Determination of Selected Perfluorinated Alkyl Substances by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry Isotope Dilution (LC/MS/MS)

References: EPA Method 537.1, Version 2, March 2020, EPA Document #: EPA/600/R-20/006

EPA Method 533, November 2019, EPA Document #: 815-B-19-020

ISO 25101, First Edition, March 2009, Reference #: ISO 25101:2009(E)

Department of Defense, Quality Systems Manual for Environmental Laboratories, Version 5.3, 2019

1. Scope and Application

Matrices: Drinking water, Non-potable Water, Tissues, Biosolids and Soil Matrices
(Drinking water is applicable for specific state regulatory requirements for this method)

Definitions: Refer to Alpha Analytical Quality Manual.

1.1 This is a liquid chromatography/tandem mass spectrometry (LC/MS/MS) method for the determination of selected perfluorinated alkyl substances (PFAS) in Non-Drinking Water and soil Matrices. Accuracy and precision data have been generated in reagent water, and finished ground and surface waters and soils for the compounds listed in Table 1.

1.2 The data report packages present the documentation of any method modification related to the samples tested. Depending upon the nature of the modification and the extent of intended use, the laboratory may be required to demonstrate that the modifications will produce equivalent results for the matrix. Approval of all method modifications is by one or more of the following laboratory personnel before performing the modification: Area Supervisor, Department Supervisor, Laboratory Director, or Quality Assurance Officer.

1.3 This method is restricted to use by or under the supervision of analysts experienced in the operation of the LC/MS/MS and in the interpretation of LC/MS/MS data. Each analyst must demonstrate the ability to generate acceptable results with this method by performing an initial demonstration of capability.

2. Summary of Method

2.1 A 250-mL water sample is fortified with extracted internal standards (EIS) and passed through a solid phase extraction (WAX) cartridge containing a mixed mode, Weak Anion Exchange, reversed phase, water-wettable polymer to extract the method analytes and isotopically-labeled compounds. The compounds are eluted from the solid phase in two fractions with methanol followed by a small amount of 2% ammonium hydroxide in methanol solution. The extract is concentrated with nitrogen in a heated water bath, and then adjusted to a 1-mL volume with 80:20% (vol/vol) methanol:water.

A 2-4 gram soil, solid, tissue or biosolid sample is fortified with extracted internal standards (EIS), diluted in methanol and agitated rigorously. An aliquot of the methanol is passed across an SPE based clean-up cartridge and the eluate collected. The extract is concentrated with nitrogen in a heated water bath, and then adjusted to a 1-mL volume with 80:20% (vol/vol) methanol:water.

2.2 A sample extract is injected into an LC equipped with a C18 column that is interfaced to an MS/MS). The analytes are separated and identified by comparing the acquired mass spectra and retention times to reference spectra and retention times for calibration standards acquired under identical LC/MS/MS conditions. The concentration of each analyte is determined by using the isotope dilution technique. Extracted Internal Standards (EIS) analytes are used to monitor the extraction efficiency of the method analytes.

2.3 Method Modifications from Reference

None.

Table 1

Parameter	Acronym	CAS
PERFLUOROALKYL ETHER CARBOXYLIC ACIDS (PFECAs)		
Tetrafluoro-2-(heptafluoropropoxy)propanoic acid	HFPO-DA	13252-13-6
4,8-dioxa-3H-perfluorononanoic acid	ADONA	919005-14-4
PERFLUOROALKYLCARBOXILIC ACIDS (PFCAs)		
Perfluorobutanoic acid	PFBA	375-22-4
Perfluoropentanoic acid	PFPeA	2706-90-3
Perfluorohexanoic acid	PFHxA	307-24-4
Perfluoroheptanoic acid	PFHpA	375-85-9
Perfluorooctanoic acid	PFOA	335-67-1
Perfluorononanoic acid	PFNA	375-95-1
Perfluorodecanoic acid	PFDA	335-76-2
Perfluoroundecanoic acid	PFUnA	2058-94-8
Perfluorododecanoic acid	PFDoA	307-55-1
Perfluorotridecanoic acid	PFTrDA	72629-94-8
Perfluorotetradecanoic acid	PFTA	376-06-7
Perfluorohexadecanoic acid	PFHxDA	67905-19-5
Perfluorooctadecanoic acid	PFODA	16517-11-6
PERFLUOROALKYL SULFONIC ACIDS (PFASs)		
Perfluoropropanesulfonic acid	PFPrS	423-41-6
Perfluorobutanesulfonic acid	PFBS	375-73-5
Perfluoropentanesulfonic acid	PFPeS	2706-91-4
Perfluorohexanesulfonic acid	PFHxS	355-46-4
Perfluoroheptanesulfonic acid	PFHpS	375-92-8
Perfluorooctanesulfonic acid	PFOS	1763-23-1
Perfluorononanesulfonic acid	PFNS	68259-12-1
Perfluorodecanesulfonic acid	PFDS	335-77-3
Perfluorododecanesulfonic acid	PFDoS	79780-39-5

Table 1 Cont.

Parameter	Acronym	CAS
CHLORO-PERFLUOROALKYLSULFONATE		
11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF3OUdS	763051-92-9
9-chlorohexadecafluoro-3-oxanone-1-sulfonic acid	9Cl-PF3ONS	756426-58-1
PERFLUOROOCTANESULFONAMIDES (FOSAs)		
Perfluorooctanesulfonamide	PFOSA	754-91-6
N-methylperfluoro-1-octanesulfonamide	NMeFOSA	31506-32-8
N-ethylperfluoro-1-octanesulfonamide	NEtFOSA	4151-50-2
TELOMER SULFONIC ACIDS		
1H,1H,2H,2H-perfluorohexanesulfonic acid (4:2)	4:2FTS	757124-72-4
1H,1H,2H,2H-perfluorooctanesulfonic acid (6:2)	6:2FTS	27619-97-2
1H,1H,2H,2H-perfluorodecanesulfonic acid (8:2)	8:2FTS	39108-34-4
1H,1H,2H,2H-perfluorododecanesulfonic acid (10:2)	10:2FTS	120226-60-0
PERFLUOROOCTANESULFONAMIDOACETIC ACIDS		
N-methyl perfluorooctanesulfonamidoacetic acid	NMeFOSAA	2355-31-9
N-ethyl perfluorooctanesulfonamidoacetic acid	NEtFOSAA	2991-50-6
NATIVE PERFLUOROOCTANESULFONAMIDOETHANOLS (FOSEs)		
2-(N-methylperfluoro-1-octanesulfonamido)-ethanol	NMeFOSE	24448-09-7
2-(N-ethylperfluoro-1-octanesulfonamido)-ethanol	NEtFOSE	1691-99-2
PERFLUOROETHER AND POLYETHER CARBOXYLIC ACIDS		
Perfluoro-3-methoxypropanoic acid	PFMPA	377-73-1
Perfluoro-4-methoxybutanoic acid	PFMBA	863090-89-5
Perfluoro(2-ethoxyethane)sulfonic acid	PFEESA	113507-82-7
Nonafluoro-3,6-dioxaheptanoic acid	NFDHA	151772-58-6

3. Reporting Limits

The reporting limit for PFAS's is 2 ng/L for aqueous samples (20 ng/L for HFPO-DA) and 1 ng/g (10 ng/g for HFPO-DA) for soil samples.

4. Interferences

4.1 PFAS standards, extracts and samples should not come in contact with any glass containers or pipettes as these analytes can potentially adsorb to glass surfaces. PFAS analyte and EIS standards commercially purchased in glass ampoules are acceptable; however, all subsequent transfers or dilutions performed by the analyst must be prepared and stored in polypropylene containers.

4.2 Method interferences may be caused by contaminants in solvents, reagents (including reagent water), sample bottles and caps, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in the chromatograms. The method analytes

in this method can also be found in many common laboratory supplies and equipment, such as PTFE (polytetrafluoroethylene) products, LC solvent lines, methanol, aluminum foil, SPE sample transfer lines, etc. All items such as these must be routinely demonstrated to be free from interferences (less than 1/3 the RL for each method analyte) under the conditions of the analysis by analyzing laboratory reagent blanks as described in Section 9.1. **Subtracting blank values from sample results is not permitted.**

4.3 Matrix interferences may be caused by contaminants that are co-extracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature of the water. Humic and/or fulvic material can be co-extracted during SPE and high levels can cause enhancement and/or suppression in the electrospray ionization source or low recoveries on the SPE sorbent. Total organic carbon (TOC) is a good indicator of humic content of the sample.

4.4 SPE cartridges can be a source of interferences. The analysis of field and laboratory reagent blanks can provide important information regarding the presence or absence of such interferences. Brands and lots of SPE devices should be tested to ensure that contamination does not preclude analyte identification and quantitation.

5. Health and Safety

5.1 The toxicity or carcinogenicity of each reagent and standard used in this method is not fully established; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. A reference file of material safety data sheets is available to all personnel involved in the chemical analysis. Additional references to laboratory safety are available in the Chemical Hygiene Plan.

5.2 All personnel handling environmental samples known to contain or to have been in contact with municipal waste must follow safety practices for handling known disease causative agents.

5.3 PFOA has been described as “likely to be carcinogenic to humans.” Pure standard materials and stock standard solutions of these method analytes should be handled with suitable protection to skin and eyes, and care should be taken not to breathe the vapors or ingest the materials.

6. Sample Collection, Preservation, Shipping and Handling

6.1 Sample Collection for Aqueous Samples

6.1.1 Samples must be collected in two (2) 250-mL high density polyethylene (HDPE) container with an unlined plastic screw cap.

6.1.2 The sample handler must wash their hands before sampling and wear nitrile gloves while filling and sealing the sample bottles. PFAS contamination during sampling can occur from a number of common sources, such as food packaging and certain foods and beverages. Proper hand washing and wearing nitrile gloves will aid in minimizing this type of accidental contamination of the samples.

6.1.3 Open the tap and allow the system to flush until the water temperature has stabilized (approximately 3 to 5 min). Collect samples from the flowing system.

6.1.4 Fill sample bottles. Samples do not need to be collected headspace free.

6.1.5 After collecting the sample and cap the bottle. Keep the sample sealed from time of collection until extraction.

6.1.6 Field Reagent Blank (FRB)

6.1.6.1 A FRB must be handled along with each sample set. The sample set is composed of samples collected from the same sample site and at the same time. At the laboratory, fill the field blank sample bottle with reagent water and preservatives, seal, and ship to the sampling site along with the sample bottles. For each FRB shipped, an empty sample bottle (no preservatives) must also be shipped. At the sampling site, the sampler must open the shipped FRB and pour the reagent water into the empty shipped sample bottle, seal and label this bottle as the FRB. The FRB is shipped back to the laboratory along with the samples and analyzed to ensure that PFAS's were not introduced into the sample during sample collection/handling.

The reagent water used for the FRBs must be initially analyzed for method analytes as a MB and must meet the MB criteria in Section 9.1.1 prior to use. This requirement will ensure samples are not being discarded due to contaminated reagent water rather than contamination during sampling.

6.2 Sample Collection for Soil and Sediment samples.

Grab samples are collected in polypropylene containers. Sample containers and contact surfaces containing PTFE shall be avoided.

6.3 Sample Preservation

Not applicable.

6.4 Sample Shipping

Samples must be chilled during shipment and must not exceed 10 °C during the first 48 hours after collection. Sample temperature must be confirmed to be at or below 10 °C when the samples are received at the laboratory. Samples stored in the lab must be held at or below 6 °C until extraction, but should not be frozen.

NOTE: Samples that are significantly above 10° C, at the time of collection, may need to be iced or refrigerated for a period of time, in order to chill them prior to shipping. This will allow them to be shipped with sufficient ice to meet the above requirements.

6.5 Sample Handling

6.5.1 Holding Times

6.5.1.1 Water samples should be extracted as soon as possible but must be extracted within 14 days. Soil samples should be extracted within 14 days. Extracts are stored at < 10 ° C and analyzed within 28 days after extraction.

7. Equipment and Supplies

7.1 SAMPLE CONTAINERS – 250-mL high density polyethylene (HDPE) bottles fitted with unlined screw caps. Sample bottles must be discarded after use.

7.2 SAMPLE JARS – 8-ounce wide mouth high density polyethylene (HDPE) bottles fitted with unlined screw caps. Sample bottles must be discarded after use.

7.3 POLYPROPYLENE BOTTLES – 4-mL narrow-mouth polypropylene bottles.

7.4 CENTRIFUGE TUBES – 50-mL conical polypropylene tubes with polypropylene screw caps for storing standard solutions and for collection of the extracts.

7.5 AUTOSAMPLER VIALS – Polypropylene 0.7-mL autosampler vials with polypropylene caps.

7.5.1 NOTE: Polypropylene vials and caps are necessary to prevent contamination of the sample from PTFE coated septa. However, polypropylene caps do not reseal, so evaporation occurs after injection. Thus, multiple injections from the same vial are not possible.

7.6 POLYPROPYLENE GRADUATED CYLINDERS – Suggested sizes include 25, 50, 100 and 1000-mL cylinders.

7.7 Auto Pipets – Suggested sizes include 5, 10, 25, 50, 100, 250, 500, 1000, 5000 and 10,000-µls.

7.8 PLASTIC PIPETS – Polypropylene or polyethylene disposable pipets.

7.9 ANALYTICAL BALANCE – Capable of weighing to the nearest 0.0001 g.

7.10 ANALYTICAL BALANCE – Capable of weighing to the nearest 0.1 g.

7.11 SOLID PHASE EXTRACTION (SPE) APPARATUS FOR USING CARTRIDGES

7.11.1 SPE CARTRIDGES – 0.5 g SPE cartridges containing a reverse phase copolymer characterized by a weak anion exchanger (WAX) sorbent phase.

7.11.2 VACUUM EXTRACTION MANIFOLD – A manual vacuum manifold with large volume sampler for cartridge extractions, or an automatic/robotic sample preparation system designed for use with SPE cartridges, may be used if all QC requirements discussed in Section 9 are met. Extraction and/or elution steps may not be changed or omitted to accommodate the use of an automated system. Care must be taken with automated SPE systems to ensure the PTFE commonly used in these systems does not contribute to unacceptable analyte concentrations in the MB (Sect. 9.1.1).

7.11.3 SAMPLE DELIVERY SYSTEM – Use of a polypropylene transfer tube system, which transfers the sample directly from the sample container to the SPE cartridge, is recommended, but not mandatory. Standard extraction manifolds come equipped with PTFE transfer tube systems. These can be replaced with 1/8” O.D. x 1/16” I.D. polypropylene or polyethylene tubing cut to an appropriate length to ensure no sample contamination from the sample transfer lines. Other types of non-PTFE tubing may be used provided it meets the MB (Sect. 9.1.1) and LCS (Sect. 9.2) QC requirements.

7.12 Extract Clean-up Cartridge – 250 mg 6ml SPE Cartridge containing graphitized polymer carbon.

7.13 EXTRACT CONCENTRATION SYSTEM – Extracts are concentrated by evaporation with nitrogen using a water bath set no higher than 65 °C.

7.14 LABORATORY OR ASPIRATOR VACUUM SYSTEM – Sufficient capacity to maintain a vacuum of approximately 10 to 15 inches of mercury for extraction cartridges.

7.15 LIQUID CHROMATOGRAPHY (LC)/TANDEM MASS SPECTROMETER (MS/MS) WITH DATA SYSTEM

7.15.1 LC SYSTEM – Instrument capable of reproducibly injecting up to 10-µL aliquots, and performing binary linear gradients at a constant flow rate near the flow rate used for development of this method (0.4 mL/min). The LC must be capable of pumping the water/methanol mobile phase without the use of a degasser which pulls vacuum on the mobile phase bottle (other types of degassers are acceptable). Degassers which pull vacuum on the mobile phase bottle will volatilize the ammonium acetate mobile phase causing the analyte peaks to shift to earlier retention times over the course of the analysis batch. The usage of a column heater is optional.

7.15.2 LC/TANDEM MASS SPECTROMETER – The LC/MS/MS must be capable of negative ion electrospray ionization (ESI) near the suggested LC flow rate of 0.4 mL/min. The system must be capable of performing MS/MS to produce unique product ions for the method analytes within specified retention time segments. A minimum of 10 scans across the chromatographic peak is required to ensure adequate precision.

7.15.3 DATA SYSTEM – An interfaced data system is required to acquire, store, reduce, and output mass spectral data. The computer software should have the capability of processing stored LC/MS/MS data by recognizing an LC peak within any given retention time window. The software must allow integration of the ion abundance of any specific ion within specified time or scan number limits. The software must be able to calculate relative response factors, construct linear regressions or quadratic calibration curves, and calculate analyte concentrations.

7.15.4 ANALYTICAL COLUMN – An LC BEH C_{18} column (2.1 x 50 mm) packed with 1.7 µm d_p C_{18} solid phase particles was used. Any column that provides adequate resolution, peak shape, capacity, accuracy, and precision (Sect. 9) may be used.

8. Reagents and Standards

8.1 GASES, REAGENTS, AND SOLVENTS – Reagent grade or better chemicals must be used.

8.1.1 REAGENT WATER – Purified water which does not contain any measurable quantities of any method analytes or interfering compounds greater than 1/3 the RL for each method analyte of interest. Prior to daily use, at least 3 L of reagent water should be flushed from the purification system to rinse out any build-up of analytes in the system's tubing.

8.1.2 METHANOL (CH_3OH, CAS#: 67-56-1) – High purity, demonstrated to be free of analytes and interferences.

8.1.3 AMMONIUM ACETATE ($NH_4C_2H_3O_2$, CAS#: 631-61-8) – High purity, demonstrated to be free of analytes and interferences.

8.1.4 ACETIC ACID (H_3CCOOH, CAS#: 64-19-7) - High purity, demonstrated to be free of analytes and interferences.

8.1.5 1M AMMONIUM ACETATE/REAGENT WATER – High purity, demonstrated to be free of analytes and interferences.

8.1.6 2mM AMMONIUM ACETATE/METHANOL:WATER (5:95) – To prepare, mix 2 ml of 1M AMMONIUM ACETATE,1 ml ACETIC ACID and 50 ml METHANOL into I Liter of REAGENT WATER.

8.1.7 Methanol/Water (80:20) – To prepare a 1 Liter bottle, mix 200 ml of REAGENT WATER with 800 ml of METHANOL.

8.1.8 AMMONIUM HYDROXIDE (NH_3, CAS#: 1336-21-6) – High purity, demonstrated to be free of analytes and interferences.

8.1.9 Sodium Acetate ($NaOOCCH_3$, CAS#: 127-09-3) – High purity, demonstrated to be free of analytes and interferences.

8.1.10 25 mM Sodium Acetate Buffer – To prepare 250mls, dissolve .625 grams of sodium acetate into 100 mls of reagent water. Add 4 mls Acetic Acid and adjust the final volume to 250 mls with reagent water.

8.1.11 NITROGEN – Used for the following purposes: Nitrogen aids in aerosol generation of the ESI liquid spray and is used as collision gas in some MS/MS instruments. The nitrogen used should meet or exceed instrument manufacturer's specifications. In addition, Nitrogen is used to concentrate sample extracts (Ultra High Purity or equivalent).

8.1.12 ARGON – Used as collision gas in MS/MS instruments. Argon should meet or exceed instrument manufacturer's specifications. Nitrogen gas may be used as the collision gas provided sufficient sensitivity (product ion formation) is achieved.

8.2 STANDARD SOLUTIONS – When a compound purity is assayed to be 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. PFAS analyte and IS standards commercially purchased in glass ampoules are acceptable; however, all subsequent transfers or dilutions performed by the analyst must be prepared and stored in polypropylene containers. Standards for sample fortification generally should be prepared in the smallest volume that can be accurately measured to minimize the addition of excess organic solvent to aqueous samples.

NOTE: Stock standards and diluted stock standards are stored at ≤4 °C.

8.2.1 ISOTOPE DILUTION Extracted Internal Standard (ID EIS) STOCK SOLUTIONS - ID EIS stock standard solutions are stable for at least 6 months when stored at 4 °C. The stock solution is purchased at a concentration of 1000 ng/mL.

8.2.2 ISOTOPE DILUTION Extracted Internal Standard PRIMARY DILUTION STANDARD (ID EIS PDS) – Prepare the ID EIS PDS at a concentration of 500 ng/mL. The ID PDS is prepared in methanol. The ID PDS is stable for 1 year when stored at ≤4 °C (table 2a).

Table 2a

Isotope Labeled Standard	Conc. of EIS Stock (ng/mL)	Vol. of EIS Stock (mL)	Final Vol. of EIS PDS (mL)	Final Conc. of EIS PDS (ng/mL)
M4PFBA	1000	1.0	2.0	500
M5PFPeA	1000	1.0	2.0	500
M5PFHxA	1000	1.0	2.0	500
M4PFHpA	1000	1.0	2.0	500
M8PFOA	1000	1.0	2.0	500
M9PFNA	1000	1.0	2.0	500
M6PFDA	1000	1.0	2.0	500
M7PFUdA	1000	1.0	2.0	500
MPFDoA	1000	1.0	2.0	500
M2PFTeDA	1000	1.0	2.0	500
M2PFHxDA	50,000	.02	2.0	500
M8FOSA	1000	1.0	2.0	500
d3-N-MeFOSAA	1000	1.0	2.0	500
d5-N-EtFOSAA	1000	1.0	2.0	500
M3PFBS	929	1.0	2.0	464.5
M3PFHxS	946	1.0	2.0	473
M8PFOS	957	1.0	2.0	478.5
M2-4:2FTS	935	1.0	2.0	467.5
M2-6:2FTS	949	1.0	2.0	474.5
M2-8:2FTS	958	1.0	2.0	479
M2,D4-10:2FTS	50,000	.04	2.0	1000
M3HFPO-DA	50,000	.4	2.0	10,000

Table 2b

Isotope Labeled Standard	Conc. of EIS Stock (ng/mL)	Vol. of EIS Stock (mL)	Final Vol. of EIS PDS (mL)	Final Conc. of EIS PDS (ng/mL)
d3-N-MeFOSA	50,000	.2	2.0	5000
d5-N-EtFOSA	50,000	.2	2.0	5000
d7-N-MeFOSE	50,000	.2	2.0	5000
d9-N-EtFOSE	50,000	.2	2.0	5000

8.2.3 ANALYTE STOCK STANDARD SOLUTION – Analyte stock standards are stable for at 1 year when stored at 4 °C. When using these stock standards to prepare a PDS, care must be taken to ensure that these standards are at room temperature and adequately vortexed.

8.2.4 Analyte Secondary Spiking Standard Prepare the spiking solution of additional add on components for project specific requirements only. ANALYTE PRIMARY SPIKING STANDARD – Prepare the spiking standard at a concentration of 500 ng/mL in methanol. The spiking standard is stable for at least two months when stored in polypropylene centrifuge tubes at room temperature.

Table 3

Analyte	Conc. of Stock (ng/mL)	Vol. of Stock (mL)	Final Vol. of PDS (mL)	Final Conc. of PDS (ng/mL)
PFBA	1000	1	2	500
PFPeA	1000	1	2	500
PFHxA	1000	1	2	500
PFHpA	1000	1	2	500
PFOA	1000	1	2	500
PFNA	1000	1	2	500
PFDA	1000	1	2	500
PFUnA	1000	1	2	500
PFDoA	1000	1	2	500
PFTrDA	1000	1	2	500
PFTA	1000	1	2	500
FOSA	1000	1	2	500
Br-NMeFOSAA	240	1	2	500
L-NMeFOSAA	760	1	2	500
Br-NEtFOSAA	225	1	2	500
L-NEtFOSAA	775	1	2	500
L-PFBS	887	1	2	443.5
L-PFPeS	941	1	2	470.5
L-PFHxSK	741	1	2	370.5
Br-PFHxSK	173	1	2	86.5
L-PFHpS	953	1	2	476.5
L-PFOSK	732	1	2	366
Br-PFOSK	196	1	2	98
L-PFNS	962	1	2	481
L-PFDS	965	1	2	482.5
4:2FTS	937	1	2	468.5
6:2FTS	951	1	2	475.5
8:2FTS	960	1	2	480
9ClPF3ONS	933	1	2	466.5
11ClPF3OUdS	943	1	2	471.5
ADONA	945	1	2	472.5
HFPO-DA	1000	1	2	500

8.2.5 Analyte Secondary Spiking Standard Prepare the spiking solution of additional add on components for project specific requirements only.

Table 4

Analyte	Conc. of IS Stock (ng/mL)	Vol. of IS Stock (mL)	Final Vol. of IS PDS (mL)	Final Conc. of IS PDS (ng/mL)
PFHxDA	50,000	0.04	4	500
PFODA	50,000	0.04	4	500
HFPO-DA	100,000	0.04	4	9500
10:2-FTS	48,300	0.04	4	482.3
PFDoS	48,400	0.04	4	484.1
PFPrS	45,800	0.04	4	457.8
PFMPA	50,000	0.04	4	500
PFMBA	50,000	0.04	4	500
PFEESA	44,500	0.04	4	444.8
NFDHA	50,000	0.04	4	500
NMeFOSA	50,000	0.4	4	5000
NMeFOSE	50,000	0.4	4	5000
NEtFOSA	50,000	0.4	4	5000
NEtFOSE	50,000	0.4	4	5000

8.2.6 LOW, MEDIUM AND HIGH LEVEL LCS – The LCS's will be prepared at the following concentrations and rotated per batch; 2 ng/L, 40 ng/L, 500 ng/l for drinking waters. The analyte PDS contains all the method analytes of interest at various concentrations in methanol. The analyte PDS has been shown to be stable for six months when stored at ≤4 °C.

8.2.7 Isotope Dilution Labeled Recovery Stock Solutions (ID REC) – ID REC Stock solutions are stable for at least 1 year when stored at 4 °C. The stock solution is purchased at a concentration of 2000 ng/mL.

8.2.8 Isotope Dilution Labeled Recovery Primary Dilution Standard (ID REC PDS) - Prepare the ID REC PDS at a concentration of 500 ng/mL. The ID REC PDS is prepared in methanol. The ID REC PDS is stable for at least 1 year when stored in polypropylene centrifuge tubes at ≤4 °C.

Table 5

Analyte	**Conc. of REC Stock (ng/mL)**	**Vol. of REC Stock (mL)**	**Final Vol. of REC PDS (mL)**	**Final Conc. of REC PDS (ng/mL)**
M2PFOA	2000	1	4	500
M2PFDA	2000	1	4	500
M3PFBA	2000	1	4	500
M4PFOS	2000	1	4	500

8.2.9 CALIBRATION STANDARDS (CAL) –

Current Concentrations (ng/mL): 0.5, 1.0, 5.0, 10.0, 50.0, 125, 150, 250, 500

Prepare the CAL standards over the concentration range of interest from dilutions of the analyte PDS in methanol containing 20% reagent water. 20 µl of the EIS PDS and REC PDS are added to the CAL standards to give a constant concentration of 10 ng/ml. The lowest concentration CAL standard must be at or

below the RL (2 ng/L), which may depend on system sensitivity. The CAL standards may also be used as CCVs (Sect. 9.8). To make calibration stock standards:

Table 6

Calibration Standard Concentration	Final Aqueous Cal STD Level Concentration	Final Soil Cal STD Level Concentration	30 compound stock added (ul)	Individual analyte Stocks added (ul)	500 ng/ml dilution added (ul)	Final Volume in MeOH/H_2O (82:20)
.5 ng/ml	2 ng/L	.25 ng/g	6.25		25	25 mls
1 ng/ml	4 ng/L	.5 ng/g	5		20	10 mls
5 ng/ml	20 ng/L	1 ng/g	25		100	10 mls
10 ng/ml	40 ng/L	5 ng/g	125	5		25 mls
50 ng/ml	200 ng/L	25 ng/g	250	10		10 mls
125 ng/ml	500 ng/L	62.5 ng/g	625	25		10 mls
150 ng/ml	600 ng/L	75 ng/g	750	30		10 mls
250 ng/ml	1000 ng/L	125 ng/g	625			5 mls
500 ng/ml	2000 ng/L	250 ng/g	1250			5 mls

9. Quality Control

The laboratory must maintain records to document the quality of data that is generated. Ongoing data quality checks are compared with established performance criteria to determine if the results of analyses meet the performance characteristics of the method.

9.1 Blank(s)

9.1.1 METHOD BLANK (MB) - A Method Blank (MB) is required with each extraction batch to confirm that potential background contaminants are not interfering with the identification or quantitation of method analytes. Prep and analyze a MB for every 20 samples. If the MB produces a peak within the retention time window of any analyte that would prevent the determination of that analyte, determine the source of contamination, and eliminate the interference before processing samples. Background contamination must be reduced to an acceptable level before proceeding. Background from method analytes or other contaminants that interfere with the measurement of method analytes must be below the RL. If the method analytes are detected in the MB at concentrations equal to or greater than this level, then all data for the problem analyte(s) must be considered invalid for all samples in the extraction batch. Because background contamination is a significant problem for several method analytes, it is highly recommended that the analyst maintains a historical record of MB data.

9.1.2 FIELD REAGENT BLANK (FRB) - The purpose of the FRB is to ensure that PFAS's measured in the Field Samples were not inadvertently introduced into the sample during sample collection/handling. Analysis of the FRB is required only if a Field Sample contains a method analyte or analytes at or above the RL. The FRB is processed, extracted and analyzed in exactly the same manner as a Field Sample.

9.2 Laboratory Control Sample (LCS) and Laboratory Control Sample Duplicates (LCSD)

9.2.1 An LCS is required with each extraction batch. The fortified concentration of the LCS may be rotated between low, medium, and high concentrations from batch to batch. Default limits of 50-150% of the true value may be used for analytes until sufficient replicates have been analyzed to generate proper control limits. Calculate the percent recovery (%*R*) for each analyte using the equation:

$$\%R = \frac{A \times 100}{B}$$

Where:

A = measured concentration in the fortified sample
B =fortification concentration.

9.2.2 Where applicable, in the absence of additional sample volume required to perform matrix specific QC, LCSD's are to be extracted and analyzed. The concentration and analyte recovery criteria for the LCSD must be the same as the batch LCS The RSD's must fall within ≤30% of the true value for medium and high level replicates, and ≤50% for low level replicates. Calculate the relative percent difference (RPD) for duplicate MSs (MS and MSD) using the equation:

$$RPD = \frac{|LCS - LCSD|}{(LCS + LCSD) / 2} \times 100$$

9.2.3 If the LCS and or LCSD results do not meet these criteria for method analytes, then all data for the problem analyte(s) must be considered invalid for all samples in the extraction batch.

9.3 Labeled Recovery Standards (REC)

The analyst must monitor the peak areas of the REC(s) in all injections during each analysis day.

9.4 Extracted Internal Standards (EIS)

9.4.1 The EIS standard is fortified into all samples, CCVs, MBs, LCSs, MSs, MSDs, FD, and FRB prior to extraction. It is also added to the CAL standards. The EIS is a means of assessing method performance from extraction to final chromatographic measurement. Calculate the recovery (%R) for the EIS using the following equation:

$$\%R = (A / B) \times 100$$

Where:

A = calculated EIS concentration for the QC or Field Sample
B = fortified concentration of the EIS.

9.4.2 Default limits of 50-150% may be used for analytes until sufficient replicates have been analyzed to generate proper control limits. A low or high percent recovery for a sample, blank, or CCV does not require discarding the analytical data but it may indicate a potential problem with future analytical data. When EIS recovery from a sample, blank, or CCV are outside control limits, check 1) calculations to locate possible errors, 2) standard solutions for degradation, 3) contamination, and 4) instrument performance. For CCVs and QC elements spiked with all target analytes, if the recovery of the corresponding target analytes meet the acceptance criteria for the EIS in question, the data can be used but all potential biases in the recovery of the EIS must be documented in the sample report. If the associated target analytes do not meet the acceptance criteria, the data must be reanalyzed.

9.5 Matrix Spike (MS)

9.5.1 Analysis of an MS is required in each extraction batch and is used to determine that the sample matrix does not adversely affect method accuracy. Assessment of method precision is accomplished by analysis of a Field Duplicate (FD) (Sect. 9.6); however, infrequent occurrence of method analytes would hinder this assessment. If the occurrence of method analytes in the samples is infrequent, or if historical trends are unavailable, a second MS, or MSD, must be prepared, extracted, and analyzed from a duplicate of the Field Sample. Extraction batches that contain MSDs will not require the extraction of a field sample duplicate. If a variety of different sample matrices are analyzed regularly, for example, drinking water from groundwater and surface water sources, method performance should be established for each. Over time, MS data should be documented by the laboratory for all routine sample sources.

9.5.2 Within each extraction batch, a minimum of one Field Sample is fortified as an MS for every 20 Field Samples analyzed. The MS is prepared by spiking a sample with an appropriate amount of the Analyte Stock Standard (Sect. 8.2.3). Use historical data and rotate through the low, mid and high concentrations when selecting a fortifying concentration. Calculate the percent recovery (%*R*) for each analyte using the equation:

$$\%R = \frac{(A - B)}{C} \times 100$$

Where:

A = measured concentration in the fortified sample
B = measured concentration in the unfortified sample
C = fortification concentration.

9.5.3 Analyte recoveries may exhibit matrix bias. For samples fortified at or above their native concentration, recoveries should range between 50-150%. If the accuracy of any analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control in the LCS, the recovery is judged to be matrix biased. The result for that analyte in the unfortified sample is labeled suspect/matrix to inform the data user that the results are suspect due to matrix effects.

9.6 Laboratory Duplicate

9.6.1 FIELD DUPLICATE OR LABORATORY FORTIFIED SAMPLE MATRIX DUPLICATE (FD or MSD) – Within each extraction batch (not to exceed 20 Field Samples), a minimum of one FD or MSD must be analyzed. Duplicates check the precision associated with sample collection, preservation, storage, and laboratory procedures. If method analytes are not routinely observed in Field Samples, an MSD should be analyzed rather than an FD.

9.6.2 Calculate the relative percent difference (*RPD*) for duplicate measurements (*FD1* and *FD2*) using the equation:

$$RPD = \frac{|FD1 - FD2|}{(FD1 + FD2) / 2} \times 100$$

9.6.3 RPDs for FDs should be ≤30%. Greater variability may be observed when FDs have analyte concentrations that are within a factor of 2 of the RL. At these concentrations, FDs should have RPDs that are ≤50%. If the RPD of any analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control in the CCV, the recovery is judged to be matrix biased. The result for that analyte in the unfortified sample is labeled suspect/matrix to inform the data user that the results are suspect due to matrix effects.

9.6.4 If an MSD is analyzed instead of a FD, calculate the relative percent difference (RPD) for duplicate MSs (MS and MSD) using the equation:

$$RPD = \frac{|MS - MSD|}{(MS + MSD) / 2} \times 100$$

9.6.5 RPDs for duplicate MSs should be ≤30% for samples fortified at or above their native concentration. Greater variability may be observed when MSs are fortified at analyte concentrations that are within a factor of 2 of the RL. MSs fortified at these concentrations should have RPDs that are ≤50% for samples fortified at or above their native concentration. If the RPD of any analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control in the LCSD where applicable, the result is judged to be matrix biased. If no LCSD is present, the associated MS and MSD are to be re-analyzed to determine if any analytical has occurred. If the resulting RPDs are still outside control limits, the result for that analyte in the unfortified sample is labeled suspect/matrix to inform the data user that the results are suspect due to matrix effects.

9.7 Initial Calibration Verification (ICV)

9.7.1 After each ICAL, analyze a QCS sample from a source different from the source of the CAL standards. If a second vendor is not available, then a different lot of the standard should be used. The QCS should be prepared and analyzed just like a CCV. Acceptance criteria for the QCS are identical to the CCVs; the calculated amount for each analyte must be ± 30% of the expected value. If measured analyte concentrations are not of acceptable accuracy, check the entire analytical procedure to locate and correct the problem.

9.8 Continuing Calibration Verification (CCV)

9.8.1 CCV Standards are analyzed at the beginning of each analysis batch, after every 10 Field Samples, and at the end of the analysis batch. See Section 10.11 for concentration requirements and acceptance criteria.

9.9 Method-specific Quality Control Samples

None

9.10 Method Sequence

- CCV-LOW
- MB
- LCS
- LCSD (where applicable)
- MS
- Duplicate or MSD
- Field Samples (1-10)
- CCV-MID
- Field Samples (11-20)
- CCV-LOW

10. Procedure

10.1 Equipment Set-up

10.1.1 This procedure may be performed manually or in an automated mode using a robotic or automatic sample preparation device. If an automated system is used to prepare samples, follow the manufacturer's operating instructions, but all extraction and elution steps must be the same as in the manual procedure. Extraction and/or elution steps may not be changed or omitted to accommodate the use of an automated system. If an automated system is used, the MBs should be rotated among the ports to ensure that all the valves and tubing meet the MB requirements (Sect. 9.1).

10.1.2 Some of the PFAS's adsorb to surfaces, including polypropylene. Therefore, the aqueous sample bottles must be rinsed with the elution solvent (Sect 10.3.4) whether extractions are performed manually or by automation. The bottle rinse is passed through the cartridge to elute the method analytes and is then collected (Sect. 10.3.4).

10.1.3 **NOTE:** The SPE cartridges and sample bottles described in this section are designed as single use items and should be discarded after use. They may not be refurbished for reuse in subsequent analyses.

10.2 Sample Preparation and Extraction of Aqueous Samples

10.2.1 Samples are preserved, collected, and stored as presented in Section 6.

The entire sample that is received must be sent through the SPE cartridge. In addition, the bottle must be solvent rinsed and this rinse must be sent through the SPE cartridge as well. The method blank (MB) and laboratory control sample (LCS) must be extracted in the same manner (i.e., must include the bottle solvent rinse). It should be noted that a water rinse alone is not sufficient. This does not apply to samples with high concentrations of PFAS that are prepared using serial dilution and not SPE.

10.2.2 Determine sample volume. Weigh all samples to the nearest 1g. If visible sediment is present, centrifuge and decant into a new 250mL HDPE bottle and record the weight of the new container.

NOTE: Some of the PFAS's adsorb to surfaces, thus the sample volume may NOT be transferred to a graduated cylinder for volume measurement.

10.2.3 The MB, LCS and FRB may be prepared by measuring 250 mL of reagent water with a polypropylene graduated cylinder or filling a 250-mL sample bottle to near the top.

10.2.4 Adjust the QC and sample pH to 3 by adding acetic acid in water dropwise.

10.2.5 Add 20 µL of the EIS PDS (Sect. 8.2.2) to each sample and QC, cap and invert to mix.

10.2.6 If the sample is an LCS, LCSD, MS, or MSD, add the necessary amount of analyte PDS (Sect. 8.2.4). Cap and invert each sample to mix.

10.3 Cartridge SPE Procedure

10.3.1 CARTRIDGE CLEAN-UP AND CONDITIONING – DO NOT allow cartridge packing material to go dry during any of the conditioning steps. Rinse each cartridge with 3 X 5 mL of 2% ammonium hydroxide in methanol, followed by 5mls of methanol. Next, rinse each cartridge with 5 mls of the 25 mM acetate buffer, followed by 15 mL of reagent water, without allowing the water to drop below the top edge of the packing. If the cartridge goes dry during the conditioning phase, the conditioning must be started over. Add 4-5 mL of reagent water to each cartridge, attach the sample transfer tubes (Sect. 7.11.3), turn on the vacuum, and begin adding sample to the cartridge.

10.3.2 SAMPLE EXTRACTON – Adjust the vacuum so that the approximate flow rate is approximately 4 mL/min. Do not allow the cartridge to go dry before all the sample has passed through.

10.3.3 SAMPLE BOTTLE AND CARTRIDGE RINSE – After the entire sample has passed through the cartridge, rinse the sample bottles with 4 ml reagent water followed by 4 ml 25 mM acetate buffer at pH 4 and draw the aliquot through the sample transfer tubes and the cartridges. Draw air or nitrogen through the cartridge for 5-10 min at high vacuum (10-15 in. Hg). NOTE: If empty plastic reservoirs are used in place of the sample transfer tubes to pass the samples through the cartridges, these reservoirs must be treated like the transfer tubes. After the entire sample has passed through the cartridge, the reservoirs must be rinsed to waste with reagent water.

10.3.4 SAMPLE BOTTLE AND CARTRIDGE ELUTION, Fraction 1 – Turn off and release the vacuum. Lift the extraction manifold top and insert a rack with collection tubes into the extraction tank to collect the extracts as they are eluted from the cartridges. Rinse the sample bottles with 12 mls of methanol and draw the aliquot through the sample transfer tubes and cartridges. Use a low vacuum such that the solvent exits the cartridge in a dropwise fashion.

NOTE: Due to the possible volatility and suspect degradation of sulfonamides and sulfonamide ethanols when exposed to heat, a portion of the methanol fraction may be retained and analyzed independently with no evaporation for these analytes.

SAMPLE BOTTLE AND CARTRIDGE ELUTION, Fraction 2 In a separate collection vial, rinse the sample bottles with 12 mL of 2% ammonium hydroxide in methanol and elute the analytes from the cartridges by pulling the 4 mL of methanol through the sample transfer tubes and the cartridges. Use a low vacuum such that the solvent exits the cartridge in a dropwise fashion.

NOTE: If empty plastic reservoirs are used in place of the sample transfer tubes to pass the samples through the cartridges, these reservoirs must be treated like the transfer tubes. After the reservoirs have been rinsed in Section 10.3.3, the elution solvent used to rinse the sample bottles must be swirled down the sides of the reservoirs while eluting the cartridge to ensure that any method analytes on the surface of the reservoirs are transferred to the extract.

CLEAN-UP CARTRIDGE ELUTION, Elute the clean-up cartridge with 8 additional mls of methanol and draw the aliquot through the cartridge. Use a low vacuum such that the solvent exits the cartridge in a dropwise fashion.

10.3.5 Fractions 1 and 2 are to be combined during the concentration stage (section10.8).

10.4 Sample Prep and Extraction Protocol for Soils, Solids and Sediments.

10.4.1 Homogenize and weigh 4 grams of sample (measured to the nearest hundredth of a gram) into a 50 ml polypropylene centrifuge tube. For laboratory control blanks and spikes, 4 grams of clean sand is used.

10.4.2 Add 40 µL of the EIS PDS (Sect. 8.2.2) to each sample and QC.

10.4.3 If the sample is an LCS, LCSD, MS, or MSD, add the necessary amount of analyte PDS (Sect. 8.2.6). Cap and invert each sample to mix.

10.4.4 To all samples, add 10 mls of methanol, cap, vortex for 25 seconds at 2500 RPM.

10.4.5 Following mixing, sonicate each sample for 30 minutes and let samples sit overnight (at least 2 hours is required for RUSH samples).

10.4.6 Centrifuge each sample at 3500RPM for 10 minutes.

10.4.7 Remove 5ml of supernatant, and reserve for clean-up.

10.5 Sample Prep and Extraction Protocol for Tissues, Oils and Biosolids.

10.5.1 Homogenize and weigh 2-8 grams of sample (measured to the nearest hundredth of a gram) into a 50 ml polypropylene centrifuge tube. For laboratory control blanks and spikes, 4 grams of clean sand is used.

10.5.2 Add 40 µL of the EIS PDS (Sect. 8.2.2) to each sample and QC.

10.5.3 If the sample is an LCS, LCSD, MS, or MSD, add the necessary amount of analyte PDS (Sect. 8.2.6). Cap and invert each sample to mix.

10.5.4 Add 100 ul of Ammonium Hydroxide.

10.5.5 To all samples, add 10 mls of methanol, cap, vortex for 25-30 seconds at 2500 RPM.

10.5.6 Following mixing, sonicate each sample for 30 minutes and let samples sit for 2 hours.

10.5.7 Centrifuge each sample at 3500RPM for 10 minutes.

10.5.8 Remove 5 mls of the supernatant, and reserve for clean-up.

10.6 Extract Clean-up: Soils, Solids and Sediment Matrices

10.6.1 CARTRIDGE CLEAN-UP AND CONDITIONING –. Rinse each cartridge with 15 mL of methanol and discard. If the cartridge goes dry during the conditioning phase, the conditioning must be started over. Attach the sample transfer tubes (Sect. 7.11.3), turn on the vacuum, and begin adding sample to the cartridge. For Soils extracts, transfer 5 mls of the MeOH eluate to the cartridge. Samples should be allowed to pass through the cartridge by gravity feed at a dropwise rate to ensure adequate contact time with the cartridge sorbent. Vacuum is only to applied if the flow of solvent through the cartridge stops.

10.6.2 Adjust the vacuum so that the approximate flow rate is 1-2 mL/min. Do not allow the cartridge to go dry before all the sample has passed through.

10.6.3 SAMPLE BOTTLE AND CARTRIDGE RINSE – After the entire sample has passed through the cartridge, rinse the sample collection vial with two 4-mL aliquots of methanol and draw each aliquot through the cartridges. Draw air or nitrogen through the cartridge for 5 min at high vacuum (10-15 in. Hg).

10.6.4 If extracts are not to be immediately evaporated, cover collection tubes and store at ambient temperature till concentration.

NOTE: Due to the possible volatility and suspect degradation of sulfonamides and sulfonamide ethanols when exposed to heat, a portion of the eluate may be retained and analyzed independently with no evaporation for these analytes.

10.7 Extract Clean-up: Tissues, Oils and Biosolids

10.7.1 CARTRIDGE CLEAN-UP AND CONDITIONING –. Stack a 500 mg WAX cartridge onto a 250 mg GCB cartridge. Rinse each cartridge set with 10 mL of 2% NH_4OH and discard. Immediately rinse each cartridge stack with 15 mls MeOH and discard, If the cartridge goes dry during the conditioning phase, the conditioning must be started over. Attach the sample transfer tubes (Sect. 7.11.3), turn on the vacuum.

10.7.2 Adjust the vacuum so that the approximate flow rate is 1-2 mL/min. Do not allow the cartridge to go dry before all the sample has passed through.

10.7.3 SAMPLE elution AND CARTRIDGE RINSE – Load 5 mls of the MeOH sample extract to the cartridge. After the entire sample has passed through the cartridge, rinse the cartridges with 5-mLs of methanol and draw through the cartridges. Immediately add and elute 2 5ml aliquots of 2% NH_4OH to the cartridges, collecting the eluate with the MeOH eluate.

If extracts are not to be immediately evaporated, cover collection tubes and store at ambient temperature till concentration.

NOTE: Due to the possible volatility and suspect degradation of sulfonamides and sulfonamide ethanols when exposed to heat, a portion of the eluate may be retained and analyzed independently with no evaporation for these analytes.

10.8 Extract Concentration

10.8.1 Concentrate the extract to dryness under a gentle stream of nitrogen in a heated water bath (60-65 °C) to remove all the water/methanol mix. Add the appropriate amount of 80:20% (vol/vol) methanol:water solution and 20 µl of the ID REC PDS (Sect. 8.2.8) to the collection vial to bring the volume to 1 mL and vortex. Transfer two aliquots with a plastic pipet (Sect. 7.8) into 2 polypropylene autosampler vials.

NOTE: It is recommended that the entire 1-mL aliquot not be transferred to the autosampler vial because the polypropylene autosampler caps do not reseal after injection. Therefore, do not store the extracts in the autosampler vials as evaporation losses can occur occasionally in these autosampler vials. Extracts can be split between 2 X 700 µl vials (Sect. 7.5).

10.9 Sample Volume Determination

10.9.1 If the level of the sample was marked on the sample bottle, use a graduated cylinder to measure the volume of water required to fill the original sample bottle to the mark made prior to extraction. Determine to the nearest 10 mL.

10.9.2 If using weight to determine volume, weigh the empty bottle to the nearest 10 g and determine the sample weight by subtraction of the empty bottle weight from the original sample weight (Sect. 10.2.2). Assume a sample density of 1.0 g/mL. In either case, the sample volume will be used in the final calculations of the analyte concentration (Sect. 11.2).

10.10 Initial Calibration - Demonstration and documentation of acceptable initial calibration is required before any samples are analyzed. After the initial calibration is successful, a CCV is required at the beginning and end of each period in which analyses are performed, and after every tenth Field Sample.

10.10.1 ESI-MS/MS TUNE

10.10.1.1 Calibrate the mass scale of the MS with the calibration compounds and procedures prescribed by the manufacturer.

10.10.1.2 Optimize the [M-H]- for each method analyte by infusing approximately 0.5-1.0 µg/mL of each analyte (prepared in the initial mobile phase conditions) directly into the MS at the chosen LC mobile phase flow rate (approximately 0.4 mL/min). This tune can be done on a mix of the method analytes. The MS parameters (voltages, temperatures, gas flows, etc.) are varied until optimal analyte responses are determined. The method analytes may have different optima requiring some compromise between the optima.

10.10.1.3 Optimize the product ion for each analyte by infusing approximately 0.5-1.0 µg/mL of each analyte (prepared in the initial mobile phase conditions) directly into the MS at the chosen LC mobile phase flow rate (approximately 0.4 mL/min). This tune can be done on a mix of the method analytes. The MS/MS parameters (collision gas pressure,

collision energy, etc.) are varied until optimal analyte responses are determined. Typically, the carboxylic acids have very similar MS/MS conditions and the sulfonic acids have similar MS/MS conditions.

10.10.2 Establish LC operating parameters that optimize resolution and peak shape. Modifying the standard or extract composition to more aqueous content to prevent poor shape is not permitted.

Cautions: LC system components, as well as the mobile phase constituents, contain many of the method analytes in this method. Thus, these PFAS's will build up on the head of the LC column during mobile phase equilibration. To minimize the background PFAS peaks and to keep background levels constant, the time the LC column sits at initial conditions must be kept constant and as short as possible (while ensuring reproducible retention times). In addition, prior to daily use, flush the column with 100% methanol for at least 20 min before initiating a sequence. It may be necessary on some systems to flush other LC components such as wash syringes, sample needles or any other system components before daily use.

10.10.3 Inject (2µl for Sciex systems, 3µl for Waters systems, 20µl for MeOH fractions) a mid-level CAL standard under LC/MS conditions to obtain the retention times of each method analyte. If analyzing for PFTA, ensure that the LC conditions are adequate to prevent co-elution of PFTA and the mobile phase interferants. These interferants have the same precursor and products ions as PFTA, and under faster LC conditions may co-elute with PFTA. Divide the chromatogram into retention time windows each of which contains one or more chromatographic peaks. During MS/MS analysis, fragment a small number of selected precursor ions ([M-H]-) for the analytes in each window and choose the most abundant product ion. For maximum sensitivity, small mass windows of ±0.5 daltons around the product ion mass were used for quantitation.

10.10.4 Inject a mid-level CAL standard under optimized LC/MS/MS conditions to ensure that each method analyte is observed in its MS/MS window and that there are at least 10 scans across the peak for optimum precision.

NOTE: PFHxS, PFOS, NMeFOSAA, and NEtFOSAA have multiple chromatographic peaks using the LC conditions in Table 7 due to chromatographic resolution of the linear and branched isomers of these compounds. Most PFAS's are produced by two different processes. One process gives rise to linear PFAS's only while the other process produces both linear and branched isomers. Thus, both branched and linear PFAS's can potentially be found in the environment. For the aforementioned compounds that give rise to more than one peak, all the chromatographic peaks observed in the standard must be integrated and the areas totaled. Chromatographic peaks in a sample must be integrated in the same way as the CAL standard.

10.10.5 Prepare a set of CAL standards as described in Section 8.2.9. The lowest concentration CAL standard must be at or below the RL (2 ng/L), which may depend on system sensitivity.

10.10.6 The LC/MS/MS system is calibrated using the isotope dilution technique. Target analytes are quantitated against their isotopically labeled analog (Extracted Internal Standard) where commercially available. If a labeled analog is not commercially available, the extracted internal standard with the closest retention time and /or closest chemical similarity is to be used. Use the LC/MS/MS data

system software to generate a linear regression or quadratic calibration curve for each of the analytes. This curve must always be forced through zero and may be concentration weighted, if necessary. Forcing zero allows for a better estimate of the background levels of method analytes. A minimum of 5 levels are required for a linear calibration model and a minimum of 6 levels are required for a quadratic calibration model.

10.10.7 CALIBRATION ACCEPTANCE CRITERIA – A linear fit is acceptable if the coefficient of determination (r^2) is greater than 0.99. When quantitated using the initial calibration curve, each calibration point, except the lowest point, for each analyte must calculate to be within 70-130% of its true value. The lowest CAL point must calculate to be within 50-150% of its true value. If these criteria cannot be met, the analyst will have difficulty meeting ongoing QC criteria. It is recommended that corrective action is taken to reanalyze the CAL standards, restrict the range of calibration, or select an alternate method of calibration (forcing the curve through zero is still required).

10.10.7.1 CAUTION: When acquiring MS/MS data, LC operating conditions must be carefully reproduced for each analysis to provide reproducible retention times. If this is not done, the correct ions will not be monitored at the appropriate times. As a precautionary measure, the chromatographic peaks in each window must not elute too close to the edge of the segment time window.

10.11 CONTINUING CALIBRATION CHECK (CCV) – Minimum daily calibration verification is as follows. Verify the initial calibration at the beginning and end of each group of analyses, and after every tenth sample during analyses. In this context, a “sample” is considered to be a Field Sample. MBs, CCVs, LCSs, MSs, FDs FRBs and MSDs are not counted as samples. The beginning CCV of each analysis batch must be at or below the RL in order to verify instrument sensitivity prior to any analyses. If standards have been prepared such that all low CAL points are not in the same CAL solution, it may be necessary to analyze two CAL standards to meet this requirement. Alternatively, the analyte concentrations in the analyte PDS may be customized to meet these criteria. Subsequent CCVs should alternate between a medium and Low concentration CAL standard.

10.11.1 Inject an aliquot of the appropriate concentration CAL standard and analyze with the same conditions used during the initial calibration.

10.11.2 Calculate the concentration of each analyte and EIS in the CCV. The calculated amount for each analyte for medium level CCVs must be within ± 30% of the true value with an allowance of 10% of the reported analytes to be greater than 30%. The calculated amount for each EIS must be within ± 50% of the true value. The calculated amount for the lowest calibration point for each analyte must be within ± 50%. If these conditions do not exist, then all data for the problem analyte must be considered invalid, and remedial action should be taken which may require recalibration. Any Field or QC Samples that have been analyzed since the last acceptable calibration verification should be reanalyzed after adequate calibration has been restored, with the following exception. If the CCV fails because the calculated concentration is greater than 130% (150% for the low-level CCV) for a particular method analyte, and Field Sample extracts show no detection for that method analyte, non-detects may be reported without re-analysis.

10.11.3 REMEDIAL ACTION – Failure to meet CCV QC performance criteria may require remedial action. Major maintenance, such as cleaning the electrospray probe, atmospheric pressure ionization source, cleaning the mass analyzer, replacing the LC column, etc., requires recalibration (Sect 10.10) and verification of sensitivity by analyzing a CCV at or below the RL (Sect 10.11).

10.12 EXTRACT ANALYSIS

10.12.1 Establish operating conditions equivalent to those summarized in Tables 7-9 of Section 16.

10.12.2 Establish an appropriate retention time window for each analyte. This should be based on measurements of actual retention time variation for each method analyte in CAL standard solutions analyzed on the LC over the course of time. A value of plus or minus three times the standard deviation of the retention time obtained for each method analyte while establishing the initial calibration can be used to calculate a suggested window size. However, the experience of the analyst should weigh heavily on the determination of the appropriate retention window size.

10.12.3 Calibrate the system by either the analysis of a calibration curve (Sect. 10.10) or by confirming the initial calibration is still valid by analyzing a CCV as described in Section 10.11.

10.12.4 Begin analyzing Field Samples, including QC samples, at their appropriate frequency by injecting the same size aliquots under the same conditions used to analyze the CAL standards.

10.12.5 At the conclusion of data acquisition, use the same software that was used in the calibration procedure to identify peaks of interest in predetermined retention time windows. Use the data system software to examine the ion abundances of the peaks in the chromatogram. Identify an analyte by comparison of its retention time with that of the corresponding method analyte peak in a reference standard.

10.12.6 The analyst must not extrapolate beyond the established calibration range. If an analyte peak area exceeds the range of the initial calibration curve, the sample should be re-extracted with a reduced sample volume in order to bring the out of range target analytes into the calibration range. If a smaller sample size would not be representative of the entire sample, the following options are recommended. Re-extract an additional aliquot of sufficient size to insure that it is representative of the entire sample. Spike it with a higher concentration of internal standard. Prior to LC/MS analysis, dilute the sample so that it has a concentration of internal standard equivalent to that present in the calibration standard. Then, analyze the diluted extract.

11. Data Evaluation, Calculations and Reporting

11.1 Complete chromatographic resolution is not necessary for accurate and precise measurements of analyte concentrations using MS/MS. In validating this method, concentrations were calculated by measuring the product ions listed in Table 9.

11.2 Calculate analyte concentrations using the multipoint calibration established in Section 10.9. Do not use daily calibration verification data to quantitate analytes in samples. Adjust final analyte concentrations to reflect the actual sample volume determined in Section 10.8

C_{ex} = (Area of target analyte * Concentration of Labeled analog) / (area of labeled analog * CF)

C_s = (C_{ex} / sample volume in ml) * 1000

C_{ex} = The concentration of the analyte in the extract
CF = calibration factor from calibration.

11.3 Prior to reporting the data, the chromatogram should be reviewed for any incorrect peak identification or poor integration.

11.4 PFHxS, PFOS, PFOA, NMeFOSAA, and NEtFOSAA have multiple chromatographic peaks using the LC conditions in Table 7 due to the linear and branch isomers of these compounds (Sect. 10.10.4.). The areas of all the linear and branched isomer peaks observed in the CAL standards for each of these analytes must be summed and the concentrations reported as a total for each of these analytes.

11.5 Calculations must utilize all available digits of precision, but final reported concentrations should be rounded to an appropriate number of significant figures (one digit of uncertainty), typically two, and not more than three significant figures.

12. Contingencies for Handling Out-of-Control Data or Unacceptable Data

12.1 Section 9.0 outlines sample batch QC acceptance criteria. If non-compliant organic compound results are to be reported, the Organic Section Head and/or the Laboratory Director, and the Operations Manager must approve the reporting of these results. The laboratory Project Manager shall be notified and may choose to relay the non-compliance to the client, for approval, or other corrective action, such as re-sampling and re-analysis. The analyst, Data Reviewer, or Department Supervisor performing the secondary review initiates the project narrative, and the narrative must clearly document the non-compliance and provide a reason for acceptance of these results.

12.2 All results for the organic compounds of interest are reportable without qualification if extraction and analytical holding times are met, preservation requirements (including cooler temperatures) are met, all QC criteria are met, and matrix interference is not suspected during extraction or analysis of the samples. If any of the below QC parameters are not met, all associated samples must be evaluated for re-extraction and/or re-analysis.

13. Method Performance

13.1 Detection Limit Study (DL) / Limit of Detection Study (LOD) / Limit of Quantitation (LOQ)

13.1.1 The laboratory follows the procedure to determine the DL, LOD, and/or LOQ as outlined in Alpha SOP ID 1732. These studies performed by the laboratory are maintained on file for review.

13.2 Demonstration of Capability Studies

13.2.1 Refer to Alpha SOP ID 1739 for further information regarding IDC/DOC Generation.

13.2.2 The analyst must make a continuing, annual, demonstration of the ability to generate acceptable accuracy and precision with this method.

14. Pollution Prevention and Waste Management

14.1 Refer to Alpha's Chemical Hygiene Plan and Hazardous Waste Management and Disposal SOP for further pollution prevention and waste management information.

14.2 This method utilizes SPE to extract analytes from water. It requires the use of very small volumes of organic solvent and very small quantities of pure analytes, thereby minimizing the potential hazards to both the analyst and the environment as compared to the use of large volumes of organic solvents in conventional liquid-liquid extractions.

14.3 The analytical procedures described in this method generate relatively small amounts of waste since only small amounts of reagents and solvents are used. The matrices of concern are finished drinking water or source water. However, laboratory waste management practices must be conducted consistent with all applicable rules and regulations, and that laboratories protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Also, compliance is required with any sewage discharge permits and regulations, particularly the hazardous waste identification rules and land disposal restrictions.

15. Referenced Documents

Chemical Hygiene Plan – ID 2124

SOP ID 1732 Detection Limit (DL), Limit of Detection (LOD) & Limit of Quantitation (LOQ) SOP

SOP ID 1739 Demonstration of Capability (DOC) Generation SOP

SOP ID 1728 Hazardous Waste Management and Disposal SOP

16. Attachments

Table 7a: LC Method Conditions

Time (min)	2 mM Ammonium Acetate (5:95 MeOH/H_2O)	100% Methanol
Initial	100.0	0.0
1.0	100.0	0.0
2.2	85.0	15.0
11	20.0	80.0
11.4	0.0	100.0
12.4	100.0	00.0
15.5	100.0	0.0
Waters Aquity UPLC ® $BEHC_{18}$ 2.1 x 50 mm packed with 1.7 µm BEH C_{18} stationary phase Flow rate of 0.4 mL/min 3 µL injection		

Table 7b: LC Method Conditions (MeOH Fraction)

Time (min)	2 mM Ammonium Acetate (5:95 MeOH/H_2O)	100% Methanol
Initial	100.0	0.0
1.0	50.0	50.0
4.5	1.0	99.0
4.95	1.0	99.0
5.0	100.0	0.0
5.5	100.0	0.0
Waters Aquity UPLC ® BEHC_{18} 2.1 x 50 mm packed with 1.7 µm BEH C_{18} stationary phase Flow rate of 0.6 mL/min 20 µL injection		

Table 8: ESI-MS Method Conditions

ESI Conditions	
Polarity	Negative ion
Capillary needle voltage	.5 kV
Cone Gas Flow	25 L/hr
Nitrogen desolvation gas	1000 L/hr
Desolvation gas temp.	500 ºC

Table 9a: Method Analyte Source, Retention Times (RTs), and EIS References

#	Analyte	Transition	RT	IS	Type
1	M3PBA	216>171	2.65		REC
2	PFBA	213 > 169	2.65	3: M4PFBA	
3	M4PFBA	217 > 172	2.65	1: M3PBA	EIS
4	PFPeA	263 > 219	5.67	5: M5PFPEA	
5	M5PFPEA	268 > 223	5.66	1: M3PBA	EIS
6	PFBS	299 > 80	6.35	7: M3PFBS	
7	M3PFBS	302 > 80	6.35	29:M4PFOS	EIS
8	FTS 4:2	327 > 307	7.47	9: M2-4:2FTS	
9	M2-4:2FTS	329 > 81	7.47	29:M4PFOS	EIS
10	PFHxA	303 > 269	7.57	11: M5PFHxA	
11	M5PFHxA	318 > 273	7.57	19:M2PFOA	EIS
12	PFPeS	349 > 80	7.88	18: M3PFHxS	
13	PFHpA	363 > 319	8.80	14: M4PFHpA	
14	M4PFHpA	367 > 322	8.80	19:M2PFOA	EIS
15	L-PFHxS	399 > 80	8.94	18: M3PFHxS	
16	br-PFHxS	399 > 80	8.72	18: M3PFHxS	
17	PFHxS Total	399 > 80	8.94	18: M3PFHxS	
18	M3PFHxS	402 > 80	8.94	29:M4PFOS	EIS
19	M2PFOA	415 > 370	9.7		REC
20	PFOA	413 > 369	9.7	23: M8PFOA	
21	br-PFOA	413 > 369	9.48	23: M8PFOA	
22	PFOA Total	413 > 369	9.7	23: M8PFOA	
23	M8PFOA	421 > 376	9.7	19: M2PFOA	EIS
24	FTS 6:2	427 > 407	9.66	25: M2-6:2FTS	
25	M2-6:2FTS	429 > 409	9.66	29:M4PFOS	EIS
26	PFHpS	449 > 80	9.78	33: M8PFOS	
27	PFNA	463 > 419	10.41	33: M9PFNA	
28	M9PFNA	472 > 427	10.41	19: M2PFOA	EIS
29	M4PFOS	501 > 80	10.45		REC
30	PFOS	499 > 80	10.45	33: M8PFOS	
31	br-PFOS	499 > 80	10.27	33: M8PFOS	
32	PFOS Total	499 > 80	10.45	33: M8PFOS	
33	M8PFOS	507 > 80	10.45	29: M4PFOS	EIS
34	FTS 8:2	527 > 507	10.99	35: M2-8:2FTS	
35	M2-8:2FTS	529 > 509	10.99	29:M4PFOS	EIS
36	M2PFDA	515 > 470	11.00		REC
37	PFDA	513 > 469	11.00	38: M6PFDA	

#	Analyte	Transition	RT	IS	Type
38	M6PFDA	519 > 474	11.00	36: M2PFDA	EIS
39	PFNS	549 > 80	11.02	33:M8PFOS	
40	br-NMeFOSAA	570 > 419	11.41	41: D3-NMeFOSAA	
41	L-NMeFOSAA	570 > 419	11.41	41: D3-NMeFOSAA	
42	NMeFOSAA total	570 > 419	11.41	41: D3-NMeFOSAA	
43	d3-NMeFOSAA	573 > 419	11.41	36: M2PFOA	EIS
44	PFOSA	498 > 78	11.48	29: M8FOSA	
45	M8FOSA	506 > 78	11.48	19: M2PFOA	EIS
46	PFUnDA	563 > 519	11.51	41: M7-PFUDA	
47	M7-PFUDA	570 > 525	11.51	36: M2PFDA	EIS
48	PFDS	599 > 80	11.51	33:M8PFOS	
49	br-NEtFOSAA	584 > 419	11.68	48: d5-NEtFOSAA	
50	L-NEtFOSAA	584 > 419	11.68	48: d5-NEtFOSAA	
51	NEtFOSAA total	584 > 419	11.68	48: d5-NEtFOSAA	
52	d5-NEtFOSAA	589 > 419	11.68	36: M2PFOA	EIS
53	PFDoA	613 > 569	11.96	50: MPFDOA	
54	MPFDOA	615 > 570	11.96	36: M2PFDA	EIS
55	PFTriA	663 > 619	12.34	53: M2PFTEDA	
56	PFTeA	713 > 669	12.6	53: M2PFTEDA	
57	M2PFTEDA	715 > 670	12.6	36: M2PFDA	EIS
58	M3HFPO-DA	329>285	7.97	19: M2PFOA	EIS
59	HFPO-DA	332>287	7.97	54: M3HFPO-DA	
60	ADONA	377>251	8.00	23: M8PFOA	
61	PFHxDA	813>769	13.20	59: M2PFHxDA	
62	PFODA	913>869	13.50	59: M2PFHxDA	
63	M2PFHxDA	815>770	13.20	36:M2PFDA	EIS
64	NEtFOSA	526>169	11.00	61: d5-NEtFOSA	
65	NMeFOSA	512>169	10.50	63: d3-NMeFOSA	
66	d3-NMeFOSA	515>169	10.50	19: M2PFOA	EIS
67	d5-NEtFOSA	531>169	11.00	19: M2PFOA	EIS
68	NMeFOSE	556>122	11.25	66: d7-NMeFOSE	
69	NEtFOSE	570>136	10.75	67: d9-NEtFOSE	
70	d7-NMeFOSE	563>126	11.25	19: M2PFOA	EIS
71	d9-NEtFOSE	579>142	10.75	19: M2PFOA	EIS
72	FTS 10:2	627>607	11.50	25: M2-8:2FTS	
73	PFDoS	699>99	12.50	33: M8PFOS	
74	9ClPF3ONS	531>351	10.23	33: M8PFOS	
75	11ClPF3OUdS	631>451	11.27	33: M8PFOS	
76	PFPrS	249>80	3.40	7: M3PFBS	

#	Analyte	Transition	RT	IS	Type
77	PFMPA	229>85	3.39	1: M3PBA	
78	PFMBA	279 .85	5.75	1: M3PBA	
79	PFEESA	315>135	6.45	18: M3PFHxS	
80	NFDHA	295>210	6.79	11: M5PFHxA	

Table 9b: Methanol Analyte Source, Retention Times (RTs), and EIS References

#	Analyte	Transition	RT	IS	Type
1	M2PFNA	472 > 427	2.55		IS
2	M2PFUdA	213 > 169	2.87		IS
3	M8FOSA	217 > 172	2.86	1: M2PFNA	EIS
4 5	FOSA	263 > 219	2.86	3: M8FOSA	
6	D3-NMeFOSA	515>169	3.22	1: M2PFNA	EIS
7	NMeFOSA	512>169	3.22	6: d3-NMeFOSA	
8	D5-NEtFOSAA	531>169	3.41	1: M2PFNA	EIS
9	NEtFOSAA	526>169	3.41	8: d5-NEtFOSA	
10	D7-NMeFOSE	563>126	3.23	1: M2PFNA	EIS
11	NMeFOSE	556>122	3.23	10: d7-NMeFOSE	
12	D9-NEtFOSE	579>142	3.40	11: M2PFNA	EIS
13	NEtFOSE	570>136	3.40	67: d9-NEtFOSE	