	PFNS	0.003403	3.4	<RL
PFEESA	0.003284	3.4	0.000434	8	PFDS	0.003221	5.0	<RL
N-MeFOSA	0.003708	5.6	0.000354	10	9Cl-PF3ONS	0.003585	2.8	<RL
N-EtFOSA	0.006732	6.7	0.000130	52	11Cl-PF3OUdS	0.004490	6.9	<RL
					N-MeFBSA	0.003487	9.4	<RL

a Ratio of result for the LCS sample divided by the result for the LLLCS sample. The expected ratio is 10.

No PFAS result above the RL was observed for RM 8692. Table 45, Table 46, and Table 47 list the results for RMs 8690, 8691, and 8693.

Table 45. RM 8690: Method AFFF01 Results, µg/g.

PFAS	Rep ₁	Rep ₂	Rep ₃	Rep ₄	Mean	SD
PFPeA	0.221	0.205	0.279	0.221	0.232	0.033
PFHxA	0.257	0.278	0.36	0.266	0.290	0.047
PFHpA	0.118	0.103	0.144	0.101	0.117	0.020
PFOA	0.414	0.450	0.521	0.380	0.441	0.060
PFPrS	0.136	0.213	0.28	0.203	0.208	0.0589
PFBS	0.560	0.512	0.708	0.505	0.571	0.094
PFPeS	0.532	0.469	0.637	0.520	0.540	0.071
PFHxS	3.20	2.62	3.71	2.99	3.13	0.46
PFHpS	0.380	0.371	0.475	0.353	0.395	0.055
PFOS	21.9	20.7	28.0	20.9	22.9	3.5
6:2 FTS	0.510	0.303	0.385	0.390	0.397	0.0852
8:2 FTS	0.039	0.031	0.049	0.049	0.0420	0.0087
PFBSA	0.039	0.034	0.048	0.036	0.0393	0.0062
PFHxSA	0.029	0.026	0.033	0.028	0.0290	0.0029

Table 46. RM 8691: Method AFFF01 Results, µg/g.

PFAS	Rep ₁	Rep ₂	Rep ₃	Rep ₄	Mean	SD
6:2 FTS	0.091	0.089	0.071	0.043	0.0735	0.0222

Table 47. RM 8693: Method AFFF01 Results, µg/g.

PFAS	Rep ₁	Rep ₂	Rep ₃	Rep ₄	Mean	SD
PFPeA	0.169	0.162	0.166	0.152	0.1623	0.0074
PFHxA	0.149	0.107	0.140	0.123	0.1298	0.0186
PFHpA	0.010	0.012	0.012	0.009	0.0108	0.0014
4:2 FTS	0.040	0.048	0.049	0.033	0.0425	0.0075
6:2 FTS	19.0	17.7	19.6	18.7	18.7	0.8

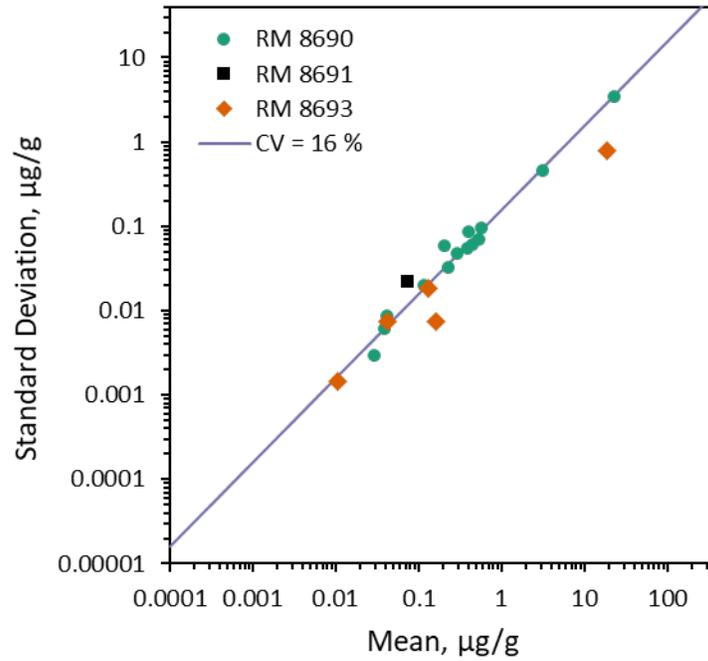


Fig. 17. Standard Deviation of Method AFFF01 Results as a Function of Mean Value.

The SD values reported using AFFF01 indicates higher variability, likely as a result of the increased sample preparation steps specified by the method as compared to the methanolic dilution used in Sections 3.1 and 3.2. However, similar concentrations were observed overall indicating that concentrations are not changing over time and RMs 8690, 8691, 8692, and 8693 are stable.

4. Consensus Analysis

The values and uncertainties of the PFAS quantified in two or more independent methods can be estimated by combining the results from all the measurement methods using the model:

$$y_{ij} = \mu + m_i + \varepsilon_{ij}; i = 1, 2, \dots, n_{mm}; j = 1, 2, \dots, n_i$$

where i indexes measurement methods, j indexes replication within a measurement method, n_{mm} represents the number of measurement methods, n_i represents the number of replications within measurement method, $m_i \stackrel{iid}{\sim} N(0, \sigma^2)$ (i.e., m_i is an independent and identically distributed Gaussian random variable centered on zero with a characteristic variance), and $\varepsilon_{ij} \stackrel{iid}{\sim} N(0, \sigma^2)$ independent of m_i .

Table 48, Table 49, Table 50, and Table 51 list consensus values, $\hat{\mu}$, and 95 % level of confidence expanded uncertainties, $U_{95}(\hat{\mu})$ for RMs 8690, 8691, 8692, and 8693. The $\hat{\mu}$ are estimates of μ calculated using the DerSimonian-Laird estimator [33]. The $U_{95}(\hat{\mu})$ are calculated using the Horn-Horn-Duncan (HHD) method for variances [34] and the bootstrap method (BS) [35]. While the HHD and BS $U_{95}(\hat{\mu})$ agree reasonably well, using the larger of the two provides a more conservative estimate.

Graphical representation of the results and consensus values for PFAS quantified in at least two studies are provided in Fig. 18.

Table 48. RM 8690: Summary Data, Consensus Means, and 95 % Expanded Uncertainties, µg/g.

PFAS	Section 3.1			Section 3.2			Section 3.3			Section 3.4			Section 3.5			$U_{95}(\hat{\mu})$		
	<i>n</i>	\bar{x}	<i>s</i>	$\hat{\mu}$	HHD	BS												
PFBA	10	0.111	0.0029	9	0.0955	0.0094							4	0.290	0.047	0.104	0.016	0.016
PFHxA	10	0.280	0.027	9	0.311	0.022	1	0.199		2	0.51	0.10	4	0.117	0.020	0.295	0.024	0.026
PFHpA	10	0.105	0.008	9	0.056	0.015							4	0.441	0.060	0.0918	0.0370	0.0362
PFOA	10	0.282	0.027	9	0.283	0.012	1	0.267					4	0.208	0.059	0.317	0.078	0.041
PFPrS	10	0.306	0.008	9	0.308	0.012	1	0.288		2	0.55	0.14	4	0.571	0.094	0.302	0.015	0.016
PFBS	10	0.477	0.010	9	0.450	0.025	1	0.446		2	0.423	0.029	4	0.540	0.071	0.473	0.032	0.031
PFPeS	10	0.384	0.019	9	0.360	0.030	1	0.411		3	0.465	0.060	4	0.395	0.055	0.409	0.085	0.049
PFHxS	10	2.63	0.11	9	2.52	0.24	4	2.66	0.14	4	3.04	0.48	4	3.13	0.46	2.64	0.089	0.124
PFHpS	10	0.450	0.019	9	0.319	0.017	1	0.308		2	0.329	0.018	4	0.395	0.055	0.315	0.225	0.156
PFOS	10	19.4	0.5	9	18.9	1.2	5	19.4	0.3	5	12.0	0.8	4	22.9	3.5	19.4	0.19	0.41
PFBSA	10	0.0380	0.0009	9	0.0304	0.0009							4	0.0393	0.0062	0.0356	0.0057	0.0065
PFHxSA	10	0.0295	0.0012	9	0.0457	0.0009							4	0.0290	0.0029	0.035	0.011	0.013
N-AP-FHxSA	10	7.77	0.55	9	8.93	0.64	18	10.7	0.9	2	11.5	0.4				9.12	1.7	1.7
N-TAmP-FHxSA	10	0.294	0.025	9	0.311	0.033	4	0.260	0.038	2	0.525	0.035				0.293	0.023	0.023
6:2 FTS	10	0.204	0.013	9	0.188	0.020							4	0.397	0.085	0.222	0.066	0.037
6:2 FTAB	10	2.40	0.17	9	3.00	0.31	8	2.13	0.22	4	2.95	0.80				2.50	0.50	0.41

n: Number of replicates, \bar{x} : Mean of replicates, *s*: Standard deviation of replicates

$\hat{\mu}$: DerSimonian-Laird consensus value, $U_{95}(\hat{\mu})$: 95 % level of confidence expanded uncertainty, HHD: Horn-Horn-Duncan estimate, BS: Boot-Strap estimate

Table 49. RM 8691: Consensus Means and 95 % Expanded Uncertainties, µg/g.

PFAS	Section 3.1			Section 3.2			Section 3.5			$U_{95}(\hat{\mu})$		
	<i>n</i>	\bar{x}	<i>s</i>	<i>n</i>	\bar{x}	<i>s</i>	<i>n</i>	\bar{x}	<i>s</i>	$\hat{\mu}$	HHD	BS
PFBA	10	0.00749	0.00015	9	0.00733	0.00040				0.00745	0.00013	0.00014
PFHxA	10	0.0152	0.0004	9	0.0154	0.0015				0.0153	0.0001	0.0004
PFBSA	10	0.00108	0.00002	9	0.00081	0.00003				0.00095	0.00027	0.00026
PFOA	10	0.02602	0.00049	9	0.0260	0.0014				0.0260		0.00040
6:2 FTS	10	0.0554	0.0013	9	0.0566	0.0024	4	0.074	0.022	0.0560	0.0012	0.0019
6:2 FTAB	10	0.00272	0.00042	9	0.00183	0.00027				0.00227	0.00088	0.00087

n: Number of replicates, \bar{x} : Mean of replicates, *s*: Standard deviation of replicates

$\hat{\mu}$: DerSimonian-Laird consensus value, $U_{95}(\hat{\mu})$: 95 % level of confidence expanded uncertainty, HHD: Horn-Horn-Duncan estimate, BS: Boot-Strap estimate

Table 50. RM 8692: Summary Data, Consensus Means and 95 % Expanded Uncertainties, µg/g.

PFAS	Section 3.1			Section 3.2			$U_{95}(\hat{\mu})$		
	<i>n</i>	\bar{x}	<i>s</i>	<i>n</i>	\bar{x}	<i>s</i>	$\hat{\mu}$	HHD	BS
PFBA	10	0.0206	0.0002	9	0.0206	0.0004	0.0206	0.00004	0.0001
PFHxA	10	0.00915	0.00030	9	0.00970	0.00057	0.0094	0.00055	0.00054
PFBSA	10	0.00107	0.00002	9	0.000816	0.000012	0.00094	0.00026	0.00025
6:2 FTS	10	0.0320	0.0028	9	0.0365	0.0020	0.0343	0.0045	0.0044
6:2 FTAB	10	0.0083	0.0010	9	0.0205	0.0013	0.014	0.012	0.012

n: Number of replicates, \bar{x} : Mean of replicates, *s*: Standard deviation of replicates

$\hat{\mu}$: DerSimonian-Laird consensus value, $U_{95}(\hat{\mu})$: 95 % level of confidence expanded uncertainty, HHD: Horn-Horn-Duncan estimate, BS: Boot-Strap estimate

Table 51. RM 8693: Summary Data, Consensus Means, and 95 % Expanded Uncertainties, µg/g.

PFAS	Section 3.1			Section 3.2			Section 3.3			Section 3.4			Section 3.5			$U_{95}(\hat{\mu})$		
	<i>n</i>	\bar{x}	<i>s</i>	$\hat{\mu}$	HHD	BS												
PFBA	10	0.0570	0.0052	9	0.0435	0.0033										0.0502	0.0136	0.0133
PFHxA	10	0.1467	0.0066	9	0.1743	0.0117							4	0.130	0.019	0.151	0.025	0.022
PFBSA	10	0.0335	0.0014	9	0.0248	0.0009										0.0291	0.0088	0.0086
PFOS							1	0.98		1	0.96							
6:2 FTS	10	12.4	0.5	9	14.5	1.0	5	15.0	0.1	5	17.2	2.3	4	18.7	0.8	15.1	2.61	1.97
6:2 FTAB	10	220	13	9	270	16	20	276	17	5	311	18				255	35	37

n: Number of replicates, \bar{x} : Mean of replicates, *s*: Standard deviation of replicates

$\hat{\mu}$: DerSimonian-Laird consensus value, $U_{95}(\hat{\mu})$: 95 % level of confidence expanded uncertainty, HHD: Horn-Horn-Duncan estimate, BS: Boot-Strap estimate

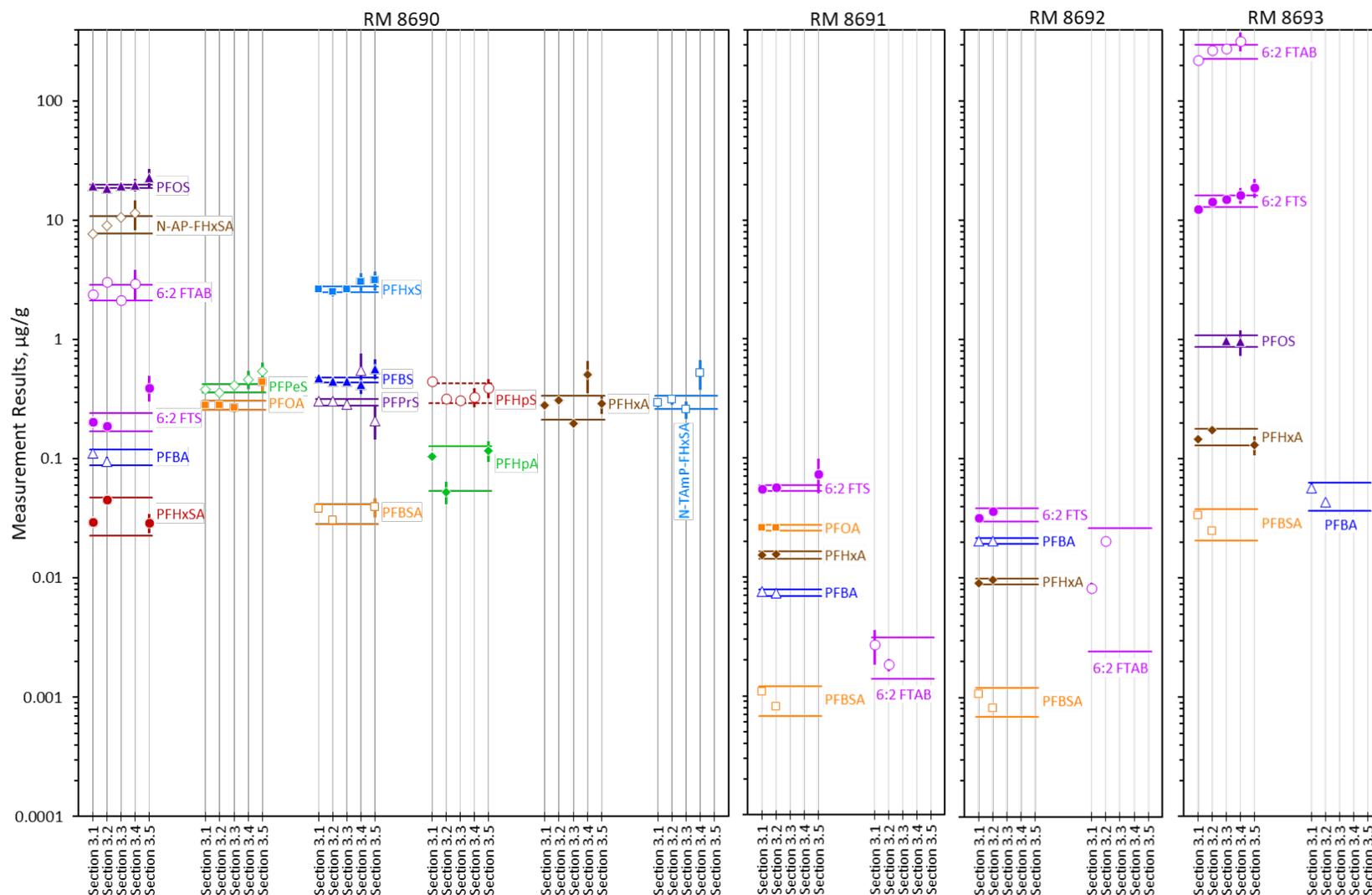


Fig. 18. Results and Consensus Values for PFAS Quantified in at Least Two Studies, µg/g.

Symbols denote mean values, error bars represent two standard deviations of the mean, horizontal lines bound an approximate 95 % level of confidence intervals about the consensus value, vertical lines connect the symbols to labels that indicate the Sections of this document that provide the detailed results.

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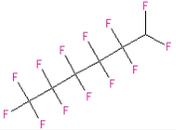
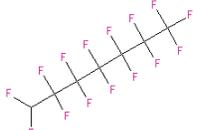
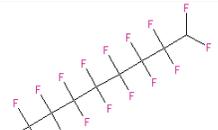
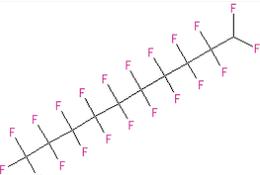
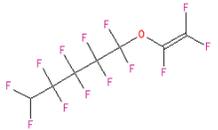
Appendix A. List of Symbols, Abbreviations, and Acronyms

AFFF	aqueous film forming foam
AGC	automatic gain control
ASV	autosampler vial
BS	bootstrap method for estimating variances
CAS	Chemical Abstract Service Registry Number
CV	coefficient of variation (relative standard deviation expressed as a percent)
DOD	Department of Defense
DTXSID	Distributed Structure-Searchable Toxicity Structural Identifier
EPA	Environmental Protection Agency
ESI	electrospray ionization
ESI-	ESI negative polarity mode
ESI+	ESI positive polarity mode
HDPE	high density polyethylene
HHD	Horn-Horn-Duncan method for estimating variances
HRMS	high resolution mass spectrometry
InChI	International Chemical Identifier
IS	internal standard
IT	injection time
LC-MS/MS	liquid chromatography tandem mass spectrometry
LC-QToF-MS	liquid chromatography quadrupole time-of-flight mass spectrometry
<i>m/z</i>	mass-to-charge
MS1	first stage of tandem mass spectrometry
MS2	second stage of tandem mass spectrometry
NIST	National Institute of Standards and Technology
NTA	non-targeted analysis
ORM	NIST Office of Reference Materials
PFAS	per- and polyfluoroalkyl substances
PTFE	polytetrafluoroethylene
RL	reporting limit
RM	Reference Material
SERDP	Strategic Environmental Research and Development Program
SD	standard deviation
SI	International System of Units
SMILES	Simplified Molecular-Input Line-Entry System
topN	maximum number of most abundant ions
WS	working standard solution

Appendix B. PFAS Names and Structures

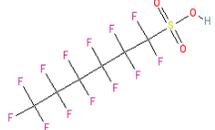
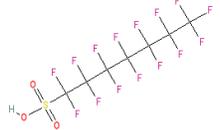
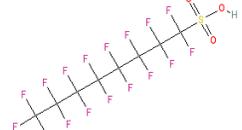
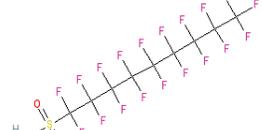
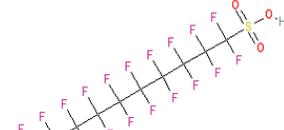
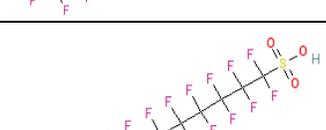
The following list connects the consensus chemical and code names used in this document with their Simplified Molecular-Input Line-Entry System (SMILES) [25] and International Chemical Identifier (InChI) [26,27] molecular structure descriptors along with a 2D diagram of the compound. When known, the unique Distributed Structure-Searchable Toxicity Structural Identifier (DTXSID) [28,29] and Chemical Abstract Service Registry Number (CAS) [30] are also provided. The compounds are listed roughly in order of increasing structural complexity.

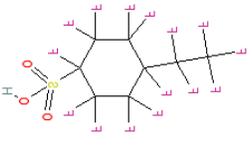
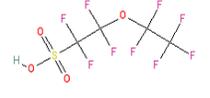
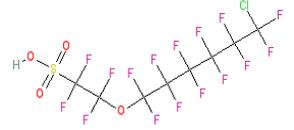
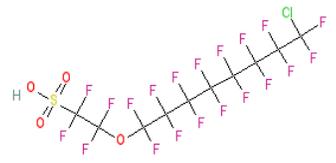
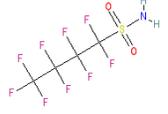
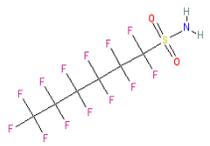
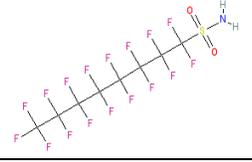
The CAS, SMILES, InChI, and the 2D diagram are for the linear (unbranched) per- or polyfluorinated moiety. The PFAS measurands referenced by these descriptors include both the linear and branched forms of these compounds.

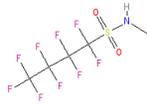
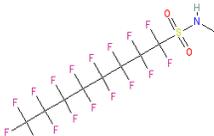
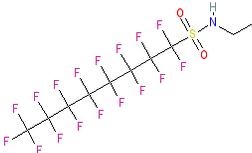
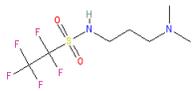
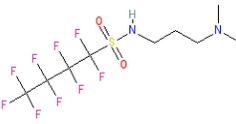
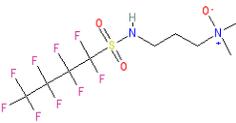
Code Name	Names	Structure
H-PFHx	1H-Perfluorohexane DTXSID20188995 CAS: 355-37-3 SMILES: FC(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F InChI=1S/C6HF13/c7-1(8)2(9,10)3(11,12)4(13,14)5(15,16)6(17,18)19/h1H	
H-PFHp	1H-fluoroheptane DTXSID80871634 CAS: 375-83-7 SMILES: FC(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F InChI=1S/C7HF15/c8-1(9)2(10,11)3(12,13)4(14,15)5(16,17)6(18,19)7(20,21)22/h1H	
H-PFO	1H-Perfluorooctane DTXSID00187143 CAS: 335-65-9 SMILES: FC(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F InChI=1S/C8HF17/c9-1(10)2(11,12)3(13,14)4(15,16)5(17,18)6(19,20)7(21,22)8(23,24)25/h1H	
H-PFD	1H-Perfluorodecane DTXSID60190951 CAS: 375-97-3 SMILES: FC(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F InChI=1S/C10HF21/c11-1(12)2(13,14)3(15,16)4(17,18)5(19,20)6(21,22)7(23,24)8(25,26)9(27,28)10(29,30)31/h1H	
PFPePFV	1,1,2,2,3,3,4,4,5,5-Decafluoro-1-((trifluorovinyl)oxy)pentane DTXSID20880967 CAS: 73928-40-2 SMILES: FC(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)OC(F)=C(F)F InChI=1S/C7HF13O/c8-1(9)2(10)21-7(19,20)6(17,18)5(15,16)4(13,14)3(11)12/h3H	

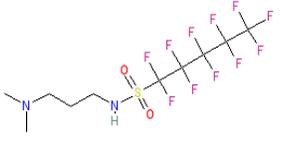
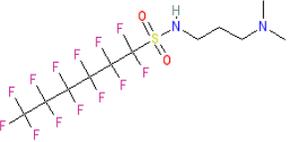
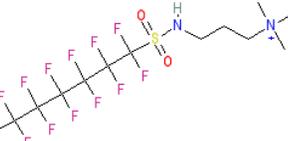
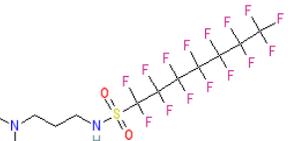
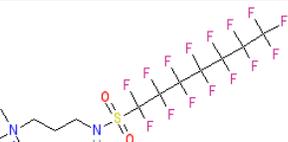
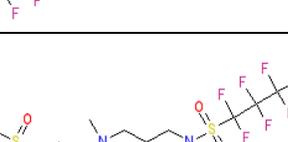
Code Name	Names	Structure
PFPEG3OMe	Fluorinated triethylene glycol monomethyl ether DTXSID40380797 CAS: 147492-57-7 SMILES: OCC(F)(F)OC(F)(F)C(F)(F)OC(F)(F)C(F)(F)OC(F)(F)F InChI=1S/C7H3F13O4/c8-2(9,1-21)22-3(10,11)4(12,13)23-5(14,15)6(16,17)24-7(18,19)20/h21H,1H2	
PFProPrVO	Perfluoro(2-propoxypropyl vinyl ether) DTXSID30880150 CAS: 1644-11-7 SMILES: FC(F)=C(F)OC(F)(F)C(F)(OC(F)(F)C(F)(F)C(F)(F)F)C(F)(F)F InChI=1S/C8F16O2/c9-1(10)2(11)25-8(23,24)4(14,6(18,19)20)26-7(21,22)3(12,13)5(15,16)17	
PFBA	Perfluorobutanoic acid DTXSID4059916 CAS: 375-22-4 SMILES: OC(=O)C(F)(F)C(F)(F)C(F)(F)F InChI=1S/C4HF7O2/c5-2(6,1(12)13)3(7,8)4(9,10)11/h(H,12,13)	
PFPeA	Perfluoropentanoic acid DTXSID6062599 CAS: 2706-90-3 SMILES: OC(=O)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F InChI=1S/C5HF9O2/c6-2(7,1(15)16)3(8,9)4(10,11)5(12,13)14/h(H,15,16)	
PFHxA	Perfluorohexanoic acid DTXSID3031862 CAS: 307-24-4 SMILES: OC(=O)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F InChI=1S/C6HF11O2/c7-2(8,1(18)19)3(9,10)4(11,12)5(13,14)6(15,16)17/h(H,18,19)	
PFHpA	Perfluoroheptanoic acid DTXSID1037303 CAS: 375-85-9 SMILES: OC(=O)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F InChI=1S/C7HF13O2/c8-2(9,1(21)22)3(10,11)4(12,13)5(14,15)6(16,17)7(18,19)20/h(H,21,22)	
PFOA	Perfluorooctanoic acid DTXSID8031865 CAS: 335-67-1 SMILES: OC(=O)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F InChI=1S/C8HF15O2/c9-2(10,1(24)25)3(11,12)4(13,14)5(15,16)6(17,18)7(19,20)8(21,22)23/h(H,24,25)	
PFNA	Perfluorononanoic acid DTXSID8031863 CAS: 375-95-1 SMILES: OC(=O)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F InChI=1S/C9HF17O2/c10-2(11,1(27)28)3(12,13)4(14,15)5(16,17)6(18,19)7(20,21)8(22,23)9(24,25)26/h(H,27,28)	

Code Name	Names	Structure
3,6-OPFHpA	Perfluoro-3,6-dioxaheptanoic acid DTXSID30382063 CAS: 151772-58-6 SMILES: OC(=O)C(F)(F)OC(F)(F)C(F)(F)OC(F)(F)F InChI=1S/C5HF9O4/c6-2(7,1(15)16)17-3(8,9)4(10,11)18-5(12,13)14/h(H,15,16)	
ADONA	4,8-Dioxa-3H-perfluorononanoic acid DTXSID40881350 CAS: 919005-14-4 SMILES: OC(=O)C(F)(F)C(F)OC(F)(F)C(F)(F)C(F)(F)OC(F)(F)F InChI=1S/C7H2F12O4/c8-1(3(9,10)2(20)21)22-5(13,14)4(11,12)6(15,16)23-7(17,18)19/h1H,(H,20,21)	
PF4OPeA	Perfluoro-3-methoxypropanoic acid DTXSID70191136 CAS: 377-73-1 SMILES: OC(=O)C(F)(F)C(F)(F)OC(F)(F)F InChI=1S/C4HF7O3/c5-2(6,1(12)13)3(7,8)14-4(9,10)11/h(H,12,13)	
PF5OHxA	Perfluoro-4-methoxybutanoic acid DTXSID60500450 CAS: 863090-89-5 SMILES: OC(=O)C(F)(F)C(F)(F)C(F)(F)OC(F)(F)F InChI=1S/C5HF9O3/c6-2(7,1(15)16)3(8,9)4(10,11)17-5(12,13)14/h(H,15,16)	
3,6-PFRHpA	Perfluoro-5-methoxypentanoic acid *InChIKey: RUWAFPLNUJKITJ-UHFFFAOYSA-N CAS: unknown SMILES: OC(=O)C(F)(F)C(F)(F)C(F)(F)C(F)(F)OC(F)(F)F InChI=1S/C6HF11O3/c7-2(8,1(18)19)3(9,10)4(11,12)5(13,14)20-6(15,16)17/h(H,18,19)	
PFPrS	Perfluoropropanesulfonic acid DTXSID30870531 CAS: 423-41-6 SMILES: O[S](=O)(=O)C(F)(F)C(F)(F)C(F)(F)F InChI=1S/C3HF7O3S/c4-1(5,2(6,7)8)3(9,10)14(11,12)13/h(H,11,12,13)	
PFBS	Perfluorobutanesulfonic acid DTXSID5030030 CAS: 375-73-5 SMILES: O[S](=O)(=O)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F InChI=1S/C4HF9O3S/c5-1(6,3(9,10)11)2(7,8)4(12,13)17(14,15)16/h(H,14,15,16)	
PFPeS	Perfluoropentanesulfonic acid DTXSID8062600 CAS: 2706-91-4 SMILES: O[S](=O)(=O)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F InChI=1S/C5HF11O3S/c6-1(7,2(8,9)4(12,13)14)3(10,11)5(15,16)20(17,18)19/h(H,17,18,19)	

Code Name	Names	Structure
PFHxS	Perfluorohexanesulfonic acid DTXSID7040150 CAS: 355-46-4 SMILES: O[S](=O)(=O)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F InChI=1S/C6HF13O3S/c7-1(8,3(11,12)5(15,16)17)2(9,10)4(13,14)6(18,19)23(20,21)22/h(H,20,21,22)	
PFHpS	Perfluoroheptanesulfonic acid DTXSID8059920 CAS: 375-92-8 SMILES: O[S](=O)(=O)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F InChI=1S/C7HF15O3S/c8-1(9,2(10,11)4(14,15)6(18,19)20)3(12,13)5(16,17)7(21,22)26(23,24)25/h(H,23,24,25)	
PFOS	Perfluorooctanesulfonic acid DTXSID3031864 CAS: 1763-23-1 SMILES: O[S](=O)(=O)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F InChI=1S/C8HF17O3S/c9-1(10,3(13,14)5(17,18)7(21,22)2)3(11,12)4(15,16)6(19,20)8(24,25)29(26,27)28/h(H,26,27,28)	
PFNS	Perfluorononanesulfonic acid DTXSID8071356 CAS: 68259-12-1 SMILES: O[S](=O)(=O)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F InChI=1S/C9HF19O3S/c10-1(11,2(12,13)4(16,17)6(20,21)8(24,25)26)3(14,15)5(18,19)7(22,23)9(27,28)32(29,30)31/h(H,29,30,31)	
PFDS	Perfluorodecanesulfonic acid DTXSID3040148 CAS: 335-77-3 SMILES: O[S](=O)(=O)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F InChI=1S/C10HF21O3S/c11-1(12,3(15,16)5(19,20)7(23,24)9(27,28)29)2(13,14)4(17,18)6(21,22)8(25,26)10(30,31)35(32,33)34/h(H,32,33,34)	
PFDoS	Perfluorododecanesulfonic acid DTXSID20873011 CAS: 79780-39-5 SMILES: O[S](=O)(=O)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F InChI=1S/C12HF25O3S/c13-1(14,3(17,18)5(21,22)7(25,26)9(29,30)11(33,34)35)2(15,16)4(19,20)6(23,24)8(27,28)10(31,32)12(36,37)41(38,39)40/h(H,38,39,40)	

Code Name	Names	Structure
PFECHS	perfluoro-4-ethylcyclohexanesulfonic acid; 1,2,2,3,3,4,5,5,6,6-decafluoro-4-(1,1,2,2,2-pentafluoroethyl)cyclohexane-1-sulfonic acid CAS: 646-83-3 SMILES: O[S](=O)(=O)C1(F)C(F)(F)C(F)(F)C(F)(C(F)(F)C(F)(F)C(F)(F)C1(F)F DTXSID70275965 InChI=1S/C8HF15O3S/c9-1(4(14,15)8(21,22)23)2(10,11)5(16,17)7(20,27(24,25)26)6(18,19)3(1,12)13/h(H,24,25,26)	
PFEESA	Perfluoro(2-ethoxyethane)sulfonic acid DTXSID50379814 CAS: 113507-82-7 SMILES: O[S](=O)(=O)C(F)(F)C(F)(F)OC(F)(F)C(F)(F)F InChI=1S/C4HF9O4S/c5-1(6,7)2(8,9)17-3(10,11)4(12,13)18(14,15)16/h(H,14,15,16)	
9Cl-PF3ONS	9-chlorohexadecafluoro-3-oxanone-1-sulfonic acid DTXSID80892506 CAS: 756426-58-1 SMILES: O[S](=O)(=O)C(F)(F)C(F)(F)OC(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)Cl InChI=1S/C8HClF16O4S/c9-5(18,19)3(14,15)1(10,11)2(12,13)4(16,17)6(20,21)29-7(22,23)8(24,25)30(26,27)28/h(H,26,27,28)	
11Cl-PF3OUdS	11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid DTXSID40892507 CAS: 763051-92-9 SMILES: O[S](=O)(=O)C(F)(F)C(F)(F)OC(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)Cl InChI=1S/C10HClF20O4S/c11-7(24,25)5(20,21)3(16,17)1(12,13)2(14,15)4(18,19)6(22,23)8(26,27)35-9(28,29)10(30,31)36(32,33)34/h(H,32)	
PFBSA	Perfluorobutanesulfonamide DTXSID30880251 CAS: 30334-69-1 SMILES: N[S](=O)(=O)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F InChI=1S/C4H2F9NO2S/c5-1(6,3(9,10)11)2(7,8)4(12,13)17(14,15)16/h(H2,14,15,16)	
PFHxSA	Perfluorohexanesulfonamide DTXSID50469320 CAS: 41997-13-1 SMILES: N[S](=O)(=O)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F InChI=1S/C6H2F13NO2S/c7-1(8,3(11,12)5(15,16)17)2(9,10)4(13,14)6(18,19)23(20,21)22/h(H2,20,21,22)	
PFOSA	Perfluorooctanesulfonamide DTXSID3038939 CAS: 754-91-6 SMILES: N[S](=O)(=O)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F InChI=1S/C8H2F17NO2S/c9-1(10,3(13,14)5(17,18)7(21,22)23)2(11,12)4(15,16)6(19,20)8(24,25)29(26,27)28/h(H2,26,27,28)	

Code Name	Names	Structure
N-MeFBSA	<i>N</i> -methylperfluorobutanesulfonamide DTXSID1071373 CAS: 68298-12-4 SMILES: CN[S](=O)(=O)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F InChI=1S/C5H4F9NO2S/c1-15-18(16,17)5(13,14)3(8,9)2(6,7)4(10,11)12/h15H,1H3	
N-MeFOSA	<i>N</i> -methylperfluorooctanesulfonamide DTXSID1067629 CAS: 31506-32-8 SMILES: CN[S](=O)(=O)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F InChI=1S/C9H4F17NO2S/c1-27-30(28,29)9(25,26)7(20,21)5(16,17)3(12,13)2(10,11)4(14,15)6(18,19)8(22,23)24/h27H,1H3	
N-EtFOSA	<i>N</i> -ethylperfluorooctanesulfonamide DTXSID1032646 CAS: 4151-50-2 SMILES: CCN[S](=O)(=O)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F InChI=1S/C10H6F17NO2S/c1-2-28-31(29,30)10(26,27)8(21,22)6(17,18)4(13,14)3(11,12)5(15,16)7(19,20)9(23,24)25/h28H,2H2,1H3	
N-AP-FESA	Perfluoroethane sulfonamido amine DTXSID201032838 CAS: 1513864-23-7 SMILES: CN(C)CCCN[S](=O)(=O)C(F)(F)C(F)(F)F InChI=1S/C7H13F5N2O2S/c1-14(2)5-3-4-13-17(15,16)7(11,12)6(8,9)10/h13H,3-5H2,1-2H3	
N-AP-FPrSA	Perfluoropropane sulfonamido amine DTXSID201032838 CAS: 1513864-23-7 SMILES: CN(C)CCCN[S](=O)(=O)C(F)(F)C(F)(F)C(F)(F)F InChI=1S/C8H13F7N2O2S/c1-17(2)5-3-4-16-20(18,19)8(14,15)6(9,10)7(11,12)13/h16H,3-5H2,1-2H3	
N-AP-FBSA	Perfluorobutane sulfonamido amine DTXSID1071666 CAS: 68555-77-1 SMILES: CN(C)CCCN[S](=O)(=O)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F InChI=1S/C9H13F9N2O2S/c1-20(2)5-3-4-19-23(21,22)9(17,18)7(12,13)6(10,11)8(14,15)16/h19H,3-5H2,1-2H3	
N-OxAmP-FBSA	Perfluorobutane sulfonamido amine oxide DTXSID50893565 CAS: 178094-76-3 SMILES: C[N+](C)([O-])CCCN[S](=O)(=O)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F InChI=1S/C9H13F9N2O3S/c1-20(2,21)5-3-4-19-24(22,23)9(17,18)7(12,13)6(10,11)8(14,15)16/h19H,3-5H2,1-2H3	

Code Name	Names	Structure
N-AP-FPeSA	Perfluoropentane sulfonamido amine DTXSID6071667 CAS: 68555-78-2 SMILES: CN(C)CCCN[S](=O)(=O)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F InChI=1S/C10H13F11N2O2S/c1-23(2)5-3-4-22-26(24,25)10(20,21)8(15,16)6(11,12)7(13,14)9(17,18)19/h22H,3-5H2,1-2H3	
N-AP-FHxSA	Perfluorohexane sulfonamido amine DTXSID7068556 CAS: 50598-28-2 SMILES: CN(C)CCCN[S](=O)(=O)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F InChI=1S/C11H13F13N2O2S/c1-26(2)5-3-4-25-29(27,28)11(23,24)9(18,19)7(14,15)6(12,13)8(16,17)10(20,21)22/h25H,3-5H2,1-2H3	
N-TAmP-FHxSA	<i>N</i> -[3-(perfluoro-1-hexanesulfonamido)propan-1-yl]- <i>N,N,N</i> -trimethylammonium DTXSID50866239 CAS: 70248-51-0 SMILES: C[N+](C)(C)CCCN[S](=O)(=O)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F InChI=1S/C12H16F13N2O2S/c1-27(2,3)6-4-5-26-30(28,29)12(24,25)10(19,20)8(15,16)7(13,14)9(17,18)11(21,22)23/h26H,4-6H2,1-3H3/q+1	
N-AP-FHpSA	Perfluoroheptane sulfonamido amine DTXSID2070506 CAS: 67584-54-7 SMILES: CN(C)CCCN[S](=O)(=O)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F InChI=1S/C12H13F15N2O2S/c1-29(2)5-3-4-28-32(30,31)12(26,27)10(21,22)8(17,18)6(13,14)7(15,16)9(19,20)11(23,24)25/h28H,3-5H2,1-2H3	
N-OxAmP-FHpSA	Perfluoroheptane sulfonamido amine oxide DTXSID50892957 CAS: 178094-74-1 SMILES: C[N+](C)([O-])CCCN[S](=O)(=O)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F InChI=1S/C12H13F15N2O3S/c1-29(2,30)5-3-4-28-33(31,32)12(26,27)10(21,22)8(17,18)6(13,14)7(15,16)9(19,20)11(23,24)25/h28H,3-5H2,1-2H3	
N-AP-FOSA	Perfluorooctane sulfonamido amine DTXSID9065443 CAS: 13417-01-1 SMILES: CN(C)CCCN[S](=O)(=O)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F InChI=1S/C13H13F17N2O2S/c1-32(2)5-3-4-31-35(33,34)13(29,30)11(24,25)9(20,21)7(16,17)6(14,15)8(18,19)10(22,23)12(26,27)28/h31H,3-5H2,1-2H3	
N-SPAmP-FPrSA	<i>N,N</i> -dimethyl- <i>N</i> -(3-perfluoroalkylsulfonamidopropan-1-yl)- <i>N</i> -(-sulfopropan-1-yl)ammonium DTXSID801032343 CAS: 2089108-65-4 SMILES: C[N+](CCCNS(C(F)(F)C(F)(F)C(F)(F)F)(=O)=O)(CCCS(O)(=O)=O)C InChI=1S/C11H19F7N2O5S2/c1-20(2,7-4-8-26(21,22)23)6-3-5-19-27(24,25)11(17,18)9(12,13)10(14,15)16/h19H,3-8H2,1-2H3/p+1	

Code Name	Names	Structure
PFASAC $n=4$	<p><i>N</i>-[3-(Dimethylamino)propyl]-<i>N</i>-[(nonafluorobutyl)sulfonyl]-beta-alanine DTXSID20882022 CAS: 172616-04-5 SMILES: CN(C)CCCN(CCC(O)=O)[S](=O)(=O)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F InChI=1S/C12H17F9N2O4S/c1-22(2)5-3-6-23(7-4-8(24)25)28(26,27)12(20,21)10(15,16)9(13,14)11(17,18)19/h3-7H2,1-2H3,(H,24,25)</p>	
PFASAC $n=5$	<p><i>N</i>-[3-(Dimethylamino)propyl]-<i>N</i>-[(undecafluoropentyl)sulfonyl]-beta-alanine DTXSID10892554 CAS: 1383438-83-2 SMILES: CN(C)CCCN(CCC(O)=O)[S](=O)(=O)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F InChI=1S/C13H17F11N2O4S/c1-25(2)5-3-6-26(7-4-8(27)28)31(29,30)13(23,24)11(18,19)9(14,15)10(16,17)12(20,21)22/h3-7H2,1-2H3,(H,27,28)</p>	
PFASAC $n=6$	<p><i>N</i>-[3-(Dimethylamino)propyl]-<i>N</i>-[(tridecafluorohexyl)sulfonyl]-beta-alanine DTXSID00882020 CAS: 141607-32-1 SMILES: CN(C)CCCN(CCC(O)=O)[S](=O)(=O)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F InChI=1S/C14H17F13N2O4S/c1-28(2)5-3-6-29(7-4-8(30)31)34(32,33)14(26,27)12(21,22)10(17,18)9(15,16)11(19,20)13(23,24)25/h3-7H2,1-2H3,(H,30,31)</p>	
PFASAC $n=7$	<p><i>N</i>-[3-(Dimethylamino)propyl]-<i>N</i>-[(pentadecafluoroheptyl)sulfonyl]-beta-alanine DTXSID40892547 CAS: 1432486-91-3 SMILES: CN(C)CCCN(CCC(O)=O)[S](=O)(=O)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F InChI=1S/C15H17F15N2O4S/c1-31(2)5-3-6-32(7-4-8(33)34)37(35,36)15(29,30)13(24,25)11(20,21)9(16,17)10(18,19)12(22,23)14(26,27)28/h3-7H2,1-2H3,(H,33,34)</p>	
PFASAC $n=8$	<p><i>N</i>-[3-(Dimethylamino)propyl]-<i>N</i>-[(heptadecafluorooctyl)sulfonyl]-beta-alanine DTXSID30892551 CAS: 1432486-92-4 SMILES: CN(C)CCCN(CCC(O)=O)[S](=O)(=O)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F InChI=1S/C16H17F17N2O4S/c1-34(2)5-3-6-35(7-4-8(36)37)40(38,39)16(32,33)14(27,28)12(23,24)10(19,20)9(17,18)11(21,22)13(25,26)15(29,30)31/h3-7H2,1-2H3,(H,36,37)</p>	

Code Name	Names	Structure
6:2 FTSHA	6:2 Fluorotelomer thioether hydroxyammonium DTXSID90892532 CAS: 88992-46-5 SMILES: C[N+](C)(C)CC(O)CSCCC(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F InChI=1S/C14H19F13NOS/c1-28(2,3)6-8(29)7-30-5-4-9(15,16)10(17,18)11(19,20)12(21,22)13(23,24)14(25,26)27/h8,29H,4-7H2,1-3H3/q+1	
6:2 FTSHA Sulfoxide	6:2 Fluorotelomer sulfoxide hydroxyammonium DTXSID50892593 CAS: 1513864-18-0 SMILES: C[N+](C)(C)CC(O)C[S](=O)CCC(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F InChI=1S/C14H19F13NO2S/c1-28(2,3)6-8(29)7-31(30)5-4-9(15,16)10(17,18)11(19,20)12(21,22)13(23,24)14(25,26)27/h8,29H,4-7H2,1-3H3/q+1	
4:2 FTSAS 4:2 FTtAoS	4:2 Fluorotelomer thioether amido sulfonic acid DTXSID00892528 CAS: 1333933-57-5 SMILES: CC(C)(C[S](O)(=O)=O)NC(=O)CCSCCC(F)(F)C(F)(F)C(F)(F)C(F)(F)F InChI=1S/C13H18F9NO4S2/c1-9(2,7-29(25,26)27)23-8(24)3-5-28-6-4-10(14,15)11(16,17)12(18,19)13(20,21)22/h3-7H2,1-2H3,(H,23,24)(H,25,26,27)	
6:2 FTSAS 6:2 FTtAoS	6:2 Fluorotelomer thioether amido sulfonic acid DTXSID90892330 CAS: 62880-95-9 SMILES: CC(C)(C[S](O)(=O)=O)NC(=O)CCSCCC(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F InChI=1S/C15H18F13NO4S2/c1-9(2,7-35(31,32)33)29-8(30)3-5-34-6-4-10(16,17)11(18,19)12(20,21)13(22,23)14(24,25)15(26,27)28/h3-7H2,1-2H3,(H,29,30)(H,31,32,33)	
6:2 FTSAS Sulfoxide 6:2 FtSiAoS	6:2 Fluorotelomer sulfinyl amido sulfonic acid; 6:2 Fluorotelomer thioether amido sulfonic acid sulfoxide DTXSID90892592 CAS: 1513864-10-2 SMILES: CC(C)(CS(O)(=O)=O)NC(=O)CCS(=O)CCC(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F InChI=1S/C15H18F13NO5S2/c1-9(2,7-36(32,33)34)29-8(30)3-5-35(31)6-4-10(16,17)11(18,19)12(20,21)13(22,23)14(24,25)15(26,27)28/h3-7H2,1-2H3,(H,29,30)(H,32,33,34)	
6:2 FtSO2AoS 6:2 FtSoAoS	6:2 Fluorotelomer sulfonyl amido sulfonic acid DTXSID90894132 CAS: 1911606-13-7 SMILES: CC(C)(CS(O)(=O)=O)NC(=O)CCS(=O)(=O)CCC(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)(F)F InChI=1S/C15H18F13NO6S2/c1-9(2,7-37(33,34)35)29-8(30)3-5-36(31,32)6-4-10(16,17)11(18,19)12(20,21)13(22,23)14(24,25)15(26,27)28/h3-7H2,1-2H3,(H,29,30)(H,33,34,35)	

