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2nd Draft Method 1633

Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS

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2nd Draft of Method 1633
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Notice

This document represents a draft of a PFAS method currently under development by the EPA Office of Water, Engineering and Analysis Division (EAD), in conjunction with the Department of Defense (DoD). **This method is not required for Clean Water Act compliance monitoring until it has been proposed and promulgated through rulemaking.**

A single-laboratory validation of the procedure has been completed and the report on the results of that study is being prepared. Historically, EAD posts draft methods on the Clean Water Act website after the single-laboratory validation report is completed. However, due to a large number of public and stakeholder requests, this method is being posted on the web before the single-laboratory validation study report is finalized. A revision of this draft method with a later publication date may be issued at that time. No procedural changes are expected as a result of the single-laboratory validation, but some of the performance data (which are presented only as examples) may change once the statistical analysis of the single-laboratory validation data is completed.

This draft method has been subjected to multiple levels of review across several EPA Program Offices. DoD began a multi-laboratory validation study of the procedure in late 2021, in collaboration with the Office of Water and the Office of Land and Emergency Management.

The Office of Water will use the results of the multi-laboratory validation study to finalize the method and add formal performance criteria. The method validation process may eliminate some of the parameters listed in this draft method.

In September 2021, the Office of Water released a draft of the method on its web site and encouraged laboratories, regulatory authorities, and other interested parties to review the method, provide feedback and comments to the Office of Water, and where appropriate, utilize it for their own purposes, with the explicit understanding that method was a draft, subject to revision.

Partly as a result of such reviews and comments, as well as questions raised to DoD during the multi-laboratory validation study, the Office of Water addressed some errors and less-than-clear aspects of the method in an errata sheet that was posted on its web site. This 2nd draft of the method incorporates the items from that errata sheet and a few other changes into one document.

The changes are indicated by the combination of red font and yellow highlighting.

Acknowledgements

This draft method was prepared under the direction of Adrian Hanley of the Engineering and Analysis Division, Office of Science and Technology, within EPA's Office of Water, in collaboration with the Department of Defense.

EPA acknowledges the support of a number of organizations in the development and validation of this draft method, including the developers of the original procedure, the Department of Defense, the members of EPA's workgroup, and EPA's support contractor staff at General Dynamics Information Technology, including:

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Disclaimer

See the notice on the title page regarding the status of this method.

Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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Revision History

June 2022, 2nd Draft

This 2nd draft of Method 1633 incorporates the following changes presented in the errata sheet prepared by the Office of Water in October 2021 and updated in February 2022, along with other changes in response to questions raised during the early stages of the multi-laboratory validation study.

Location	Rationale for the change
4.2.2	Some SPE manifold components may be too large to fit in commonly used ultrasonic baths. Where size is not a concern, sonication is encouraged as an effective approach to cleaning these parts.
6.3.4	The description of the blade and shaft of the originally listed device is incorrect, it has a PTFE bearing that makes it inappropriate for use in a PFAS method. Use of the device itself is never called out in the procedure after this section. Therefore, the description was deleted, but the subsection number has been retained for the time being to avoid renumbering any subsequent sections until the final version of the method is prepared.
6.9.3	Since the initial release of the draft method, the supplier has discontinued the snap cap that was cited. The replacement shown is currently available and does not contain PTFE.
7.1.2	Erroneous description of the use of the solution was deleted.
7.1.9	Description of the use of the solution was added.
7.3.5	New "Note" regarding the standards was added.
7.4	The description of the mass calibration solution was made vendor agnostic in keeping with changes to Section 10.1.
7.5	Naming this solution the bile salt interference check standard simplifies later discussions of its use. The potential interference with PFOS from bile salts is affected by the mobile phase used for the LC separation and laboratories choosing to use a mobile phase other than that specified in the draft procedure need to make the adjustments described here. The concentration of the solution also has been lowered so it does not overwhelm the PFOS peak.
8.2.3	The change addresses the discrepancy between Sections 8.2 and 8.5.
8.3.2	The change addresses the discrepancy between Sections 8.2 and 8.5.
9.1.2.2 (c)	Section 1.6 does not exist in the draft method, and Section 1.5 is the correct citation.
9.2.2	Responds to questions about the example values in Table 6. Also see the discussion of Table 6 below.
10.1	A large portion of the description of the mass calibration process in Section 10.1 and all of its subsections was revised in response to comments from some of the laboratories in the validation study, relative to the existing mass calibration procedures from some instrument manufacturers. The changes make the procedure more vendor-agnostic, while still accomplishing the intended goal.
10.2.2.5	Naming this solution the bile salt interference check standard also simplifies discussions of its use. The potential interference with PFOS from bile salts is affected by the mobile phase used for the LC separation and laboratories choosing to use a mobile phase other than that specified in the draft procedure need to make the adjustments described here. Also added the requirement to run the bile salt interference check standard when initially establishing the chromatography conditions, regardless of sample matrices.

10.3.3.4	This new subsection was added to address questions about monitoring the areas of the non-extracted internal standards in each sample.
10.3.5	Naming this solution the bile salt interference check standard simplifies these discussions of its use. Added the requirement to run the bile salt interference check standard when initially establishing the chromatography conditions, regardless of sample matrices.
11.2.5	Clarified that Section 11.2 addresses processing of aqueous samples, not extracts.
11.3.8	Based on questions raised by the laboratories in the DoD multi-laboratory validation study and alternatives supported by data, the discussion of the dilution of solid sample extracts was revised to clarify that flexibility in the specifics is allowed.
11.3.9	Additional discussion of the extract concentration procedure added in keeping with the changes in Subsection 11.3.8.
13.1	Making the discussion more generic in the context of the bile salt interference check changes noted above.
13.3, #9	Making the discussion more generic in the context of the bile salt interference check changes noted above.
14.3.5	This new subsection was added to more accurately describe the calculation of ion abundance ratios for the target analytes.
14.9	In conjunction with the addition of 10.3.3.4, additional material and an equation were added here to address questions about monitoring the areas of the non-extracted internal standards in each sample.
15.1	There is no subsection 15.1.5 in the draft method and subsection 15.1.4 is the correct citation.
15.1.3	This subsection was revised in conjunction with the addition of Section 14.3.5 to address ion abundance ratios.
15.1.4	The requirements in subsection 15.1.1 were omitted from the original text.
15.3.1	The incorrect dilution solvent was called out in the first draft procedure. Also, the explanation of how to deal with quantification of the analytes in diluted extracts was not clear nor correct. Finally, the changes address samples where the diluted extract analysis does not meet the requirements. This becomes two new paragraphs within Section 15.3.1.
15.4.2.4	Responds to questions from laboratories.
19.10	The placeholder for the report on the single-laboratory method validation study was replaced with the actual citation.
Table 1	
$^{13}C_2$-4:2FTS	The full name of this labeled EIS compound was corrected.
Table 2	
PFHxS	The quantitation and confirmation ions for this analyte were reversed in the table.
$^{13}C_3$-PFHxS	The quantitation and confirmation ions for this analyte were reversed in the table.
$^{13}C_8$-PFOS	The quantitation and confirmation ions for this analyte were reversed in the table.
$^{13}C_3$-HFPO-DA	The parent ion mass was incorrect in the table.
Notes	A new note was added to explain the "NA" entries in this table.
Table 6	Commenters noted that they could not reproduce the Minimum Level (ML) values in the table from the details in the method. An explanatory footnote was added below the table.

Table 7	The mass calibration verification citation was updated to reflect the changes to Section 10.1 described above.
Glossary	Signal-to-noise ratio - The definition was corrected to refer to measuring the mean height of the noise, not the width.

Table of Contents

2nd DRAFT Method 1633 - Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS

1.0 Scope and Application

1.1 Method 1633 is for use in the Clean Water Act (CWA) for the determination of the per- and polyfluoroalkyl substances (PFAS) in Table 1 in aqueous, solid (soil, biosolids, sediment) and tissue samples by liquid chromatography/mass spectrometry (LC-MS/MS).

1.2 The method calibrates and quantifies PFAS analytes using isotopically labeled standards. Where linear and branched isomers are present in the sample and either qualitative or quantitative standards containing branched and linear isomers are commercially available, the PFAS analyte is reported as a single analyte consisting of the sum of the linear and branched isomer concentrations.

1.3 The instrumental portion of this method is for use only by analysts experienced with LC-MS/MS or under the close supervision of such qualified persons. Each laboratory that uses this method must demonstrate the ability to generate acceptable results using the procedure in Section 9.2.

1.4 By their very nature, many components of PFAS present analytical challenges unique to this class of analytes. For example, PFAS analytes readily adhere to the walls of the sample containers and may also stratify in the container. EPA has included procedures in the method that must be employed to address such challenges (see Section 11.0 and Appendices A and B).

1.5 This method is "performance-based," which means that modifications may be made without additional EPA review to improve performance (e.g., overcome interferences, or improve the sensitivity, accuracy, or precision of the results) *provided that* all performance criteria in this method are met. Requirements for establishing equivalency are in Section 9.1.2 and include 9.1.2.2c. For CWA uses, additional flexibility is described at 40 CFR 136.6. Changes in performance, sensitivity, selectivity, precision, recovery, etc., that result from modifications within the scope of 40 CFR Part 136.6, and Section 9.0 of this method must be documented, as well as how these modifications compare to the specifications in this method. Changes outside the scope of 40 CFR Part 136.6 and Section 9.0 of this method may require prior review or approval.

2.0 Summary of Method

Environmental samples are prepared and extracted using method-specific procedures. Sample extracts are subjected to cleanup procedures designed to remove interferences. Analyses of the sample extracts are conducted by LC-MS/MS in the multiple reaction monitoring (MRM) mode. Sample concentrations are determined by isotope dilution or extracted internal standard quantification (see Section 10.3) using isotopically labeled compounds added to the samples before extraction.

2.1 Extraction

2.1.1 Aqueous samples are spiked with isotopically labeled standards, extracted using solid-phase extraction (SPE) cartridges and undergo cleanup using carbon before analysis.

2.1.2 Solid samples are spiked with isotopically labeled standards, extracted into basic methanol, and cleaned up by carbon and SPE cartridges before analysis.

2.1.3 Tissue samples are spiked with isotopically labeled standards, extracted in potassium hydroxide and acetonitrile followed by basic methanol, and cleaned up by carbon and SPE cartridges before analysis.

2.2 This method measures the analytes as either their anions or neutral forms. The default approach for Clean Water Act uses of the method is to report the analytes in their acid or neutral forms, using the equations in Section 15.2, although the differences between the anion and acid form concentrations are minimal (See Table 8). Other project-specific reporting schemes may be used where required.

2.3 Individual PFAS analytes are identified through peak analysis of the quantification and confirmation ions, where applicable.

2.4 Quantitative determination of target analyte concentrations is made with respect to an isotopically labeled PFAS standard; the concentrations are then used to convert raw peak areas in sample chromatograms to final concentrations.

2.5 Results for target analytes are recovery corrected by the method of quantification (i.e., either isotope dilution or extracted internal standard quantification, see Section 10.3). Isotopically labeled compound recoveries are determined by comparison to the responses of one of seven non-extracted internal standards (a.k.a., the "recovery" standards) and are used as general indicators of overall analytical quality.

2.6 The quality of the analysis is assured through reproducible calibration and testing of the extraction, cleanup, and LC-MS/MS systems.

3.0 Definitions

Definitions are provided in the glossary at the end of this method.

4.0 Contamination and Interferences

4.1 Solvents, reagents, glassware, and other sample processing hardware may yield artifacts and elevated baselines causing misinterpretation of chromatograms. Specific selection of reagents and solvents may be required.

4.2 Clean all equipment prior to, and after each use to avoid PFAS cross-contamination. Typical cleaning solvents used include water, methanol, and methanolic ammonium hydroxide. The residual PFAS content of disposable plasticware and filters must be verified by batch/lot number and may be used without cleaning if PFAS levels are less than half the Minimum Level (ML, see Table 6).

4.2.1 All glass equipment that is used in the preparation or storage of reagents is cleaned by washing with detergent and baking in a kiln or furnace (Section 6.2.2). After detergent washing, glassware should be rinsed immediately with reagent water. Prior to use, baked glassware must be solvent rinsed and then air dried. A solvent rinse procedure using methanolic ammonium hydroxide (1%), toluene, and methanol is recommended.

4.2.2 All parts of the SPE manifold must be cleaned between samples with methanolic ammonium hydroxide (1%) and air dried prior to use. Sonication with methanolic ammonium hydroxide (1%) may be used for components that will fit in an ultrasonic bath.

Smaller parts, like the needles, adapters, reservoirs, and stopcocks associated with the manifold, require rinsing with tap water prior to manual cleaning or sonicating with methanolic ammonium hydroxide (1%) and air drying. When in use, after loading the samples but prior to elution procedures, the chamber must be rinsed with methanolic ammonium hydroxide (1%).

4.2.3 All equipment used in the filleting, dissecting, shucking, compositing, and homogenization of tissue must be cleaned with detergent and hot water, then rinsed with ultra-pure water followed by a series of solvent rinses. A typical solvent rinse procedure would be acetone, followed by toluene, and then dichloromethane.

4.3 All materials used in the analysis must be demonstrated to be free from interferences by running method blanks (Section 9.5) at the beginning and with each sample batch (samples started through the extraction process on a given analytical batch to a maximum of 20 field samples).

4.3.1 The reference matrix must simulate, as closely as possible, the sample matrix being tested. Ideally, the reference matrix should not contain PFAS in detectable amounts but should contain potential interferents in the concentrations expected to be found in the samples to be analyzed.

4.3.2 For tissue, chicken breast or other similar animal tissue (see Section 7.2.3) may be used as the reference matrix. The laboratory must verify that the source product used does not contain PFAS in detectable amounts.

4.3.3 When a reference matrix that simulates the sample matrix under test is not available, reagent water (Section 7.2.1) can be used to simulate water samples and Ottawa sand and/or reagent-grade sand (Section 7.2.2) can be used to simulate soils.

4.4 Interferences co-extracted from samples will vary considerably from source to source, depending on the diversity of the site being sampled. Interfering compounds may be present at concentrations several orders of magnitude higher than the native PFAS. Because low levels of PFAS are measured by this method, elimination of interferences is essential. The cleanup steps given in Section 12.0 can be used to reduce or eliminate these interferences and thereby permit reliable determination of the PFAS at the levels shown in Table 6. The most frequently encountered interferences are fluoropolymers; however, when analyzing whole fish samples, bile salts (e.g., Taurodeoxycholic Acid [TDCA]) can interfere in the chromatography. For this reason, analysis of a standard containing TDCA is required as part of establishing the initial chromatographic conditions (see Sections 10.2.2.5 and 10.3.5).

4.5 Each piece of reusable glassware may be numbered to associate that glassware with the processing of a particular sample. This may assist the laboratory in tracking possible sources of contamination for individual samples, identifying glassware associated with highly contaminated samples that may require extra cleaning, and determining when glassware should be discarded.

5.0 Safety

5.1 The toxicity or carcinogenicity of each chemical used in this method has not been precisely determined; however, each compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level.

5.1.1 PFOA has been described as likely to be carcinogenic to humans. Pure standards should be handled by trained personnel, with suitable protection to skin and eyes, and care should be taken not to breathe the vapors or ingest the materials.

5.1.2 It is recommended that the laboratory purchase dilute standard solutions of the analytes in this method. However, if primary solutions are prepared, they must be prepared in a hood, following universal safety measures.

5.2 The laboratory is responsible for maintaining a current awareness file of Occupational Safety and Health Administration (OSHA) regulations regarding the safe handling of the chemicals specified in this method. A reference file of safety data sheets (SDS) should also be made available to all personnel involved in these analyses. It is also suggested that the laboratory perform personal hygiene monitoring of each analyst who uses this method and that the results of this monitoring be made available to the analyst. Additional information on laboratory safety can be found in References 1-4. The references and bibliography at the end of Reference 3 are particularly comprehensive in dealing with the general subject of laboratory safety.

5.3 Samples suspected to contain these compounds are handled using essentially the same techniques employed in handling radioactive or infectious materials. Well-ventilated, controlled access laboratories are required. Assistance in evaluating the health hazards of particular laboratory conditions may be obtained from certain consulting laboratories and from State Departments of Health or Labor, many of which have an industrial health service. Each laboratory must develop a strict safety program for handling these compounds.

5.3.1 Facility – When finely divided samples (dusts, soils, dry chemicals) are handled, all operations (including removal of samples from sample containers, weighing, transferring, and mixing) should be performed in a glove box demonstrated to be leak tight or in a fume hood demonstrated to have adequate air flow. Gross losses to the laboratory ventilation system must not be allowed. Handling of the dilute solutions normally used in analytical and animal work presents no inhalation hazards except in the case of an accident.

5.3.2 Protective equipment – Disposable plastic gloves, apron or lab coat, safety glasses or mask, and a glove box or fume hood adequate for radioactive work should be used. During analytical operations that may give rise to aerosols or dusts, personnel should wear respirators equipped with activated carbon filters. Eye protection (preferably full-face shields) must be worn while working with exposed samples or pure analytical standards. Latex gloves are commonly used to reduce exposure of the hands.

5.3.3 Training – Workers must be trained in the proper method of removing contaminated gloves and clothing without contacting the exterior surfaces.

5.3.4 Personal hygiene – Hands and forearms should be washed thoroughly after each manipulation and before breaks (coffee, lunch, and shift).

5.3.5 Confinement – Isolated work areas posted with signs, segregated glassware and tools, and plastic absorbent paper on bench tops will aid in confining contamination.

5.3.6 Waste Handling – Good technique includes minimizing contaminated waste. Plastic bag liners should be used in waste cans. Janitors and other personnel should be trained in the safe handling of waste.

5.3.7 Laundry – Clothing known to be contaminated should be collected in plastic bags. Persons that convey the bags and launder the clothing should be advised of the hazard and trained in proper handling. The clothing may be put into a washer without contact if the launderer knows of the potential problem. The washer should be run through a cycle before being used again for other clothing.

5.4 Biosolids samples may contain high concentrations of biohazards and must be handled with gloves and opened in a fume hood or biological safety cabinet to prevent exposure. Laboratory staff should know and observe the safety procedures required in a microbiology laboratory that handles pathogenic organisms when handling biosolids samples.

6.0 Equipment and Supplies

Note: *Brand names, suppliers, and part numbers are for illustration purposes only and no endorsement is implied. Equivalent performance may be achieved using apparatus and materials other than those specified here. Meeting the performance requirements of this method is the responsibility of the laboratory.*

6.1 Sampling equipment for discrete or composite sampling

6.1.1 Sample bottles and caps

Note: *Do not use PTFE-lined caps on sample containers.*

6.1.1.1 Liquid samples (waters, sludges, and similar materials containing < 50 mg solids per sample) – Sample bottle, HDPE, with linerless HDPE or polypropylene caps.

Note: *At least two aliquots of aqueous samples are collected to allow sufficient volume for the determination of percent solids and for pre-screening analysis. One aliquot should be collected in a 500-mL container while the second aliquot may be collected in a smaller sample container (e.g., 250-mL or 125-mL).*

6.1.1.2 Solid samples (soils, sediments, and biosolids that contain more than 50 mg solids) – Sample bottle or jar, wide-mouth, HDPE, 500-mL, with linerless HDPE or polypropylene caps.

6.1.1.3 Tissue samples – Sample jar, wide-mouth HDPE, 100-mL, with linerless HDPE or polypropylene caps.

6.1.2 Compositing equipment – Automatic or manual compositing system incorporating containers cleaned per bottle cleaning procedure above. Only HDPE tubing must be used. If the sampler uses a peristaltic pump, a minimum length of compressible silicone rubber tubing may be used in the pump only. Before use, the tubing must be thoroughly rinsed with methanol, followed by repeated rinsing with reagent water to minimize sample contamination. An integrating flow meter is used to collect proportional composite samples.

6.2 Equipment for glassware cleaning

Note: *If blanks from bottles or other glassware show no detectable PFAS contamination when using fewer cleaning steps than required above, unnecessary cleaning steps and equipment may be eliminated.*

6.2.1 Laboratory sink with overhead fume hood

6.2.2 Kiln – Capable of reaching 450 ºC within 2 hours and maintaining 450 - 500 ºC ± 10 ºC, with temperature controller and safety switch (Cress Manufacturing Co., Santa Fe Springs, CA, B31H, X31TS, or equivalent). For safety, the kiln or furnace should be vented outside the laboratory, or to a trapping system.

6.3 Equipment for sample preparation

6.3.1 Polyethylene gloves

6.3.2 Laboratory fume hood (of sufficient size to contain the sample preparation equipment listed below)

6.3.3 Glove box (optional)

6.3.4 *Text deleted, but Section number retained for the time being to avoid other numbering changes*

6.3.5 Meat grinder – Hobart, or equivalent, with 3- to 5-mm holes in inner plate

6.3.6 Equipment for determining percent moisture

6.3.6.1 Oven – Capable of maintaining a temperature of 110 ± 5 ºC

6.3.6.2 Desiccator

6.3.7 Balances

6.3.7.1 Analytical – Capable of weighing 0.1 mg

6.3.7.2 Top loading – Capable of weighing 10 mg

6.3.8 Aluminum foil

6.3.9 Disposable spoons, 10 mg, polypropylene or stainless steel

6.3.10 Ultrasonic mixer (sonicator)

6.3.11 HDPE bottles, with linerless HDPE or polypropylene caps – 60 mL

6.3.12 pH Paper, range 0-14 - (Whatman® Panpeha™ or equivalent), 0.5-unit readability

6.3.13 Analog or digital vortex mixer, single or multi-tube (Fisher Scientific 02-215-452, or equivalent)

6.3.14 Volumetric flasks, Class A

6.3.15 Disposable polypropylene collection tubes (13 x 100 mm, 8 mL)

6.3.16 Variable speed mixing table (Fisherbrand™ Nutating mixer or equivalent)

6.4 Filtration

6.4.1 Silanized glass wool (Sigma-Aldrich, Cat # 20411 or equivalent) – store in a clean glass jar and rinsed with methanol (2 times) prior to use.

6.4.2 Disposable syringe filter, 25-mm, 0.2-µm Nylon membrane, PALL/Acrodisc or equivalent

6.4.3 Glass fiber filter, 47 mm, 1 µm, PALL A/E or equivalent

6.5 Centrifuge apparatus

6.5.1 Centrifuge (Thermo Scientific Legend RT+, 16 cm rotor, or equivalent), capable of reaching at least 3000 rpm

6.5.2 Centrifuge tubes – Disposable polypropylene centrifuge tubes (50 mL)

6.6 Pipettes

6.6.1 Norm-Ject® syringe (or equivalent), polypropylene/HDPE, 5 mL

6.6.2 Variable volume pipettes with disposable HDPE or polypropylene tips (10 µL to 5 mL) – used for preparation of calibration standards and spiked samples.

6.6.3 Disposable glass pipets

6.6.4 Calibrated mechanical pipettes or Hamilton graduated syringes

6.7 Solid-phase extraction

6.7.1 Solid-phase extraction (SPE) cartridges (Waters Oasis WAX 150 mg, Cat # 186002493 or equivalent). The SPE sorbent must have a pKa above 8 so that it remains positively charged during the extraction.

Note: *SPE cartridges with a different bed volume (e.g., 500 mg) may be used; however, the laboratory must demonstrate that the bed volume does not negatively affect analyte absorption and elution, by performing the initial demonstration of capability analyses described in Section 9.2.*

6.7.2 Vacuum manifold for SPE Cartridges (Waters™ extraction manifold #WAT200607 or equivalent)

6.8 Evaporation

6.8.1 Automatic or manual solvent evaporation system (TurboVap® LV or equivalent)

6.8.2 Evaporation/concentrator tubes: 60 mL clear glass vial, 30 x 125 mm, without caps (Wheaton Cat # W226060 or equivalent). Cover with foil if required.

6.9 Vials

6.9.1 Snap cap/crimp top vials, 300 µL, polypropylene (12 x 32 mm) – used in sample pre-screening (DWK Life Sciences Cat # 225180 or equivalent)

6.9.2 Polypropylene crimp/snap vials, 1 mL (Agilent Cat # 5182-0567 or equivalent)

6.9.3 Clear snap cap, polyethylene, 11 mm (Fisher Scientific # 03-375-24E, or equivalent)

6.9.4 Single step filter vials (Restek Thomson SINGLE StEP® Standard Filter Vials, 0.2 µm Nylon membrane, with Black Preslit caps Cat # 25891 or equivalent) – used in sample pre-screening.

6.10 Instrument

6.10.1 Ultra high-performance liquid chromatograph (UPLC also called UHPLC) or high-performance liquid chromatograph (HPLC) equipped with tandem quadrupole mass spectrometer (Waters Xevo TQ-S Micro or equivalent)

6.10.2 C18 column, 1.7 µm, 50 x 2.1 mm (Waters Acquity UPLC® BEH or equivalent)

6.10.3 Guard column (Phenomenex Kinetex® Evo C18 or equivalent)

6.10.4 Trap/delay column (Purospher Star RP-18 endcapped [3 µm] Hibar® RT 50-4 or equivalent)

6.11 Bottles, HDPE or glass, with linerless HDPE or polypropylene caps. Various sizes. To store prepared reagents.

7.0 Reagents and Standards

7.1 Reagents

Reagents prepared by the laboratory may be stored in either glass or HDPE containers. Proper cleaning procedures (Section 4.2) must be followed prior to using the containers.

7.1.1 Acetic acid – ACS grade or equivalent, store at room temperature

7.1.2 Acetic acid (0.1%) – dissolve acetic acid (1 mL) in reagent water (1 L), store at room temperature, replace after 3 months.

7.1.3 Acetonitrile – UPLC grade or equivalent, verified before use, store at room temperature

7.1.4 Ammonium acetate – (Caledon Ultra LC/MS grade, or equivalent), store at 2-8 °C, replace 2 years after opening date

7.1.5 Ammonium hydroxide – certified ACS+ grade or equivalent, 30% in water, store at room temperature

7.1.6 Aqueous ammonium hydroxide (3%) – add ammonium hydroxide (10 mL, 30%) to reagent water (90 mL), store at room temperature, replace after 3 months

7.1.7 Methanolic ammonium hydroxide

7.1.7.1 Methanolic ammonium hydroxide (0.3%) – add ammonium hydroxide (1 mL, 30%) to methanol (99 mL), store at room temperature, replace after 1 month

7.1.7.2 Methanolic ammonium hydroxide (1%) – add ammonium hydroxide (3.3 mL, 30%) to methanol (97 mL), store at room temperature, replace after 1 month

7.1.7.3 Methanolic ammonium hydroxide (2%) – add ammonium hydroxide (6.6 mL, 30%) to methanol (93.4 mL), store at room temperature, replace after 1 month

7.1.8 Methanolic potassium hydroxide (0.05 M) – add 3.3 g of potassium hydroxide to 1 L of methanol, store at room temperature, replace after 3 months

7.1.9 Methanol with 4% water, 1% ammonium hydroxide and 0.625% acetic acid – add ammonium hydroxide (3.3 mL, 30%), reagent water (1.7 mL) and acetic acid (0.625 mL) to methanol (92 mL), store at room temperature, replace after 1 month. This solution is used to prepare the instrument blank (Section 7.3.6) and is used to dilute the extracts of samples that exceed the calibration range (see Section 15.3).

7.1.10 Eluent A – Acetonitrile, Caledon Ultra LCMS grade or equivalent

7.1.11 Eluent B – 2 mM ammonium acetate in 95:5 water/acetonitrile. Dissolve 0.154 g of ammonium acetate (Section 7.1.4) in 950 mL of water and 50 mL of acetonitrile (Caledon Ultra LCMS grade, or equivalent). Store at room temperature, shelf life 2 months.

7.1.12 Formic acid – (greater than 96% purity or equivalent), verified by lot number before use, store at room temperature

7.1.13 Formic acid

7.1.13.1 Formic acid (aqueous, 0.1 M) – dissolve formic acid (4.6 g) in reagent water (1 L), store at room temperature, replace after 2 years

7.1.13.2 Formic acid (aqueous, 0.3 M) – dissolve formic acid (13.8 g) in reagent water (1 L), store at room temperature, replace after 2 years

7.1.13.3 Formic acid (aqueous, 5% v/v) – mix 5 mL formic acid with 95 mL reagent water, store at room temperature, replace after 2 years

7.1.13.4 Formic acid (aqueous, 50% v/v) – mix 50 mL formic acid with 50 mL reagent water, store at room temperature, replace after 2 years

7.1.13.5 Formic acid (methanolic 1:1, 0.1 M formic acid/methanol) – mix equal volumes of methanol and 0.1 M formic acid, store at room temperature, replace after 2 years

7.1.14 Methanol – (HPLC grade or better, 99.9% purity), verified by lot number before use, store at room temperature

7.1.15 Potassium hydroxide – certified ACS or equivalent, store at room temperature, replace after 2 years

7.1.16 Reagent water – Laboratory reagent water, test by lot/batch number for residual PFAS content

7.1.17 Carbon – EnviCarb® 1-M-USP or equivalent, verified by lot number before use, store at room temperature. Loose carbon allows for better adsorption of interferent organics.

Note: *The single-laboratory validation laboratory achieved better performance with loose carbon than carbon cartridges. Loose carbon will be used for the multi-laboratory validation to set statistically based method criteria. Once the method is multi-laboratory validated, laboratories will have the flexibility to use carbon cartridges, as long as all method QC criteria are met.*

7.1.18 Toluene – HPLC grade, verified by lot number before use. Store at room temperature.

7.1.19 Acetone – Pesticide grade, verified by lot number before use in rinsing tissue dissection and processing equipment.

7.1.20 Dichloromethane (methylene chloride) – Pesticide grade, verified by lot number before use in rinsing tissue dissection and processing equipment.

7.2 Reference matrices – Matrices in which PFAS and interfering compounds are not detected by this method. These matrices are to be used to prepare the batch QC samples (e.g., method blank, and ongoing precision and recovery sample).

7.2.1 Reagent water – purified water, Type I

7.2.2 Solids reference matrix – Ottawa or reagent-grade sand

7.2.3 Tissue reference matrix – chicken breast or similar animal tissue

7.3 Standard solutions – Prepare from materials of known purity and composition or purchase as solutions or mixtures with certification to their purity, concentration, and authenticity. Observe the safety precautions in Section 5.

Purchase of commercial standard solutions or mixtures is highly recommended for this method; however, when these are not available, preparation of stock solutions from neat materials may be necessary. If the chemical purity is 98% or greater, the weight may be used without correction to calculate the concentration of the standard. Dissolve an appropriate amount of assayed reference material in the required solvent. For example, weigh 10 to 20 mg of an individual compound to three significant figures in a 10-mL ground-glass-stoppered volumetric flask and fill to the mark with the required solvent. Once the compound is completely dissolved, transfer the solution to a clean vial and cap.

When not being used, store standard solutions in the dark at less than 4 °C unless the vendor recommends otherwise in screw-capped vials with foiled-lined caps. Place a mark on the vial at the

level of the solution so that solvent loss by evaporation can be detected. Replace the solution if solvent loss has occurred.

Note: *Native PFAS standards are available from several suppliers. Isotopically labeled compounds are available from Cambridge Isotope Laboratories and Wellington Laboratories, but may also be available from other suppliers. Listing of these suppliers does not constitute a recommendation or endorsement for use. All diluted solutions must be stored in glass or HDPE containers that have been thoroughly rinsed with methanol.*

^{18}O-mass labeled perfluoroalkyl sulfonates may undergo isotopic exchange with water under certain conditions, which lowers the isotopic purity of the standards over time.

The laboratory must maintain records of the certificates for all standards for traceability purposes. Copies of the certificates must be provided as part of the data packages in order to check that proper calculations were performed.

7.3.1 Extracted Internal Standard (EIS) – (a.k.a. isotopically labeled compound) Prepare the EIS solution containing the isotopically labeled compounds listed in Table 3 as extracted internal standards in methanol from prime stocks. An aliquot of EIS solution, typically 50 µL, is added to each sample prior to extraction. Table 3 presents the nominal amounts of EIS compounds added to each sample. The list of isotopically labeled compounds in Table 3 represents the compounds that were available at the time this method was validated. Other isotopically labeled compounds may be used as they become available.

7.3.2 Non-Extracted Internal Standard (NIS) – The NIS solution containing the isotopically labeled compounds listed in Table 3 as non-extracted internal standards is prepared in methanol from prime stock. An aliquot of NIS solution, typically 50 µL, is added to each sample prior to instrumental analysis. Table 3 presents the nominal amounts of NIS compounds added to each sample.

7.3.3 Native Standards Solution – Prepare a spiking solution, containing the method analytes listed in Table 4, in methanol from prime stocks. The solution is used to prepare the calibration standards and to spike the known reference QC samples that are analyzed with every batch. Quantitative standards containing a mixture of branched and linear isomers must be used for method analytes if they are commercially available. Currently, these include PFOS, PFHxS, NMeFOSAA, and NEtFOSAA.

7.3.4 Calibration standard solutions – A series of calibration solutions containing the target analytes and the ^{13}C-, ^{18}O-, and deuterium-labeled extracted internal standards (EIS) and non-extracted internal standards (NIS) is used to establish the initial calibration of the analytical instrument. The concentration of the method analytes in the solutions varies to encompass the working range of the instrument, while the concentrations of the EIS and NIS remain constant. The calibration solutions are prepared using methanol, methanolic ammonium hydroxide (2%), water, acetic acid and the method analyte and isotopically labeled compound standard solutions. After dilution, the final solution will match the solvent mix of sample extracts, which contain methanol with 4% water, 1% ammonium hydroxide and 0.625% acetic acid (Section 7.1.9). Calibration standard solutions do not undergo solid phase extraction/cleanup.

Concentrations for seven calibration solutions are presented in Table 4. A minimum of six contiguous calibrations standards are required for a valid analysis when using a linear calibration model, with at least five of the six calibration standards being within the

quantitation range (e.g., from the LOQ to the highest calibration standard). If a second-order calibration model is used, then a minimum of seven calibration standards are required, with at least six of the seven calibration standards within the quantitation range. The lowest level calibration standard must meet a signal-to-noise ratio of 3:1 and be at a concentration less than or equal to the Limit of Quantitation (LOQ). All initial calibration requirements listed in Table 7 must be met. An instrument sensitivity check (ISC) standard at the concentration of the lowest calibration standard within the quantitation range is required to be analyzed at the beginning of the analytical run (Section 10.3.3.1 and Section 13.3). A mid-level calibration solution is analyzed at least every ten samples or less, on an ongoing basis for the purpose of calibration verification. A mid-level calibration verification (CV) standard must also be analyzed after all sample analyses in order to bracket the analytical batch.

Note: *Additional calibration standards, at levels lower than the lowest calibration standard listed in the method, may be added to accommodate a lower limit of quantitation if the instrument sensitivity allows. Calibration standards at the high end of the calibration may be eliminated if the linearity of the instrument is exceeded or at the low end if those calibration standards do not meet the S/N ratio criterion of 3:1, as long as the required number of calibration points is met. All analytes with commercially available stable isotope analogues must be quantified using isotope dilution.*

7.3.5 Qualitative Standards – Standards that contain mixtures of the branched and linear isomers of the method analytes and that are used for comparison against suspected branched isomer peaks in field samples. These qualitative standards are **not** required for those analytes where the quantitative standards in Section 7.3.3 already contain the branched and linear isomers. Qualitative standards that are currently commercially available include PFOA, PFNA, PFOSA, NMeFOSA, NEtFOSA, NEtFOSE, and NMeFOSE.

Note: *During the multi-laboratory validation study, laboratories reported that NMeFOSA was an impurity in the branched isomer qualitative standard for NMeFOSE and NEtFOSA was an impurity in the branched isomer qualitative standard for NEtFOSE supplied for the study. Those impurities did not preclude the use of these standards, but laboratories should be aware of the possibility.*

7.3.6 Instrument Blank – During the analysis of a batch of samples, a solvent blank is analyzed after samples containing high level of target compounds (e.g., calibration, CV) to monitor carryover from the previous injection. The injection blank consists of the solution in Section 7.1.9 fortified with the EIS and NIS for quantitation purposes.

7.3.7 Stability of solutions – Standard solutions used for quantitative purposes (Sections 7.3.1 through 7.3.5) should be assayed periodically (e.g., every 6 months) against certified standard reference materials (SRMs) from the National Institute of Science and Technology (NIST), if available, or certified reference materials from a source that will attest to the authenticity and concentration, to assure that the composition and concentrations have not changed.

7.4 Mass calibration solution – Use the mass calibration solution specified by the instrument manufacturer.

7.5 Bile salt interference check standard containing Taurodeoxycholic Acid (TDCA) or Sodium taurodeoxychloate hydrate – (Sigma Aldrich 580221-5GM, or equivalent). This standard is used to evaluate the chromatographic program relative to the risk of an interference from bile salts in tissue

samples when using acetonitrile as the mobile phase in the instrument. Prepare solution at a concentration of 1 µg/mL in the same solvent as the calibration standards. If using other mobile phases and analyzing tissues, it will be necessary to evaluate taurochenodeoxycholic acid (TCDCA) and tauroursodeoxycholic acid (TUDCA) as well.

8.0 Sample Collection, Preservation, Storage, and Holding Times

8.1 Collect samples in HDPE containers following conventional sampling practices (Reference 5). All sample containers must have linerless HDPE or polypropylene caps. Other sample collection techniques, or sample volumes may be used, if documented.

8.2 Aqueous samples

8.2.1 Samples that flow freely are collected as grab samples or in refrigerated bottles using automatic sampling equipment. Collect 500 mL of sample (other than leachates) in an HDPE bottle. Do not fill the bottle past the shoulder, to allow room for expansion during frozen storage.

Note: *Collect at least two aliquots of all aqueous samples to allow sufficient volume for the determination of percent solids and for pre-screening analysis. That second aliquot may be collected in a smaller sample container (e.g., 250-mL or 125-mL).*

Because the target analytes are known to bind to the interior surface of the sample container, the entire aqueous sample that is collected must be prepared and analyzed and subsampling avoided whenever possible. Therefore, if a sample volume smaller than 500 mL is to be used for analysis, collect the sample in an appropriately sized HDPE container.

8.2.2 Leachate samples from landfills can present significant challenges and therefore only 100 mL of sample is collected for the analysis. Collect two 100-mL leachate sample aliquots in a similar manner as described in Section 8.2.1, using appropriately sized containers.

8.2.3 Maintain all aqueous samples protected from light at 0 - 6 ºC from the time of collection until shipped to the laboratory. Samples must be shipped as soon as practical with sufficient ice to maintain the sample temperature below 6 ºC during transport and be received by the laboratory within 48 hours of collection. The laboratory must confirm that the sample temperature is 0 - 6 ºC upon receipt. Once received by the laboratory, the samples may be stored at ≤ -20 ºC, or at 0 - 6 ºC, until sample preparation. However, the allowable holding time for samples depends on the storage temperature, as described in Section 8.5.

8.3 Solid (soil, sediment, biosolids), excluding tissue

8.3.1 Collect samples as grab samples using wide-mouth jars and fill no more than ¾ full (see Section 6.1.1.2 for container size and type).

8.3.2 Maintain solid samples protected from light (in HDPE containers) at 0 - 6 ºC from the time of collection until receipt at the laboratory. The laboratory must confirm that the sample temperature is 0 - 6 ºC upon receipt. Once received by the laboratory, the samples may be stored at ≤ -20 ºC or at 0 - 6 ºC, until sample preparation. However, the allowable holding time for samples depends on the storage temperature, as described in Section 8.5.

8.4 Fish and other tissue samples

The nature of the tissues of interest may vary by project. Field sampling plans and protocols should explicitly state the samples to be collected and if any processing will be conducted in the field (e.g., filleting of whole fish or removal of organs). All field procedures must involve materials and equipment that have been shown to be free of PFAS.

8.4.1 Fish may be cleaned, filleted, or processed in other ways in the field, such that the laboratory may expect to receive whole fish, fish fillets, or other tissues for analysis.

8.4.2 If whole fish are collected, wrap the fish in aluminum foil or food-grade polyethylene tubing, and maintain at 0 - 6 ºC from the time of collection until receipt at the laboratory, to a maximum time of 24 hours. If a longer transport time is necessary, freeze the sample before shipping. Ideally, fish should be frozen upon collection and shipped to the laboratory on dry ice.

8.4.3 Once received by the laboratory, the samples must be maintained protected from light at ≤ -20 ºC until prepared. Store unused samples in HDPE containers or wrapped in aluminum foil at ≤ -20 ºC.

8.5 Holding times

8.5.1 Aqueous samples (including leachates) should be analyzed as soon as possible; however, samples may be held in the laboratory for up to 90 days from collection, when stored at ≤ -20 ºC and protected from the light. When stored at 0 - 6 ºC and protected from the light, aqueous samples may be held for up to 28 days, with the caveat that issues were observed with certain perfluorooctane sulfonamide ethanols and perfluorooctane sulfonamidoacetic acids after 7 days. These issues are more likely to elevate the observed concentrations of other PFAS compounds via the transformation of these precursors if they are present in the sample.

8.5.2 Solid samples (soils and sediments) and tissue samples may be held for up to 90 days, if stored by the laboratory in the dark at either 0 - 6 ºC or ≤ -20 ºC, with the caveat that samples may need to be extracted as soon as possible if NFDHA is an important analyte.

8.5.3 Biosolids samples may be held for up to 90 days, if stored by the laboratory in the dark at 0 - 6 ºC or at -20 ºC. Because microbiological activity in biosolids samples at 0 - 6 ºC may lead to production of gases which may cause the sample to be expelled from the container when it is opened, as well as producing noxious odors, EPA recommends that samples be frozen if they need to be stored for more than a few days before extraction.

8.5.4 Store sample extracts in the dark at less than 0 - 4 ºC until analyzed. If stored in the dark at less than 0 - 4 ºC, sample extracts may be stored for up to 90 days, with the caveat that issues were observed for some ether sulfonates after 28 days. These issues may elevate the observed concentrations of the ether sulfonates in the extract over time. Samples may need to be extracted as soon as possible if NFDHA is an important analyte.

9.0 Quality Control

9.1 Each laboratory that uses this method is required to operate a formal quality assurance program (Reference 6). The minimum requirements of this program consist of an initial demonstration of

laboratory capability, analysis of samples spiked with isotopically labeled compounds to evaluate and document data quality, and analysis of standards and blanks as tests of continued performance. Laboratory performance is compared to established performance criteria to determine if the results of analyses meet the performance characteristics of the method.

If the method is to be applied to a sample matrix other than water (e.g., soils, biosolids, tissue), the appropriate alternative reference matrix (Sections 7.2.2 - 7.2.3) is substituted for the reagent water matrix (Section 7.2.1) in all performance tests.

9.1.1 The laboratory must make an initial demonstration of the ability to generate acceptable precision and recovery with this method. This demonstration is given in Section 9.2.

9.1.2 In recognition of advances that are occurring in analytical technology, and to overcome matrix interferences, the laboratory is permitted certain options to improve separations or lower the costs of measurements. These options include alternative extraction, concentration, and cleanup procedures, and changes in sample volumes, columns, and detectors. Alternative determinative techniques and changes that degrade method performance, are *not* allowed without prior review and approval.

Note: *For additional flexibility to make modifications without prior EPA review, see 40 CFR Part 136.6.*

9.1.2.1 Each time a modification is made to this method, the laboratory is required to repeat the procedure in Section 9.2. If calibration will be affected by the change, the instrument must be recalibrated per Section 10. Once the modification is demonstrated to produce results equivalent or superior to results produced by this method as written, that modification may be used routinely thereafter, so long as the other requirements in this method are met (e.g., isotopically labeled compound recovery).

9.1.2.2 The laboratory is required to maintain records of any modifications made to this method. These records include the following, at a minimum:

a) The names, titles, business addresses, and telephone numbers of the analyst(s) that performed the analyses and modification, and of the quality control officer that witnessed and will verify the analyses and modifications.

b) A listing of pollutant(s) measured, by name and CAS Registry number.

c) A narrative stating reason(s) for the modifications (see Section 1.5).

d) Results from all quality control (QC) tests comparing the modified method to this method, including:

i. Calibration (Section 10)
ii. Calibration verification (Section 14.3)
iii. Initial precision and recovery (Section 9.2.1)
iv. Isotopically labeled compound recovery (Section 9.3)
v. Analysis of blanks (Section 9.5)
vi. Accuracy assessment (Section 9.4)

e) Data that will allow an independent reviewer to validate each determination by tracing the instrument output (peak height, area, or other signal) to the final result. These data are to include:
   i. Sample numbers and other identifiers
   ii. Extraction dates
   iii. Analysis dates and times
   iv. Analysis sequence/run chronology
   v. Sample weight or volume (Section 11)
   vi. Extract volume prior to each cleanup step (Section 12)
   vii. Extract volume after each cleanup step (Section 12)
   viii. Final extract volume prior to injection (Section 12)
   ix. Injection volume (Section 13.3)
   x. Dilution data, differentiating between dilution of a sample or an extract (Section 15.3)
   xi. Instrument
   xii. Column (dimensions, liquid phase, solid support, film thickness, etc.)
   xiii. Operating conditions (temperatures, temperature program, flow rates)
   xiv. Detector (type, operating conditions, etc.)
   xv. Chromatograms, printer tapes, and other recordings of raw data
   xvi. Quantitation reports, data system outputs, and other data to link the raw data to the results reported

9.1.2.3 Alternative columns and column systems – If a column or column system other than those specified in this method is used, that column or column system must meet all the requirements of this method.

Note: *The use of alternative columns or programs will likely result in a different elution order.*

9.1.3 Analyses of method blanks are required on an on-going basis to demonstrate the extent of background contamination in any reagents or equipment used to prepare and analyze field samples (Section 4.3). The procedures and criteria for analysis of a method blank are described in Section 9.5.

9.1.4 The laboratory must spike all samples with isotopically labeled compounds to monitor method performance. This test is described in Section 9.3. When results of these spikes indicate atypical method performance for samples, the samples are diluted to evaluate whether the performance issue is caused by the sample matrix. Procedures for dilution are given in Section 15.3.

9.1.5 The laboratory must, on an ongoing basis, demonstrate that the analytical system is in control through calibration verification and the analysis of ongoing precision and recovery standards (OPR), spiked at low (LLOPR) and mid-level, and blanks. These procedures are given in Sections 14.1 through 14.7.

9.1.6 The laboratory must maintain records to define the quality of data generated. Development of accuracy statements is described in Section 9.4.

9.2 Initial Demonstration of Capability

9.2.1 Initial precision and recovery (IPR) – To establish the ability to generate acceptable precision and recovery, the laboratory must perform the following operations for each sample matrix type to which the method will be applied by that laboratory.

9.2.1.1 Extract, concentrate, and analyze four aliquots of the matrix type to be tested (Section 7.2.1 through 7.2.3), spiked with 200 µL of the native standard solution (Section 7.3.3), 50 µL of the EIS solution (Section 7.3.1), and 50 µL of NIS solution (Section 7.3.2). At least one method blank, matching the matrix being analyzed, must be prepared with the IPR batch. In the event that more than one MB was prepared and analyzed with the IPR batch, all blank results must be reported. All sample processing steps that are to be used for processing samples, including preparation and extraction (Sections 11.2 – 11.4), cleanup (Section 12.0) and concentration (Section 12.0), must be included in this test.

9.2.1.2 Using results of the set of four analyses, compute the average percent recovery (R) of the extracts and the relative standard deviation (RSD) of the concentration for each target and EIS compound.

9.2.1.3 For each native and isotopically labeled compound, compare RSD and % recovery with the corresponding limits for initial precision and recovery in Table 5. If RSD and R for all compounds meet the acceptance criteria, system performance is acceptable, and analysis of blanks and samples may begin. If, however, any individual RSD exceeds the precision limit or any individual R falls outside the range for recovery, system performance is unacceptable for that compound. Correct the problem and repeat the test (Section 9.2).

9.2.2 Method detection limit (MDL) - Each laboratory must also establish MDLs for all the analytes using the MDL procedure at 40 CFR Part 136, Appendix B. An MDL determination must be performed for all compounds. The minimum level of quantification (ML) can be calculated by multiplying the MDL by 3.18 and rounding the result to the nearest 1, 2 or 5 x 10^n, where n is zero or an integer (see the Glossary for alternative derivations). Example matrix-specific method detection limits are listed in Table 6.

9.3 To assess method performance on the sample matrix, the laboratory must spike all samples with the isotopically labeled compound standard solution (Section 7.3.1) and all sample extracts with the NIS spiking solution (Section 7.3.2).

9.3.1 Analyze each sample according to the procedures in Sections 11.0 through 16.0.

9.3.2 Compute the percent recovery of the isotopically labeled compound using the non-extracted internal standard method (Section 15.2) and the equation in Section 14.5.2.

9.3.3 The recovery of each isotopically labeled compound must be within the limits in Tables 9 and 10 (*once the tables are finalized*). If the recovery of any compound falls outside of these limits, method performance is unacceptable for that compound in that sample. Additional cleanup procedures must then be employed to attempt to bring the recovery within the normal range. If the recovery cannot be brought within the normal range after all cleanup procedures have been employed, water samples are diluted, and smaller amounts of soils, biosolids, sediments, and other matrices are prepared and analyzed, per Section 15.3.

9.4 Recovery of isotopically labeled compounds from samples must also be assessed and records maintained.

9.4.1 After the analysis of 30 samples of a given matrix type (water, soil, biosolids, tissues, etc.) for which the isotopically labeled compounds pass the tests in Section 9.3, compute the R

and the standard deviation of the percent recovery (S_R) for the isotopically labeled compounds only. Express the assessment as a percent recovery interval from R - $2S_R$ to R + $2S_R$ for each matrix. For example, if R = 90% and S_R = 10% for five analyses of soil, the recovery interval is expressed as 70 to 110%.

9.4.2 Update the accuracy assessment for each isotopically labeled compound in each matrix on a regular basis (e.g., after each five to ten new measurements).

9.5 Method blanks – A method blank is analyzed with each sample batch (Section 4.3) to demonstrate freedom from contamination. The matrix for the method blank must be similar to the sample matrix for the batch (e.g., reagent water blank [Section 7.2.1], solids matrix blank [Section 7.2.2], or tissue blank [Section 7.2.3]).

9.5.1 Analyze the cleaned extract (Section 12.0) of the method blank aliquot before the analysis of the OPRs (Section 14.5).

9.5.2 If any PFAS is found in the blank at 1) at a concentration greater than the ML for the analyte, 2) at a concentration greater than one-third the regulatory compliance limit, or 3) at a concentration greater than one-tenth the concentration in a sample in the extraction batch, whichever is greatest, analysis of samples must be halted, and the problem corrected. Other project-specific requirements may apply; therefore, the laboratory may adopt more stringent acceptance limits for the method blank at their discretion. If the contamination is traceable to the extraction batch, samples affected by the blank must be re-extracted and analyzed, provided enough sample volume is available and the sample are still within holding time.

If continued re-testing results in repeated blank contamination, the laboratory must document and report the failures (e.g., as qualifiers on results), unless the failures are not required to be reported as determined by the regulatory/control authority. Results associated with blank contamination for an analyte regulated in a discharge cannot be used to demonstrate regulatory compliance. QC failures do not relieve a discharger or permittee of reporting timely results.

9.6 The specifications contained in this method can be met if the apparatus used is calibrated properly and then maintained in a calibrated state. The standards used for initial calibration (Section 10.3), calibration verification (Sections 14.2 and 14.3), and for initial (Section 9.2.1) and ongoing (Section 14.5) precision and recovery may be prepared from the same source; however, the use of a secondary source for calibration verification is highly recommended whenever available. If standards from a different vendor are not available, a different lot number from the same vendor can be considered a secondary source. A LC-MS/MS instrument will provide the most reproducible results if dedicated to the settings and conditions required for determination of PFAS by this method.

9.7 Depending on specific program requirements, field replicates may be collected to determine the precision of the sampling technique, and spiked samples may be required to determine the accuracy of the analysis when the extracted internal standard method is used.

9.8 Matrix spikes generally are not required for isotope dilution methods because any deleterious effects of the matrix should be evident in the recoveries of the isotopically labeled compounds spiked into every sample. However, because some of the compounds are quantified by a non-analogous isotopically labeled compounds (e.g., PFPeS is quantified by $^{13}C_3$-PFHxS), the analysis of matrix spike samples may help diagnose matrix interferences for specific compounds.

10.0 Calibration and Standardization

10.1 Mass Calibration

The mass spectrometer must undergo mass calibration to ensure accurate assignments of m/z's by the instrument. This mass calibration must be performed at least annually to maintain instrument sensitivity and stability. Mass calibration must be repeated on an as-needed basis (e.g., QC failures, ion masses fall outside of the required mass window, major instrument maintenance, or if the instrument is moved). Mass calibration must be performed using the calibration compounds and procedures prescribed by the manufacturer.

Multiple Reaction Monitoring (MRM) analysis is required to achieve better sensitivity than full-scan analysis. The default parent ions, quantitation ions (Q1), and confirmation (Q2) ions that were monitored during the single-laboratory validation of this method are listed in Table 2 for each native analyte, EIS, and NIS.

10.1.1 During the development of this method, instrumental parameters were optimized for the precursor and product ions listed on Table 2. Product ions other than those listed may be selected; however, the use of ions with lower mass or common ions that may not provide sufficient discrimination between analytes of interest and co-eluting interferences must be avoided.

10.1.2 Optimize the response of the precursor ion $[M-H]^-$ or $[M-CO_2]^-$ for each method analyte following the manufacturer's guidance. MS parameters (e.g., source voltages, source and desolvation temperatures, gas flow, etc.) must be methodically changed until optimal analyte responses are determined. Typically, carboxylic acids have similar MS/MS conditions and sulfonic acids have similar MS/MS conditions. However, since analytes may have different optimal parameters, some compromise on the final operating conditions may be required.

10.1.3 Establish suitable operating conditions using the manufacturer's instructions and use the table below of MS conditions used during the development of this method as guidance.

Operating Conditions for Waters Acquity UPLC, TQ-S Xevo MS/MS

Injection volume	2.0 µL	(This is the default volume, and may be changed to improve performance)
MS/MS Conditions	Source Temp (ºC)	140
	Desolvation Temp (°C)	500
	Capillary Voltage (kV)	0.70
	Cone Gas (L/h)	~70
	Desolvation gas (L/h)	~800

10.1.4 As noted above, perform the mass calibration following the instrument manufacturer's instructions, using the calibrant prescribed by the manufacturer.

10.1.5 Regardless of the calibrant used, mass calibration is judged on the basis of the presence or absence of the exact calibration masses (e.g., a limit on the number of masses that are "missed"). If peaks are missing or not correctly identified, adjust the MS/MS, and repeat the test. Only after the MS/MS is properly calibrated may standards, blanks, and samples be analyzed.

10.1.6 Mass spectrometer optimization – Prior to measurements of a given analyte the mass spectrometer must be separately optimized for that analyte.

10.1.6.1 Using the post-column pump, separately infuse a solution containing 2 - 5 µg/mL of each compound in methanol into the MS.

10.1.6.2 Optimize sensitivity for the product ion m/z for each compound. Precursor-product ion m/z's other than those listed may be used provided requirements in this method are met.

10.1.6.3 After MS calibration and optimization and LC-MS/MS calibration, the same LC-MS/MS conditions must be used for analysis of all standards, blanks, IPR and OPR standards, and samples.

10.1.7 Mass Calibration Verification

The mass calibration must be verified prior to the analysis of any standards and samples and after each subsequent mass calibration. Each laboratory must follow the instructions for their instrument software to confirm the mass calibration, mass resolution, and peak relative response.

10.1.7.1 Check the instrument mass resolution to ensure that it is at least unit resolution. Inject a mid-level CAL standard under LC-MS/MS conditions to obtain the retention times of each method analyte. 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. The product ions (also the quantitation ions) chosen during method development are listed in Table 2, although these will be instrument dependent. Unit resolution must meet the manufacturer's criteria.

10.1.7.2 Check the mass calibration by measuring the amount of peak drift from the expected masses. If the peak apex has shifted more than approximately 0.2 Da, then the instrument will need to be recalibrated following the manufacturer's instructions.

10.2 Chromatographic conditions

10.2.1 The chromatographic conditions should be optimized for compound separation and sensitivity. The same optimized operating conditions must be used for the analysis of all standards, blanks, IPR and OPR standards, and samples. The following table gives the suggested chromatographic conditions for this method using the specified instrument and column. Different instruments may require slightly different operating conditions. **Modification of the solvent composition of the standard or extract by increasing the aqueous content to prevent poor peak shape is not permitted.** The peak shape of early eluting compounds may be improved by increasing the volume of the injection loop or increasing the aqueous content of the initial mobile phase composition.

General LC Conditions

Column Temp (°C) 40

Max Pressure (bar) 1100.0

LC Gradient Program

Time (min)	Flow mixture [1,2]	Flow Rate Program	Gradient Curve
0.0	2% eluent A, 98% eluent B	0.35 mL/min	Initial
0.2	2% eluent A, 98% eluent B	0.35 mL/min	2
4.0	30% eluent A, 70% eluent B	0.40 mL/min	7
7.0	55% eluent A, 45% eluent B	0.40 mL/min	8
9.0	75% eluent A, 25% eluent B	0.40 mL/min	8
10.0	95% eluent A, 5% eluent B	0.40 mL/min	6
10.4	2% eluent A, 98% eluent B	0.40 mL/min	10
11.8	2% eluent A, 98% eluent B	0.40 mL/min	7
12.0	2% eluent A, 98% eluent B	0.35 mL/min	1

[1] Eluent A = Acetonitrile

[2] Eluent B = 2 mM ammonium acetate in 95:5 water/acetonitrile

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Note: *LC system components, as well as the mobile phase constituents, may contain many of the analytes in this method. Thus, these PFAS will build up on the head of the LC column during mobile phase equilibration. To minimize the background PFAS peaks and to keep baseline 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, priming the mobile phase and flushing the column with at least 90% methanol before initiating a sequence may reduce background contamination.*

10.2.2 Retention time calibration

10.2.2.1 Inject compound solution(s) to determine its retention time. The laboratory may want to inject compounds separately the first time they perform the calibration. All native compounds for which there is an isotopically labeled analog will elute slightly before or with the labeled analog. Store the retention time (RT) for each compound in the data system.

10.2.2.2 Once RT windows have been confirmed for each analyte, once per ICAL and at the beginning of the analytical sequence, the position of each method analyte, EIS analyte, and NIS analyte peaks shall be set using the midpoint standard of the ICAL curve when ICAL is performed. When ICAL is not performed, the initial CV retention times or the midpoint standard of the ICAL curve can be used to establish the RT window position.

10.2.2.3 Method analyte, EIS analyte, and NIS analyte RTs must fall within 0.4 minutes of the predicted retention times from the midpoint standard of the ICAL or initial daily CV, whichever was used to establish the RT window position for the analytical batch. All branched isomer peaks identified in either the calibration standard or the qualitative (technical grade) standard must fall within in the retention time window for that analyte.

10.2.2.4 For all method analytes with exact corresponding isotopically labeled analogs, method analytes must elute within 0.1 minutes of the associated EIS.

10.2.2.5 When establishing the chromatographic conditions, it is important to consider the potential interference of bile salts during analyses of tissue samples. Inject the

bile salt interference check standard containing TDCA (see Section 7.5 if the mobile phase is not acetonitrile) during the retention time calibration process and adjust the conditions to ensure that TDCA (or TCDCA and TUDCA) does not coelute with any of the target analytes, EIS, or NIS standards. Analytical conditions must be set to allow a separation of at least 1 minute between the bile salts and the retention time window of PFOS as described in Section 7.3.3. This evaluation is required when establishing the chromatographic conditions for the method, **regardless of the sample matrices to be analyzed**.

10.3 Initial calibration

Initial calibration is performed using a series of at least six solutions, with at least five of the six calibration standards being within the quantification. (If a second-order calibration model is used, then one additional concentration is required.) The initial calibration solutions contain the entire suite of isotopically labeled compounds, NISs, and target compounds. Calibration is verified with a calibration verification (CV) standard at least once every ten field samples or less, by analysis of a mid-level calibration solution. Calibration verification uses the mean RRs or RFs determined from the initial calibration to calculate the analyte concentrations in the verification standard.

Note: *Six calibration standards is the minimum number that must be used in the initial calibration; however, the laboratory may use more standards, as long as the criteria in Section 10.3.3.3 can be met.*

Prior to the analysis of samples, and after the mass calibration check has met all criteria in Section 10.1.4, each LC-MS/MS system must be calibrated at a minimum of 6 standard concentrations (Section 7.3.4 and Table 4). This method procedure calibrates and quantifies 40 PFAS target analytes, using the isotopically labeled compounds added to the sample prior to extraction, by one of two approaches:

- True isotope dilution quantification (ID), whereby the response of the target compound is compared to the response of its isotopically labeled analog. Twenty-four target compounds are quantified in this way.
- Extracted internal standard quantification (EIS), whereby the response of the target compound is compared to the response of the isotopically labeled analog of another compound with chemical and retention time similarities. Sixteen target compounds are quantified in this way.

10.3.1 Initial calibration frequency

Each LC-MS/MS system must be calibrated whenever the laboratory takes corrective action that might change or affect the initial calibration criteria, or if either the CV or Instrument Sensitivity Check (ISC) acceptance criteria have not been met.

10.3.2 Initial calibration procedure

Prepare calibration standards containing the native compounds, EISs, and NISs, at the concentrations described in Table 4. Analyze each calibration standard by injecting 2.0 µL (this volume may be changed to improve performance).

Note: *The same injection volume must be used for all standards, samples, blanks, and QC samples.*

10.3.3 Initial calibration calculations

10.3.3.1 Instrument sensitivity

Sufficient instrument sensitivity is established if a signal-to-noise ratio ≥ 3:1 can be achieved when analyzing the lowest concentration standard within the quantitation range that the laboratory includes in its assessment of calibration linearity (Table 4).

10.3.3.2 Response Ratios (RR) and Response Factors (RF)

The response ratio (RR) for each compound calibrated by isotope dilution is calculated according to the equation below, separately for each of the calibration standards, using the areas of the quantitation ions (Q1) with the m/z shown in Table 2. RR is used for the 24 compounds quantified by true isotope dilution.

$$RR = \frac{Area_n \, M_l}{Area_l \, M_n}$$

where:

$Area_n$ = The measured area of the Q1 m/z for the native (unlabeled) PFAS

$Area_l$ = The measured area at the Q1 m/z for the corresponding isotopically labeled PFAS added to the sample before extraction

M_l = The mass of the isotopically labeled compound in the calibration standard

M_n = The mass of the native compound in the calibration standard

Similarly, the response factor (RF) for each unlabeled compound calibrated by extracted internal standard is calculated according to the equation below. RF is used for the 16 compounds quantified by extracted internal standard.

$$RF = \frac{Area_s \, M_{EIS}}{Area_{EIS} \, M_s}$$

where:

$Area_s$ = The measured area of the Q1 m/z for the target (unlabeled) PFAS

$Area_{EIS}$ = The measured area at the Q1 m/z for the isotopically labeled PFAS used as the extracted internal standard (EIS)

M_{EIS} = The mass of the isotopically labeled PFAS used as the extracted internal standard (EIS) in the calibration standard

M_s = The mass of the target (unlabeled) PFAS in the calibration standard

A response factor (RF_s) is calculated for each isotopically labeled compound in the calibration standard using the equation below. RF_s is used for the 24 isotopically labeled compounds quantified by non-extracted internal standard.

$$RF_s = \frac{Area_l \, M_{NIS}}{Area_{NIS} \, M_l}$$

where:

$Area_l$ = The measured area of the Q1 m/z for the isotopically labeled PFAS standard added to the sample before extraction

$Area_{NIS}$ = The measured area at the Q1 m/z for the isotopically labeled PFAS used as the non-extracted internal standard (NIS)

M_{NIS} = The mass of the isotopically labeled compound used as the non-extracted internal standard (NIS) in the calibration standard

M_l = The mass of the isotopically labeled PFAS standard added to the sample before extraction

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Note: *Other calculation approaches may be used, such as linear regression or non-linear regression, based on the capability of the data system used by the laboratory.*

10.3.3.3 Instrument Linearity

One of the following two approaches must be used to evaluate the linearity of the instrument calibration:

Option 1: Calculate the relative standard deviation (RSD) of the RR or RF values of the six initial calibration standards for each native compound and isotopically labeled compound. The RSD must be ≤ 20% to establish instrument linearity.

Option 2: Calculate the relative standard error (RSE) of the six initial calibration standards for each native compound and isotopically labeled compound. The RSE for all method analytes must be ≤ 20% to establish instrument linearity.

10.3.3.4 Non-extracted Internal Standard Area

Each time an initial calibration is performed, use the data from all the initial calibration standards used to meet the linearity test in Section 10.3.3.3 to calculate the mean area response for each of the NIS compounds, using the equation below.

$$Mean\ Area_{NIS_i} = \frac{\sum Area_{NIS_i}}{n}$$

where:

$Area_{NIS_i}$ = Area counts for the *ith* NIS, where *i* ranges from 1 to 7, for the seven NIS compounds listed in Table 1

n = The number of ICAL standards (the default value is n = 6). If a different number of standards is used for the ICAL, for example, to increase the calibration range or by dropping a point at either end of the range to meet the linearity criterion, change 6 to match the actual number of standards used)

Record the mean areas for each NIS for use in evaluating results for sample analyses (see Section 14.9). There is no acceptance criterion associated with the mean NIS area data.

10.3.4 Initial calibration corrective actions

If the instrument sensitivity or the instrument linearity criteria for initial calibration are not met, inspect the system for problems and take corrective actions to achieve the criteria. This may require the preparation and analysis of fresh calibration standards. All initial calibration criteria must be met before any samples or required blanks are analyzed.

10.3.5 Bile salts interference check

The laboratory must analyze a bile salt interference check standard (see Section 7.5) after the initial calibration as a check on the chromatographic conditions, even if tissue samples are not going to be run. If an interference is present, the chromatographic conditions must be modified to eliminate the interference from the bile salts (e.g., changing the retention time of the bile salts such that they fall outside the retention time window for any of the linear or branched PFOS isomers in the standard described in Section 7.3.3 by at least one minute), and the initial calibration repeated. If tissue sample analyses are not being conducted, this check may be skipped.

11.0 Sample Preparation and Extraction

For aqueous samples that contain particles and solid samples, percent solids are determined using the procedures in Section 11.1. This section describes the sample preparation procedures for aqueous samples with < 50 mg solids (Section 11.2), solid (soil, sediment or biosolid) samples (Section 11.3) and tissue samples (Section 11.4).

Note: *It is highly recommended that the laboratory pre-screen all samples prior to performing the analysis (see Appendix A). For aqueous samples, use the secondary container provided for percent solids to perform the pre-screening. If high levels of PFAS are present in the sample, a lower volume is required for analysis.*

The laboratory may subsample the aqueous samples as described in Appendix B; however, subsampling must meet project-specific requirements. The laboratory must notify the client before proceeding with subsampling. Once the laboratory becomes familiar with the levels of PFAS in the samples for their clients, the samples should be collected in the appropriate sample container size to avoid subsampling. The sample data report must state when subsampling has been employed.

Do not use any fluoropolymer articles or task wipes in these extraction procedures. Use only HDPE or polypropylene wash bottles and centrifuge tubes. Reagents and solvents for cleaning syringes may be kept in glass containers.

11.1 Determination of percent solids

11.1.1 Determination of percent suspended solids – Aqueous liquids and multi-phase samples consisting of mainly an aqueous phase

11.1.1.1 Desiccate and weigh a glass fiber filter (Section 6.4.3) to three significant figures.

11.1.1.2 Filter 10.0 ± 0.02 mL of well-mixed sample through the filter.

11.1.1.3 Dry the filter a minimum of 12 hours at 110 ± 5 ºC and cool in a desiccator.

11.1.1.4 Calculate percent solids as follows:

$$\%\ solids = \frac{weight\ of\ sample\ aliquot\ after\ drying\ (g) - weight\ of\ filter\ (g)}{10\ g}\ x\ 100$$

11.1.2 Solids (excluding tissues)

11.1.2.1 Weigh 5 to 10 g of sample to three significant figures in a tared beaker.

11.1.2.2 Dry a minimum of 12 hours at 110 ± 5 ºC, and cool in a desiccator.

11.1.2.3 Calculate percent solids as follows:

$$\%\ solids = \frac{weight\ of\ sample\ aliquot\ after\ drying\ (g)}{weight\ of\ sample\ aliquot\ before\ drying\ (g)}\ x\ 100$$

11.2 Aqueous sample processing

This method is applicable to aqueous samples containing up to 50 mg of suspended solids per sample. The procedure requires the preparation of the entire sample. Smaller sample volumes may be analyzed for samples containing solids greater than specified for this method, or when unavoidable due to high level of PFAS; however, subsampling should be avoided whenever possible. Typical sample size is 500 mL; however, sample size may be up to 1000 mL. The sample is to be analyzed in its entirety and should not be filtered. Leachate samples are analyzed using a 100-mL sample volume. Therefore, they must not be included in the same sample preparation batch as aqueous samples analyzed which are analyzed using 500-mL sample volumes.

11.2.1 Homogenize the sample by inverting the sample 3 – 4 times and allowing the sample to settle. Do not filter the sample. The standard procedure is to analyze the entire sample, plus a basic methanol rinse of the container.

11.2.2 The volume of the aqueous sample analyzed is determined by weighing the full sample bottle and then the empty sample bottle (see Section 12.2). Weigh each sample bottle (with the lid) to 0.1 g.

11.2.3 Prepare a method blank and two OPRs using PFAS-free water in HDPE bottles. Select a volume of water that is typical of the samples in the batch. Spike one OPR sample with native standard solution (Section 7.3.3) at 2x the LOQ (LLOPR). This aliquot will serve to verify the LOQ. Spike the other OPR sample at the concentration of the mid-level calibration point. This aliquot will serve as the traditional OPR.

Note: *If matrix spikes are required for a specific project, spike the field sample bottles designated for use as MS/MSD samples with native standard solution (Section 7.3.3) at a concentration 3 to 5 times the background concentration determined during screening of the unspiked sample. If screening was not performed, then spike those samples at the concentration of the mid-level calibration point.*

11.2.4 Spike an aliquot of EIS solution (Section 7.3.1) directly into the sample in the original bottle (or subsampled bottle) as well as to the bottles prepared for the QC samples. Mix by swirling the sample container.

11.2.5 Check that the pH is 6.5 ± 0.5. If necessary, adjust pH with 50% formic acid (Section 7.1.13.4) or ammonium hydroxide (or with 5% formic acid [Section 7.1.13.3] and 3% aqueous ammonium hydroxide [Section 7.1.6.2]). The sample is now ready for solid-phase extraction (SPE) and cleanup (Section 12.0).

11.3 Solid sample processing (excluding tissues)

Use a stainless spoon to mix the sample in its original jar. If it is impractical to mix the sample within its container transfer the sample to a larger container. Remove rocks, invertebrates, and foreign objects. Vegetation can either be removed from the sample before homogenization or cut into small pieces and included in the sample, based on project requirements. Mix the sample thoroughly, stirring from the bottom to the top and in a circular motion along the sides of the jar, breaking particles to less than 1 mm by pressing against the side of the container. The homogenized sample should be even in colour and have no separate layers. Store the homogenized material in its original container or in multiple smaller containers. Determine the percent solids as per Section 11.1.2.

Note: *The maximum sample weight for sediment or soil is 5 g dry weight. The maximum sample weight for biosolids is 0.5 g dry weight.*

Small amounts of the reagent water used for method blanks (10% of sample weight or less) can be added to unusually dry samples. This is an option, not a requirement.

11.3.1 Weigh out an aliquot of solid sample, not dried (aliquot should provide 5 g dry weight for soil and sediment or 0.5 g dry-weight for biosolids) into a 50-mL polypropylene centrifuge tube. Because biosolids samples are analyzed with a 0.5-g sample, they must not be included in the same sample preparation batch as solid samples analyzed with 5-g sample masses.

11.3.2 Prepare batch QC samples using 5 g of reference solid (Section 7.2.2) wetted with 2.5 g of reagent water for the method blank and two OPRs (use 0.5 g of reference solid with 0.25 g of reagent water for biosolid sample batches). The addition of reagent water to the sand provides a matrix closer in composition to real-world samples. Spike one OPR sample with native standard solution (Section 7.3.3) at 2x the LOQ (LLOPR). This aliquot will serve to verify the LOQ. Spike the other OPR sample at the concentration of the mid-level calibration point. This aliquot will serve as the traditional OPR.

Note: *If matrix spikes are required for a specific project, spike the field sample aliquots designated for MS/MSD samples with native standard solution (Section 7.3.3) at the concentration 3 to 5 times the background concentration determined during screening of the unspiked sample. If screening was not performed, then spike those samples at the concentration of the mid-level calibration point.*

11.3.3 Spike an aliquot of EIS solution (Section 7.3.1) directly into each centrifuge tube containing the aliquoted field and QC samples. Vortex the sample to disperse the standard and allow to equilibrate for at least 30 minutes.

11.3.4 Add 10 mL of 0.3% methanolic ammonium hydroxide (Section 7.1.7.1) to each centrifuge tube. Vortex to disperse, then shake for 30 minutes on a variable speed mixing table. Centrifuge at 2800 rpm for 10 minutes and transfer the supernatant to a clean 50-mL polypropylene centrifuge tube.

11.3.5 Add 15 mL of 0.3% methanolic ammonium hydroxide (Section 7.1.7.1) to the remaining solid sample in each centrifuge tube. Vortex to disperse, then shake for 30 minutes on a variable speed mixing table. Centrifuge at 2800 rpm for 10 minutes and decant the supernatant from the second extraction into the centrifuge tube with the supernatant from the first extraction.

11.3.6 Add another 5 mL of 0.3% methanolic ammonium hydroxide (Section 7.1.7.1) to the remaining sample in each centrifuge tube. Shake by hand to disperse, centrifuge at 2800 rpm for 10 minutes and decant the supernatant from the third extraction into the centrifuge tube with supernatant from the first and second extractions.

11.3.7 Using a 10-mg scoop, add 10 mg of carbon (Section 7.1.17) to the combined extract, mix by occasional hand shaking for no more than five minutes and then centrifuge at 2800 rpm for 10 minutes. Immediately decant the extract into a 60-mL glass evaporation or concentrator tube.

11.3.8 The laboratory has the option to dilute the extract to approximately 35 mL with reagent water. (Some laboratories may prefer not to add any additional water, therefore, this dilution is optional.) A separate concentrator tube marked at the 35-mL level may be kept for a visual reference to get the approximate volume. Samples containing more than 50% water may yield extracts that are greater than 35 mL in volume; therefore, do not add water to these. Determine the water content in the sample as follows (percent moisture is determined from the % solids):

$$Water\ Content\ in\ Sample = \frac{Sample\ Weight\ (g) \times Moisture\ (\%)}{100} + any\ water\ added\ in\ 11.3.2\ and\ 11.3.8$$

11.3.9 Concentrate each extract at approximately 55 ºC with a N_2 flow of approximately 1.2 L/min to a final volume that is based on the water content of the sample (*see table below*). Allow extracts to concentrate for 25 minutes, then mix (by vortex if the volume is < 20 mL or using a glass pipette if the volume is > 20 mL). Continue concentrating and mixing every 10 minutes until the extract has been reduced to the required volume as specified in the table below. If the extract volume appears to stop dropping, the concentration must be stopped and the volume at which it was stopped recorded. The concentrated extract must still contain some methanol, about 5-10 mL. The pre-cleanup extract in 11.3.10 should contain no more than 20% methanol. The laboratory has flexibility to modify the volumes used to achieve this goal. Some laboratories may prefer not to add water in Section 11.3.8. The following table provides guidance to help determine the final extract volume, based on the water content of the original solid sample.

Water Content in Sample*	Concentrated Final Volume
< 5 g	7 mL
5 - 8 g	8 mL
8 - 9 g	9 mL
9 - 10 g	10 mL

* Based on the % solids result determined in Section 11.1.2.3, and including any water added to the sample in Sections 11.3.2 or the extract in Section 11.3.8.

A good rule of thumb is to make the "Concentrated Final Volume" 7 - 10 mL above the "Water Content in Sample" value.

Note: *Slowly concentrating extracts, in 1-mL increments, is necessary to prevent excessive concentration and the loss of neutral compounds (methyl and ethyl FOSEs and FOSAs) and other highly volatile compounds. The extract must be concentrated to remove the methanol as excess methanol present during SPE clean-up results in poor recovery of C_{13} and C_{14} carboxylic acids and C_{10} and C_{12} sulfonates.*

If all of the methanol is evaporated, the aforementioned neutral compounds are likely to have poor recovery, if too much methanol is in the final concentrated extract, then the aforementioned longer-chain compounds are likely to have poor recovery.

11.3.10 Add 40 - 50 mL of reagent water to the extract and vortex. Check that the pH is 6.5 ±0.5 and adjust as necessary with 50% formic acid (Section 7.1.13.4) or 30% ammonium hydroxide (or with 5% formic acid [Section 7.1.13.3] and 3% aqueous ammonium hydroxide [Section 7.1.6.2]). The extracts are ready for SPE and cleanup (Section 12.0).

11.4. Tissue sample processing

Prior to processing tissue samples, the laboratory must determine the exact tissue to be analyzed. Common requests for analysis of fish tissue include whole fish with the skin on, whole fish with the skin removed, edible fish fillets (filleted in the field or by the laboratory), specific organs, and other portions. Once the appropriate tissue has been determined, the samples must be prepared and homogenized.

If the laboratory must dissect the whole fish to obtain the appropriate tissue for analysis, cover the benchtop with clean aluminum foil and use clean processing equipment (knives, scalpels, tweezers) to dissect each sample to prevent cross-contamination. Samples should be handled in a semi-thawed state for compositing and/or homogenization. All tissue comprising a sample is collected in a stainless-steel bowl during grinding, then mixed using a stainless-steel spoon. Homogenized samples must be stored in clean HDPE containers and stored frozen for subsequent use.

If using a grinder, after the entire sample has been processed, mix the ground tissue with a spoon, transfer back to the grinder, and repeat the grinding at least two more times until the homogenize tissue has a consistent texture and color.

11.4.1 For each sample, weigh a 2-g aliquot of homogenized tissue into a 15-mL polypropylene centrifuge tube. Reseal the container with the remaining homogenized portion of the sample and return it to frozen storage in the event that it needs to be used for reanalysis.

Note: *The default sample weight for tissue is 2 g wet weight; however, a 1-g sample may be used. Higher sample weights are not recommended for this method.*

11.4.2 Prepare the batch QC samples using 2 g of reference tissue matrix (Section 7.2.3) for the method blank and two OPRs. Spike one OPR sample with native standard solution (Section 7.3.3) at 2x the LOQ (LLOPR). This aliquot will serve to verify the LOQ. Spike the other OPR sample at the concentration of the mid-level calibration point. This aliquot will serve as the traditional OPR.

Note: *If matrix spikes are required for a specific project, spike the field sample aliquots designated as MS/MSD samples with native standard solution (Section 7.3.3) at the concentration 3 to 5 times the background concentration determined during screening of the unspiked sample. If screening was not performed, then spike those samples at the concentration of the mid-level calibration point.*

11.4.3 Spike an aliquot of EIS solution (Section 7.3.1) directly into each field and QC sample. Vortex and allow to equilibrate for at least 30 minutes.

11.4.4 Add 10 mL of 0.05M KOH in methanol (Section 7.1.8) to each sample. Vortex to disperse the tissue then place tubes on a variable speed mixing table to extract for at least 16 hours.

Centrifuge at 2800 rpm for 10 minutes and collect the supernatant in a 50-mL polypropylene centrifuge tube.

11.4.5 Add 10 mL of acetonitrile to remaining tissue in the 15-mL centrifuge tube, vortex to mix and disperse the tissue. Sonicate for 30 minutes. Centrifuge at 2800 rpm for 10 minutes and collect the supernatant, adding it to the 50-mL centrifuge tube containing the initial extract.

11.4.6 Add 5 mL of 0.05M KOH in methanol (Section 7.1.8) to the remaining sample in each centrifuge tube. Vortex to disperse the tissue and hand mix briefly. Centrifuge at 2800 rpm for 10 minutes and collect the supernatant, adding it to the 50-mL centrifuge tube containing the first two extracts.

11.4.7 Using a 10-mg scoop, add 10 mg of carbon (Section 7.1.17) to the combined extract, mix by occasional hand shaking over a period of no more than five minutes and then centrifuge at 2800 rpm for 10 minutes. Immediately decant the extract into a 60-mL glass evaporation or concentrator tube.

11.4.8 Add 1 mL of reagent water to each evaporation/concentrator tube, set the evaporator/concentrator to 55 ºC with a N_2 flow of 1.2 L/min and concentrate the extract to 2.5 mL (only ~1 mL of the methanol should remain).

11.4.9 Add reagent water to each evaporation/concentrator tube to dilute the extracts to 50 mL. Check that the pH = 6.5 ± 0.5 and adjust as needed with 50% formic acid (Section 7.1.13.4) or ammonium hydroxide (or with 5% formic acid [Section 7.1.13.3] and 3% aqueous ammonium hydroxide [7.1.6.2]). The extracts are ready for SPE and cleanup (Section 12.0).

12.0 Extraction, Cleanup, and Concentration

Samples of all matrices (and the associated batch QC) must undergo SPE and carbon cleanup to remove interferences (Section 12.1). Sample elution as well as any further extract treatment is matrix specific and may be found in Sections 12.2 through 12.4.

Note: *Carbon cleanup is required. Carbon cleanup may remove analytes if the sample has a very low organic carbon content (this is unusual for non-drinking water environmental samples). This will be apparent if the isotope dilution standard recoveries are significantly higher on the reanalysis. If the laboratory can demonstrate that the carbon cleanup is detrimental to the sample analysis (by comparing results when skipping the carbon cleanup during reanalysis), then the carbon cleanup may be skipped for that specific sample.*

12.1 All sample matrices

12.1.1 Pack clean silanized glass wool to half the height of the WAX SPE cartridge barrel (Section 6.7.1).

12.1.2 Set up the vacuum manifold with one WAX SPE cartridge plus a reservoir and reservoir adaptor for each cartridge for each sample and QC aliquot.

12.1.3 Pre-condition the cartridges by washing them with 15 mL of 1% methanolic ammonium hydroxide (Section 7.1.7.2) followed by 5 mL of 0.3M formic acid (Section 7.1.13.2) (do

not use the vacuum for this step). Do not allow the WAX SPE to go dry. Discard the wash solvents.

12.1.4 Pour the sample into the reservoir (do not use a pipette), taking care to avoid splashing while loading. Adjust the vacuum and pass the sample through the cartridge at 5 mL/min. Retain the empty sample bottle and allow it to air dry for later rinsing (Section 12.2.2). Discard eluate.

Note: *For aqueous samples, in the event the SPE cartridge clogs during sample loading, place a second pre-conditioned cartridge and continue loading the remaining sample aliquot using the same reservoir. Proceed to Section 12.1.5.*

12.1.5 Rinse the walls of the reservoir with 5 mL reagent water (twice) followed by 5 mL of 1:1 0.1M formic acid/methanol (Section 7.1.13.5) and pass those rinses through the cartridge using vacuum. Dry the cartridge by pulling air through for 15 seconds. Discard the rinse solution. Continue to the elution and concentration steps based on the matrix (see Section 12.2 – Aqueous, Section 12.3 – Solids, and Section 12.4 – Tissue).

12.2 Elution and extract concentration of aqueous samples

Note: *If two cartridges were used, perform Sections 12.2.1 through 12.2.3 with each cartridge. Filter the eluates through a 25-mm, 0.2-µm syringe filter. Combine both sets of filtered eluates into a clean tube, add the NIS solution, and vortex to mix. Transfer 350 µL of the filtered extract into a 1-mL polypropylene microvial and mark the level. Add another 350-µL portion and using a gentle stream of nitrogen (water bath at 40 ºC), concentrate to the 350-µL mark and submit for LC-MS/MS analysis. This concentration step is only applicable to situations where two SPE cartridges were eluted, each with 5 mL of elution solvent.*

12.2.1 Place clean collection tubes (13 x 100 mm polypropylene) inside the manifold, ensuring that the extract delivery needles do not touch the walls of the tubes. DO NOT add NIS to these collection tubes.

12.2.2 Rinse the inside of the sample bottle with 5 mL of 1% methanolic ammonium hydroxide (Section 7.1.7.2), then, using a glass pipette, transfer the rinse to the SPE reservoir, washing the walls of the reservoir. Use vacuum to pull the elution solvent through the cartridge and into the collection tubes.

Note: *Air dry the empty sample bottle after the rinse is transferred. Weigh the empty bottle with the cap on and subtract from the weight with the sample determined in Section 11.2.2.*

12.2.3 Add 25 µL of concentrated acetic acid to each sample eluted in the collection tubes and vortex to mix. Add 10 mg of carbon (Section 7.1.17) to each sample and batch QC extract, using a 10-mg scoop. Hand-shake occasionally for no more than 5 minutes. It is important to minimize the time the sample extract is in contact with the carbon. Immediately vortex (30 seconds) and centrifuge at 2800 rpm for 10 minutes.

12.2.4 Add NIS solution (Section 7.3.2) to a clean collection tube. Place a syringe filter (25-mm filter, 0.2-µm nylon membrane) on a 5-mL polypropylene syringe. Take the plunger out and carefully decant the sample supernatant into the syringe barrel. Replace the plunger and filter the entire extract into the new collection tube containing the NIS. Vortex to mix

and transfer a portion of the extract into a 1-mL polypropylene microvial for LC-MS/MS analysis. Cap the collection tube containing the remaining extract and store at 0 - 4 °C.

12.3 Elution and extract concentration of solid samples

12.3.1 Add NIS solution (Section 7.3.2) to a clean collection tube (13 x 100 mm polypropylene) for each sample and QC aliquot and place them into the manifold rack, ensuring the extract delivery needles are not touching the walls of the tubes.

12.3.2 Rinse the inside of the evaporation/concentrator tube using 5 mL of 1% methanolic ammonium hydroxide (Section 7.1.7.2), then, using a glass pipette, transfer the rinse to the reservoir, washing the walls of the reservoir. Use the vacuum to pull the elution solvent through the cartridge and into the collection tubes.

12.3.3 Add 25 µL of concentrated acetic acid to each sample extract in its collection tube and swirl to mix. Place a syringe filter (25-mm filter, 0.2-µm nylon membrane) on a 5 mL polypropylene syringe. Take the plunger out and carefully decant ~1 mL of sample extract into the syringe barrel. Replace the plunger and filter into a 1-mL polypropylene microvial for LC-MS/MS analysis. Cap the collection tube containing the remaining extract and store at 0 - 4 °C.

12.4 Elution and extract concentration of tissue samples

12.4.1 Add NIS solution (Section 7.3.2) to clean collection tubes (13 x 100 mm, polypropylene) for each sample and QC aliquot. Place the tubes into the manifold rack and ensure the extract delivery needles are not touching the walls of the tubes.

12.4.2 Rinse the inside of the evaporation/concentrator tube using 5 mL of 1% methanolic ammonium hydroxide (Section 7.1.7.2), then, using a glass pipette, transfer the rinse to the reservoir, washing the walls of the reservoir. Use the vacuum to pull the elution solvent through the cartridge and into the collection tubes.

12.4.3 Add 25 µL of concentrated acetic acid to each sample extract. Place a syringe filter (25-mm filter, 0.2-µm nylon membrane) on a 5-mL polypropylene syringe. Take the plunger out and carefully decant an aliquot (~1 mL) of the sample extract into the syringe barrel. Replace the plunger and filter into a 1-mL polypropylene microvial for LC-MS/MS analysis. Cap the collection tube containing the remaining extract and store at 0 - 4 °C.

13.0 Instrumental Analysis

Analysis of sample extracts for PFAS by LC-MS/MS is performed on an ultrahigh performance liquid chromatograph coupled to a triple quadrupole mass spectrometer, running manufacturer's software. The mass spectrometer is run with unit mass resolution in the multiple reaction monitoring (MRM) mode.

13.1 Perform mass calibration (Section 10.1), establish the operating conditions (Section 10.2), and perform an initial calibration (Section 10.3) prior to analyzing samples. If tissue samples are to be analyzed during the analytical shift, repeat the analysis of the bile salt interference check standard in Section 10.3.5 before analyzing any tissue samples.

13.2 Only after all performance criteria are met may blanks, MDLs, IPRs/OPRs, and samples be analyzed.

13.3 After a successful initial calibration has been completed, the analytical sequence for a batch of samples analyzed during the same time period is as follows. The volume injected for samples and QCs must be identical to the volume used for calibration (Section 10.2.3). Standards and sample extracts must be brought to room temperature and vortexed prior to aliquoting into an instrument vial in order to ensure homogeneity of the extract.

1. Instrument Blank
2. Instrument Sensitivity Check (see Section 10.3.3.1)
3. Calibration Verification Standard
4. Qualitative Identification Standards
5. Instrument Blank
6. Method Blank
7. Low-level OPR (LLOPR)
8. OPR
9. Bile salt interference check standard (Section 7.5) (only required during analytical sequences in which tissue samples are being analyzed)
10. Samples (10 or fewer)
11. Calibration Verification Standard
12. Instrument Blank
13. Samples (10 or fewer)
14. Calibration Verification Standard
15. Instrument Blank

If the results are acceptable, the closing calibration verification solution (#14 above) may be used as the opening solution for the next analytical sequence.

13.4 If the response exceeds the calibration range for any sample, extracts are diluted as per Section 15.3 to bring all target responses within the calibration range.

Note: *If the analytes that exceed the calibration range in the original analysis are known to not be of concern for the specific project (e.g., are not listed in a discharge permit), then the laboratory may consult with the client regarding the possibility of reporting that sample from the undiluted analysis.*

14.0 Performance Tests during Routine Operations

The following performance tests must be successfully completed as part of each routine instrumental analysis shift described in Section 13.3 above.

14.1 MS resolution – A mass calibration must be performed prior to analysis of the calibration curve. LC-MS/MS system performance is checked by performing an MS resolution verification after the mass calibration. MS resolution must be verified prior to any samples or QC as per Section 10.1. If the requirements in Section 10.1 cannot be met, the problem must be corrected before analyses can proceed. If any of the samples in the previous shift may be affected by poor mass resolution, the extracts of those samples must be re-analyzed.

14.2 Instrument sensitivity check

The signal-to-noise ratio of the ISC standard (Section 7.3.4) must be greater than or equal to 3:1. If the requirements cannot be met, the problem must be corrected before analyses can proceed.

Note: *An interim limit of 70-130% for 90% of the native and isotopically labeled compounds should be used, with the other recoveries achieving 50-150%.*

14.3 Calibration verification (CV)

After a passing MS resolution (Section 14.1) and a successful initial calibration (Section 10.3.3.3) is achieved, prior to the analysis of any samples, analyze a mid-level calibration standard (Section 7.3.4).

14.3.1 The calibration is verified by analyzing a CV standard at the beginning of each analytical sequence, every ten samples or less, and at the end of the analytical sequence.

14.3.2 Calculate concentration for each native and isotopically labeled compound in the CV using the equation in Section 15.2.

14.3.3 The recovery of native and isotopically labeled compounds for the CVs must be within 70 - 130%.

14.3.4 If the CV criterion in Section 14.3.3 is not met, recalibrate the LC-MS/MS instrument according to Section 10.3.

14.3.5 Ion abundance ratios

Using the data from the CV standard, compute the ion abundance ratio for each target analyte listed with a confirmation ion mass in Table 2, using the equation below. These ion abundance ratios will be used a part of the qualitative identification criteria in Section 15.1.

$$IAR = \frac{Area_{Q1}}{Area_{Q2}}$$

where:

IAR = Ion abundance ratio

$Area_{Q1}$ = The measured area of the Q1 m/z for the analyte in the mid-point calibration standard or daily CV standard, depending on the analyte concentration, as described in Section 15.1.3

$Area_{Q2}$ = The measured area of the Q2 m/z for the analyte in the mid-point calibration standard or daily CV standard, depending on the analyte concentration, as described in Section 15.1.3

Note: *Six of the analytes in Table 2 do not produce confirmation ions, so the IAR does not apply.*

Pending completion of the multi-laboratory validation study, construct an acceptance window for the IAR of each target analyte as 50% to 150% of the IAR in the mid-point calibration standard or daily CV standard as applicable per section 15.1.3.

14.4 Retention times and resolution

14.4.1 For all method analytes with exact corresponding isotopically labeled analogs, method analytes must elute within ± 0.1 minutes of the associated EIS.

14.4.2 The retention times of each native and isotopically labeled compound must be within ± 0.4 minutes of the ICAL or CV used to establish the RT windows for the samples and batch QC.

14.5 Ongoing precision and recovery (OPR)

14.5.1 After verification, analyze the extract of the OPR (Sections 12.2.4, 12.3.3, and 12.4.3) prior to analysis of samples from the same batch to ensure the analytical process is under control.

14.5.2 Compute the percent recovery of the native compounds by the appropriate quantification method depending on the compound (Section 10.3). Compute the percent recovery of each isotopically labeled compound by the non-extracted internal standard method (Sections 1.2 and 10.3).

$$Recovery\ (\%) = \frac{Concentration\ found\ (ng/mL)}{Concentration\ spiked\ (ng/mL)}\ x\ 100$$

14.5.3 For the native compounds and isotopically labeled compounds, compare the recovery to the OPR limits given in Table 5. If all compounds meet the acceptance criteria, system performance is acceptable, and analysis of blanks and samples may proceed. If, however, any individual concentration falls outside of the given range, the extraction/concentration processes are not being performed properly for that compound. In this event, correct the problem, re-prepare, extract, and clean up the sample batch and repeat the ongoing precision and recovery test.

14.5.4 If desired, add results that pass the specifications in Section 14.5.3 to initial and previous ongoing data for each compound in each matrix. Update QC charts to form a graphic representation of continued laboratory performance. Develop a statement of laboratory accuracy for each compound in each matrix type by calculating the average percent recovery (R) and the standard deviation of percent recovery (SR). Express the accuracy as a recovery interval from R - 2SR to R + 2SR. For example, if R = 95% and SR = 5%, the accuracy is 85 to 105%.

14.6 Instrument blank – At the beginning of the analytical sequence and after the analysis of high concentration samples (e.g., highest calibration standard, CV), analyze an instrument blank to ensure no instrument contamination has occurred.

14.7 Method blank – After the analysis of the solvent blank and prior to the analysis of samples, analyze a method blank (Section 9.5).

14.8 A qualitative identification standard (Section 7.3.5) containing all available isomers (branched and linear) is analyzed once daily at the beginning of the analytical sequence, to confirm the retention time of each linear and known branched isomer or isomer group.

14.9 Instrument sensitivity (optional)

This step is recommended as a follow-up step if the ISC does not meet criteria. Calculate the ratio of the NIS peak areas from the QC and field samples relative to the mean area of the corresponding NIS in the most recent initial calibration to check for possible bad injections of NIS solution or loss of instrument sensitivity.

$$Area\ Ratio_{NIS_i}(\%) = 100 \times \frac{Area\ of\ NIS_i\ in\ the\ Sample}{Mean\ Area_{NIS_i}}$$

where:

Area of NIS_i in the Sample = Observed area counts for NIS_i in the sample

Mean $Area_{NISi}$ = The mean area counts for the corresponding NIS from the most recent initial calibration, calculated as described in Section 10.3.3.4

i = Indicates each of the seven NIS compounds listed in Table 1

The NIS areas in the field samples and QC samples should be within 50 – 200% of the mean area of that NIS in the initial calibration standards. If the areas are low for all the field samples and QC samples in the batch, it suggests a loss of instrument sensitivity, while low areas in only some field or QC samples suggests a possible bad injection.

15.0 Data Analysis and Calculations

15.1 Qualitative determination and peak identification

A native or isotopically labeled compound is identified in a standard, blank, sample, or QC sample when all of the criteria in Sections 15.1.1 through 15.1.4 are met.

15.1.1 Peak responses must be at least three times the background noise level (S/N 3:1). If the S/N ratio is not met due to high background noise, the laboratory must correct the issue (e.g., perform instrument troubleshooting to check and if needed, replace, the transfer line, column, detector, liner, filament, etc.). If the S/N ratio is not met but the background is low, then the analyte is to be considered a non-detect.

15.1.2 Target analyte, EIS analyte, and NIS analyte RTs must fall within ± 0.4 minutes of the predicted retention times from the midpoint standard of the ICAL or initial daily CV, whichever was used to establish the RT window position for the analytical batch. The retention time window used must be of sufficient width to detect earlier-eluting branched isomers. For all method analytes with exact corresponding isotopically labeled analogs, method analytes must elute within ± 0.1 minutes of the associated EIS.

15.1.3 The laboratory must follow the identification requirements specified by the client for the project. In the event there are no project-specific requirements, the following general requirements apply. For concentrations at or above the method LOQ, the total quantification ion (Q1) response to the total confirmation ion (Q2) response ratio must fall within ± 50% of the ratio observed in the mid-point initial calibration standard. If project-specific requirements involve reporting sample concentrations below the LOQ or ML, the response ratio must also fall within ± 50% of the ratio observed in the initial daily CV (see Section 14.3.5).

The total response of all isomers (branched and linear) in the quantitative standards should be used to define ratio. In samples, the total response should include only the branched isomer peaks that have been identified in either the quantitative or qualitative standard (see Section 7.3 regarding records of traceability of all standards). If standards (either quantitative or qualitative) are not available for purchase, only the linear isomer can be identified and quantitated in samples. The ratio requirement does not apply for PFBA, PFPeA, NMeFOSE, NEtFOSE, PFMPA, and PFMBA because suitable (not detectable or inadequate S/N) secondary transitions (Q2) are unavailable.

15.1.4 If the field sample result does not all meet the criteria stated in Sections 15.1.1 through 15.1.3, and all sample preparation avenues (e.g., extract cleanup, sample dilution, etc.) have been exhausted, the result may only be reported with a data qualifier alerting the data user that the result could not be confirmed because it did not meet the method-required criteria and therefore should be considered an estimated value. If the criteria listed above are not met for the standards, the laboratory must stop analysis of samples and correct the issue.

15.2 Quantitative determination

Concentrations of the target analytes are determined with respect to the extracted internal standard (EIS) which is added to the sample prior to extraction. The EIS is quantitated with respect to a non-extracted internal standard (NIS), as shown in Table 2, using the response ratios or response factors from the most recent multi-level initial calibration (Section 10.3). Other equations may be used if the laboratory demonstrates that those equations produce the same numerical result as produced by the equations below.

For the native analytes:

$$Concentration\ (ng/L\ or\ ng/g) = \frac{Area_n\ M_l}{Area_l(\overline{RR}\ or\ \overline{RF})} \times \frac{1}{W_S}$$

where:

$Area_n$ = The measured area of the Q1 m/z for the native (unlabeled) PFAS

$Area_l$ = The measured area at the Q1 m/z for the isotopically labeled PFAS (EIS). *See note below.*

M_l = The mass of the isotopically labeled compound added (ng)

$\overline{RR}$ = Average response ratio used to quantify target compounds by the isotope dilution method

$\overline{RF}$ = Average response factor used to quantify target compounds by the extracted internal standard method

W_S = Sample volume (L) or weight (g)

Note: *For better accuracy, PFTrDA is quantitated using the average of the areas of labeled compounds $^{13}C_2$-PFTeDA and $^{13}C_2$-PFDoA.*

And for the EIS analytes:

$$Concentration\ (ng/L\ or\ ng/g) = \frac{Area_l\ M_{NIS}}{Area_{NIS}\overline{RF_s}} \times \frac{1}{W_S}$$

where:

$Area_l$ = The measured area at the Q1 m/z for the isotopically labeled PFAS (EIS)

$Area_{NIS}$ = The measured area of the Q1 m/z for the non-extracted internal standard (NIS)

M_{NIS} = The mass of the added non-extracted internal standard (NIS) compound (ng)

W_S = Sample volume (L) or weight (g)

$\overline{RF_s}$ = Average response factor used to quantify the isotopically labeled compound by the non-extracted internal standard method

Results for native compounds are recovery corrected by the method of quantification. Extracted internal standard (EIS) recoveries are determined similarly against the non-extracted internal standard (NIS) and are used as general indicators of overall analytical quality.

The instrument measures the target analytes as either their anions or neutral forms. **The default approach for Clean Water Act uses of the method is to report the analytes in their acid or neutral forms,** using the following equation to convert the concentrations:

$$C_{Acid} = C_{Anion} \times \frac{MW_{Acid}}{MW_{Anion}}$$

where:

C_{Anion} = The analyte concentration in anion form
MW_{Acid} = The molecular weight of the acid form
MW_{Anion} = The molecular weight of the anion form

15.3 Sample dilutions

15.3.1 If the Q1 area for any compound exceeds the calibration range of the system, dilute a subsample of the sample extract with the methanolic ammonium hydroxide and acetic acid solution in Section 7.1.9 by a factor no greater than 10x and analyze the diluted extract. If the responses for each EIS in the diluted extract meet the S/N and retention time requirements in Sections 15.1.1 and 15.1.2, and the EIS recoveries from the analysis of the diluted extract are greater than 5%, then the compounds associated with those EISs may be quantified using isotope dilution. Use the EIS recoveries from the original analysis to select the dilution factor, with the objective of keeping the EIS recoveries in the dilution above that 5% lower limit (i.e., if the EIS recovery of the affected analyte in the undiluted analysis is 50%, then the sample cannot be diluted more than 10:1; if the if the EIS recovery of the affected analyte in the undiluted analysis is 30%, then the sample cannot be diluted more than 6:1). Adjust the compound concentrations, detection limits, and minimum levels to account for the dilution.

If the EIS responses in the diluted extract do not meet those S/N and retention time requirements, then the compound cannot be measured reliably by isotope dilution in the diluted extract. In such cases, the laboratory must take a smaller aliquot of any affected aqueous sample and dilute it to 500 mL with reagent water and analyze the diluted aqueous sample, or analyze a smaller aliquot of soil, biosolid, sediment, or tissue sample. Adjust the compound concentrations, detection limits, and minimum levels to account for the dilution.

If a dilution greater than 10x is indicated, then the laboratory must prepare and analyze a diluted aqueous sample or a smaller aliquot of a solid sample.

15.3.2 If the recovery of any isotopically labeled compound is outside of the acceptance limits (Table 5), a diluted aqueous sample or smaller aliquot (for solids and tissue) must be analyzed (Section 15.3.1). If the recovery of any isotopically labeled compound in the diluted sample is outside of the normal range, the method does not apply to the sample being analyzed and the result may not be reported or used for permitting or regulatory compliance purposes. In this case, an alternative column could be employed to resolve the interference. If all cleanup procedures in this method and an alternative column have been employed and isotopically labeled compound recovery remains outside of the normal range, extraction and/or cleanup procedures that are beyond this scope of this method will be required to analyze the sample.

15.4 Reporting of analytical results (acid/neutral forms)

The data reporting practices described here are focused on NPDES monitoring needs and may not be relevant to other uses of the method. For analytes reported in their acid form, use the equations in Section 15.2 and the analyte names Table 1. For analytes reported in their anion form, see Table 8 for the appropriate names and CAS Registry Numbers.

15.4.1 Report results for aqueous samples in ng/L. Report results for solid samples in ng/g, on a dry-weight basis, and report the percent solids for each sample separately. Report results for tissue samples in ng/g, on a wet-weight basis. Other units may be used if required in a permit or for a project. Report all QC data with the sample results.

15.4.2 Reporting level

Unless specified otherwise by a regulatory authority or in a discharge permit, results for analytes that meet the identification criteria are reported down to the concentration of the ML established by the laboratory through calibration of the instrument (see the glossary for the derivation of the ML). EPA considers the terms "reporting limit," "quantitation limit," "limit of quantitation," and "minimum level" to be synonymous.

15.4.2.1 Report a result for each analyte in each field sample or QC standard at or above the ML to 3 significant figures. Report a result for each analyte found in each field sample or QC standard below the ML as "<ML," where ML is the concentration of the analyte at the ML, or as required by the regulatory/control authority or permit.

15.4.2.2 Report a result for each analyte in a blank at or above the MDL to 2 significant figures. Report a result for each analyte found in a blank below the MDL as "<MDL," where MDL is the concentration of the analyte at the MDL, or as required by the regulatory/control authority or permit.

15.4.2.3 Report a result for an analyte found in a sample or extract that has been diluted at the least dilute level at which the area at the quantitation m/z is within the calibration range (e.g., above the ML for the analyte and below the highest calibration standard) and with isotopically labeled compound recoveries within their respective QC acceptance criteria. This may require reporting results for some analytes from different analyses.

15.4.2.4 Report recoveries of all associated EIS compounds for all field samples and QC standards. If a sample extract was diluted and analyzed, report the EIS recoveries from both the original analysis and the analysis of the dilution.

15.4.3 Results from tests performed with an analytical system that is not in control (i.e., that does not meet acceptance criteria for any QC tests in this method) must be documented and reported (e.g., as a qualifier on results), unless the failure is not required to be reported as determined by the regulatory/control authority. Results associated with a QC failure cannot be used to demonstrate regulatory compliance. QC failures do not relieve a discharger or permittee of reporting timely results. If the holding time would be exceeded for a reanalysis of the sample, the regulatory/control authority should be consulted for disposition.

16.0 Method Performance

Routine method performance is validated through analysis of matrix-specific reference samples, including spikes and certified reference materials. Ongoing method performance is monitored through QC samples analyzed alongside samples. The parameters monitored include percent recovery of isotopically labeled compounds, blank concentrations, and native compound recoveries.

This method is being validated, and performance specifications will be developed using data from DoD's interlaboratory validation study (Reference 10). A summary of the single-laboratory performance is presented in Tables 5, 9, and 10.

17.0 Pollution Prevention

17.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Many opportunities for pollution prevention exist in laboratory operation. EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address waste generation. When wastes cannot be reduced feasibly at the source, EPA recommends recycling as the next best option.

17.2 The compounds in this method are used in extremely small amounts and pose little threat to the environment when managed properly. Standards should be prepared in volumes consistent with laboratory use to minimize the disposal of excess volumes of expired standards.

17.3 For information about pollution prevention that may be applied to laboratories and research institutions, consult *Less is Better: Laboratory Chemical Management for Waste Reduction* (Reference 7).

18.0 Waste Management

18.1 The laboratory is responsible for complying with all Federal, State, and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions, and to protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Compliance is also required with any sewage discharge permits and regulations. An overview of requirements can be found in *Environmental Management Guide for Small Laboratories* (Reference 8).

18.2 Samples at pH < 2 or pH > 12, are hazardous and must be handled and disposed of as hazardous waste or neutralized and disposed of in accordance with all federal, state, and local regulations. It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions.

18.3 For further information on waste management, consult *The Waste Management Manual for Laboratory Personnel* and *Less is Better-Laboratory Chemical Management for Waste Reduction*, (Reference 9).

19.0 References

1. "Working with Carcinogens," Department of Health, Education, & Welfare, Public Health Service, Centers for Disease Control, NIOSH, Publication 77-206, August 1977, NTIS PB-277256.

2. "OSHA Safety and Health Standards, General Industry," OSHA 2206, 29 CFR 1910.

3. "Safety in Academic Chemistry Laboratories," ACS Committee on Chemical Safety, 1979.

4. "Standard Methods for the Examination of Water and Wastewater," 18th edition and later revisions, American Public Health Association, 1015 15th St, NW, Washington, DC 20005, 1-35: Section 1090 (Safety), 1992.

5. "Standard Practice for Sampling Water," ASTM Annual Book of Standards, ASTM, 1916 Race Street, Philadelphia, PA 19103-1187, 1980.

6. "Handbook of Analytical Quality Control in Water and Wastewater Laboratories," USEPA EMSL, Cincinnati, OH 45268, EPA 600/4-79-019, April 1979.

7. "Less is Better: Laboratory Chemical Management for Waste Reduction," American Chemical Society, 1993. Available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street NW, Washington, DC 20036.

8. "Environmental Management Guide for Small Laboratories," USEPA, Small Business Division, Washington DC, EPA 233-B-00-001, May 2000.

9. "The Waste Management Manual for Laboratory Personnel," American Chemical Society, 1990. Available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street NW, Washington, DC 20036.

10. Willey, J., R. Anderson, A. Hanley, M. Mills, C. Hamilton, T. Thompson, and A. Leeson. 2021. "Report on the Single-Laboratory Validation of PFAS by Isotope Dilution LC-MS/MS," Strategic Environmental Research and Development Program (SERDP) Project ER19-1409.

11. *DoD interlaboratory study reference will be added here.*

12. DoD QSM (US Department of Defense Quality Systems Manual for Environmental Laboratories, version 5.3, 2019).

13. Woudneh, Million B., Bharat Chandramouli, Coreen Hamilton, Richard Grace, 2019, "Effects of Sample Storage on the Quantitative Determination of 29 PFAS: Observation of Analyte Interconversions during Storage," Environmental Science and Technology 53(21): 12576-12585.

20.0 Tables, Diagrams, Flowcharts, and Validation Data

Table 1. Names, Abbreviations, and CAS Registry Numbers for Target PFAS, Extracted Internal Standards and Non-extracted Internal Standards[1]

Target Analyte Name	Abbreviation	CAS Number
Perfluoroalkyl carboxylic acids		
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	PFTeDA	376-06-7
Perfluoroalkyl sulfonic acids		
Acid Form		
Perfluorobutanesulfonic acid	PFBS	375-73-5
Perfluoropentansulfonic 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
Fluorotelomer sulfonic acids		
1*H*,1*H*, 2*H*, 2*H*-Perfluorohexane sulfonic acid	4:2FTS	757124-72-4
1*H*,1*H*, 2*H*, 2*H*-Perfluorooctane sulfonic acid	6:2FTS	27619-97-2
1*H*,1*H*, 2*H*, 2*H*-Perfluorodecane sulfonic acid	8:2FTS	39108-34-4
Perfluorooctane sulfonamides		
Perfluorooctanesulfonamide	PFOSA	754-91-6
N-methyl perfluorooctanesulfonamide	NMeFOSA	31506-32-8
N-ethyl perfluorooctanesulfonamide	NEtFOSA	4151-50-2
Perfluorooctane sulfonamidoacetic acids		
N-methyl perfluorooctanesulfonamidoacetic acid	NMeFOSAA	2355-31-9
N-ethyl perfluorooctanesulfonamidoacetic acid	NEtFOSAA	2991-50-6
Perfluorooctane sulfonamide ethanols		
N-methyl perfluorooctanesulfonamidoethanol	NMeFOSE	24448-09-7
N-ethyl perfluorooctanesulfonamidoethanol	NEtFOSE	1691-99-2
Per- and Polyfluoroether carboxylic acids		
Hexafluoropropylene oxide dimer acid	HFPO-DA	13252-13-6
4,8-Dioxa-3*H*-perfluorononanoic acid	ADONA	919005-14-4
Perfluoro-3-methoxypropanoic acid	PFMPA	377-73-1
Perfluoro-4-methoxybutanoic acid	PFMBA	863090-89-5
Nonafluoro-3,6-dioxaheptanoic acid	NFDHA	151772-58-6

Table 1. Names, Abbreviations, and CAS Registry Numbers for Target PFAS, Extracted Internal Standards and Non-extracted Internal Standards[1]

Target Analyte Name	Abbreviation	CAS Number
Ether sulfonic acids		
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	9Cl-PF3ONS	756426-58-1
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF3OUdS	763051-92-9
Perfluoro(2-ethoxyethane)sulfonic acid	PFEESA	113507-82-7
Fluorotelomer carboxylic acids		
3-Perfluoropropyl propanoic acid	3:3FTCA	356-02-5
2*H*,2*H*,3*H*,3*H*-Perfluorooctanoic acid	5:3FTCA	914637-49-3
3-Perfluoroheptyl propanoic acid	7:3FTCA	812-70-4
EIS Compounds		
Perfluoro-n-[$^{13}C_4$]butanoic acid	$^{13}C_4$-PFBA	NA
Perfluoro-n-[$^{13}C_5$]pentanoic acid	$^{13}C_5$-PFPeA	
Perfluoro-n-[1,2,3,4,6-$^{13}C_5$]hexanoic acid	$^{13}C_5$-PFHxA	
Perfluoro-n-[1,2,3,4-$^{13}C_4$]heptanoic acid	$^{13}C_4$-PFHpA	
Perfluoro-n-[$^{13}C_8$]octanoic acid	$^{13}C_8$-PFOA	
Perfluoro-n-[$^{13}C_9$]nonanoic acid	$^{13}C_9$-PFNA	
Perfluoro-n-[1,2,3,4,5,6-$^{13}C_6$]decanoic acid	$^{13}C_6$-PFDA	
Perfluoro-n-[1,2,3,4,5,6,7-$^{13}C_7$]undecanoic acid	$^{13}C_7$-PFUnA	
Perfluoro-n-[1,2-$^{13}C_2$]dodecanoic acid	$^{13}C_2$-PFDoA	
Perfluoro-n-[1,2-$^{13}C_2$]tetradecanoic acid	$^{13}C_2$-PFTeDA	
Perfluoro-1-[2,3,4-$^{13}C_3$]butanesulfonic acid	$^{13}C_3$-PFBS	
Perfluoro-1-[1,2,3-$^{13}C_3$]hexanesulfonic acid	$^{13}C_3$-PFHxS	
Perfluoro-1-[$^{13}C_8$]octanesulfonic acid	$^{13}C_8$-PFOS	
Perfluoro-1-[$^{13}C_8$]octanesulfonamide	$^{13}C_8$-PFOSA	
N-methyl-d_3-perfluoro-1-octanesulfonamidoacetic acid	D_3-NMeFOSAA	
N-ethyl-d_5-perfluoro-1-octanesulfonamidoacetic acid	D_5-NEtFOSAA	
1*H*,1*H*,2*H*,2*H*-Perfluoro-1-[1,2-$^{13}C_2$]hexane sulfonic acid	$^{13}C_2$-4:2FTS	
1*H*,1*H*,2*H*,2*H*-Perfluoro-1-[1,2-$^{13}C_2$]octane sulfonic acid	$^{13}C_2$-6:2FTS	
1*H*,1*H*,2*H*,2*H*-Perfluoro-1-[1,2-$^{13}C_2$]decane sulfonic acid	$^{13}C_2$-8:2FTS	
Tetrafluoro-2-heptafluoropropoxy-$^{13}C_3$-propanoic acid	$^{13}C_3$-HFPO-DA	
N-methyl-d_7-perfluorooctanesulfonamidoethanol	D_7-NMeFOSE	
N-ethyl-d_9-perfluorooctanesulfonamidoethanol	D_9-NEtFOSE	
N-ethyl-d_5-perfluoro-1-octanesulfonamide	D_5-NEtFOSA	
N-methyl-d_3-perfluoro-1-octanesulfonamide	D_3-NMeFOSA	
NIS Compounds		
Perfluoro-n-[2,3,4-$^{13}C_3$]butanoic acid	$^{13}C_3$-PFBA	NA
Perfluoro-n-[1,2,3,4-$^{13}C_4$]octanoic acid	$^{13}C_4$-PFOA	
Perfluoro-n-[1,2-$^{13}C_2$]decanoic acid	$^{13}C_2$-PFDA	
Perfluoro-n-[1,2,3,4-$^{13}C_4$]octanesulfonic acid	$^{13}C_4$-PFOS	
Perfluoro-n-[1,2,3,4,5-$^{13}C_5$] nonanoic acid	$^{13}C_5$-PFNA	
Perfluoro-n-[1,2-$^{13}C_2$]hexanoic acid	$^{13}C_2$-PFHxA	
Perfluoro-1-hexane[$^{18}O_2$]sulfonic acid	$^{18}O_2$-PFHxS	

[1] The target analyte names are for the acid and neutral forms of the analytes. See Table 8 for the names and CASRN of the corresponding anion forms, where applicable.

NA Not assigned a CASRN

Table 2. Analyte Ions Monitored, Extracted Internal Standard, and Non-extracted Internal Standard Used for Quantification

Abbreviation	Example Retention Time [1]	Parent Ion Mass	Quantification Ion Mass	Confirmation Ion Mass	Typical Ion Ratio	Quantification Reference Compound
Target Analytes						
PFBA	1.96	212.8	168.9	NA	NA	$^{13}C_4$-PFBA
PFPeA	4.18	263.0	219.0	68.9	NA	$^{13}C_5$-PFPeA
PFHxA	4.81	313.0	269.0	118.9	13	$^{13}C_5$-PFHxA
PFHpA	5.32	363.1	319.0	169.0	3.5	$^{13}C_4$-PFHpA
PFOA	6.16	413.0	369.0	169.0	3.0	$^{13}C_8$-PFOA
PFNA	6.99	463.0	419.0	219.0	4.9	$^{13}C_9$-PFNA
PFDA	7.47	512.9	469.0	219.0	5.5	$^{13}C_6$-PFDA
PFUnA	7.81	563.1	519.0	269.1	6.9	$^{13}C_7$-PFUnA
PFDoA	8.13	613.1	569.0	319.0	10	$^{13}C_2$-PFDoA
PFTrDA[2]	8.53	663.0	619.0	168.9	6.7	avg.$^{13}C_2$-PFTeDA and$^{13}C_2$-PFDoA
PFTeDA	8.96	713.1	669.0	168.9	6.0	$^{13}C_2$-PFTeDA
PFBS	4.79	298.7	79.9	98.8	2.1	$^{13}C_3$-PFBS
PFPeS	5.38	349.1	79.9	98.9	1.8	$^{13}C_3$-PFHxS
PFHxS	6.31	398.7	79.9	98.9	1.9	$^{13}C_3$-PFHxS
PFHpS	7.11	449.0	79.9	98.8	1.7	$^{13}C_8$-PFOS
PFOS	7.59	498.9	79.9	98.8	2.3	$^{13}C_8$-PFOS
PFNS	7.92	548.8	79.9	98.8	1.9	$^{13}C_8$-PFOS
PFDS	8.28	599.0	79.9	98.8	1.9	$^{13}C_8$-PFOS
PFDoS	9.14	699.1	79.9	98.8	1.9	$^{13}C_8$-PFOS
4:2FTS	4.67	327.1	307.0	80.9	1.7	$^{13}C_2$-4:2FTS
6:2FTS	5.81	427.1	407.0	80.9	1.9	$^{13}C_2$-6:2FTS
8:2FTS	7.28	527.1	507.0	80.8	3.0	$^{13}C_2$-8:2FTS
PFOSA	8.41	498.1	77.9	478.0	47	$^{13}C_8$-PFOSA
NMeFOSA	9.70	511.9	219.0	169.0	0.66	D_3-NMeFOSA
NEtFOSA	9.94	526.0	219.0	169.0	0.63	D_5-NEtFOSA
NMeFOSAA	7.51	570.1	419.0	483.0	2.0	D_3-NMeFOSAA
NEtFOSAA	7.65	584.2	419.1	526.0	1.2	D_5-N-EtFOSAA
NMeFOSE	9.57	616.1	58.9	NA	NA	D_7-NMeFOSE
NEtFOSE	9.85	630.0	58.9	NA	NA	D_9-NEtFOSE
HFPO-DA	4.97	284.9	168.9	184.9	1.95	$^{13}C_3$-HFPO-DA
ADONA	5.79	376.9	250.9	84.8	2.8	$^{13}C_3$-HFPO-DA
9Cl-PF3ONS	7.82	530.8	351.0	532.8→353.0	3.2	$^{13}C_3$-HFPO-DA
11Cl-PF3OUdS	8.62	630.9	450.9	632.9→452.9	3.0	$^{13}C_3$-HFPO-DA
3:3FTCA	3.89	241.0	177.0	117.0	1.70	$^{13}C_5$-PFPeA
5:3FTCA	5.14	341.0	237.1	217.0	1.16	$^{13}C_5$-PFHxA
7:3FTCA	6.76	441.0	316.9	336.9	0.69	$^{13}C_5$-PFHxA
PFEESA	5.08	314.8	134.9	82.9	9.22	$^{13}C_5$-PFHxA
PFMPA	3.21	229.0	84.9	NA	NA	$^{13}C_5$-PFPeA
PFMBA	4.53	279.0	85.1	NA	NA	$^{13}C_5$-PFPeA
NFDHA	4.84	295.0	201.0	84.9	1.46	$^{13}C_5$-PFHxA
Extracted Internal Standards						
$^{13}C_4$-PFBA	1.95	216.8	171.9	NA		$^{13}C_3$-PFBA
$^{13}C_5$-PFPeA	4.18	268.3	223.0	NA		$^{13}C_2$-PFHxA
$^{13}C_5$-PFHxA	4.80	318.0	273.0	120.3		$^{13}C_2$-PFHxA
$^{13}C_4$-PFHpA	5.32	367.1	322.0	NA		$^{13}C_2$-PFHxA
$^{13}C_8$-PFOA	6.16	421.1	376.0	NA		$^{13}C_4$-PFOA

Table 2. Analyte Ions Monitored, Extracted Internal Standard, and Non-extracted Internal Standard Used for Quantification

Abbreviation	Example Retention Time [1]	Parent Ion Mass	Quantification Ion Mass	Confirmation Ion Mass	Typical Ion Ratio	Quantification Reference Compound
$^{13}C_9$-PFNA	6.99	472.1	427.0	NA		$^{13}C_5$-PFNA
$^{13}C_6$-PFDA	7.47	519.1	474.1	NA		$^{13}C_2$-PFDA
$^{13}C_7$-PFUnA	7.81	570.0	525.1	NA		$^{13}C_2$-PFDA
$^{13}C_2$-PFDoA	8.13	615.1	570.0	NA		$^{13}C_2$-PFDA
$^{13}C_2$-PFTeDA	8.96	715.2	670.0	NA		$^{13}C_2$-PFDA
$^{13}C_3$-PFBS	4.78	302.1	79.9	98.9		$^{18}O_2$-PFHxS
$^{13}C_3$-PFHxS	6.30	402.1	79.9	98.9		$^{18}O_2$-PFHxS
$^{13}C_8$-PFOS	7.59	507.1	79.9	98.9		$^{13}C_4$-PFOS
$^{13}C_2$-4:2FTS	4.67	329.1	80.9	309.0		$^{18}O_2$-PFHxS
$^{13}C_2$-6:2FTS	5.82	429.1	80.9	409.0		$^{18}O_2$-PFHxS
$^{13}C_2$-8:2FTS	7.28	529.1	80.9	509.0		$^{18}O_2$-PFHxS
$^{13}C_8$-PFOSA	8.41	506.1	77.8	NA		$^{13}C_4$-PFOS
D_3-NMeFOSA	9.70	515.0	219.0	NA		$^{13}C_4$-PFOS
D_5-NEtFOSA	9.94	531.1	219.0	NA		$^{13}C_4$-PFOS
D_3-NMeFOSAA	7.51	573.2	419.0	NA		$^{13}C_4$-PFOS
D_5-NEtFOSAA	7.65	589.2	419.0	NA		$^{13}C_4$-PFOS
D_7-NMeFOSE	9.56	623.2	58.9	NA		$^{13}C_4$-PFOS
D_9-NEtFOSE	9.83	639.2	58.9	NA		$^{13}C_4$-PFOS
$^{13}C_3$-HFPO-DA	4.97	286.9	168.9	184.9		$^{13}C_2$-PFHxA
Non-Extracted Internal Standards						
$^{13}C_3$-PFBA	1.95	216.0	172.0	NA		
$^{13}C_2$-PFHxA	4.80	315.1	270.0	119.4		
$^{13}C_4$-PFOA	6.16	417.1	172.0	NA		
$^{13}C_5$-PFNA	6.99	468.0	423.0	NA		
$^{13}C_2$-PFDA	7.47	515.1	470.1	NA		
$^{18}O_2$-PFHxS	6.30	403.0	83.9	NA		
$^{13}C_4$-PFOS	7.59	502.8	79.9	98.9		

[1] Times shown are in decimal minute units. Example retention times are based on the instrument operating conditions and column specified in Section 10.2.

[2] For improved accuracy, PFTrDA is quantitated using the average areas of the labeled compounds $^{13}C_2$-PFTeDA and $^{13}C_2$-PFDoA.

NA= These analytes do not produce a confirmation ion mass.

Table 3. Nominal Masses of Spike Added to Samples or Extracts

Analyte	Amount Added (ng)
Extracted Internal Standards	
$^{13}C_4$-PFBA	40
$^{13}C_5$-PFPeA	20
$^{13}C_5$-PFHxA	10
$^{13}C_4$-PFHpA	10
$^{13}C_8$-PFOA	10
$^{13}C_9$-PFNA	5
$^{13}C_6$-PFDA	5
$^{13}C_7$-PFUnA	5
$^{13}C_2$-PFDoA	5
$^{13}C_2$-PFTeDA	5
$^{13}C_3$-PFBS	10
$^{13}C_3$-PFHxS	10
$^{13}C_8$-PFOS	10
$^{13}C_2$-4:2FTS	20
$^{13}C_2$-6:2FTS	20
$^{13}C_2$-8:2FTS	20
$^{13}C_8$-PFOSA	10
D_3-NMeFOSA	10
D_5-NEtFOSA	10
D_3-NMeFOSAA	20
D_5-NEtFOSAA	20
D_7-NMeFOSE	100
D_9-NEtFOSE	100
$^{13}C_3$-HFPO-DA	40
Non-extracted Internal Standards	
$^{13}C_3$-PFBA	20
$^{13}C_2$-PFHxA	10
$^{13}C_4$-PFOA	10
$^{13}C_5$-PFNA	5
$^{13}C_2$-PFDA	5
$^{18}O_2$-PFHxS	10
$^{13}C_4$-PFOS	10

Table 4. Calibration Solutions (ng/mL)

Compound	CS1 (LOQ)	CS2	CS3	CS4 (CV[1])	CS5	CS6	CS7[2]
Perfluoroalkyl carboxylic acids							
PFBA	0.8	2	5	10	20	50	250
PFPeA	0.4	1	2.5	5	10	25	125
PFHxA	0.2	0.5	1.25	2.5	5	12.5	62.5
PFHpA	0.2	0.5	1.25	2.5	5	12.5	62.5
PFOA	0.2	0.5	1.25	2.5	5	12.5	62.5
PFNA	0.2	0.5	1.25	2.5	5	12.5	62.5
PFDA	0.2	0.5	1.25	2.5	5	12.5	62.5
PFUnA	0.2	0.5	1.25	2.5	5	12.5	62.5
PFDoA	0.2	0.5	1.25	2.5	5	12.5	62.5
PFTrDA	0.2	0.5	1.25	2.5	5	12.5	62.5
PFTeDA	0.2	0.5	1.25	2.5	5	12.5	62.5
Perfluoroalkyl sulfonic acids							
PFBS	0.2	0.5	1.25	2.5	5	12.5	62.5
PFPeS	0.2	0.5	1.25	2.5	5	12.5	62.5
PFHxS	0.2	0.5	1.25	2.5	5	12.5	62.5
PFHpS	0.2	0.5	1.25	2.5	5	12.5	62.5
PFOS	0.2	0.5	1.25	2.5	5	12.5	62.5
PFNS	0.2	0.5	1.25	2.5	5	12.5	62.5
PFDS	0.2	0.5	1.25	2.5	5	12.5	62.5
PFDoS	0.2	0.5	1.25	2.5	5	12.5	62.5
Fluorotelomer sulfonic acids							
4:2FTS	0.8	2	5	10	20	50	NA
6:2FTS	0.8	2	5	10	20	50	NA
8:2FTS	0.8	2	5	10	20	50	NA
Perfluorooctane sulfonamides							
PFOSA	0.2	0.5	1.25	2.5	5	12.5	62.5
NMeFOSA	0.2	0.5	1.25	2.5	5	12.5	62.5
NEtFOSA	0.2	0.5	1.25	2.5	5	12.5	62.5
Perfluorooctane sulfonamidoacetic acids							
NMeFOSAA	0.2	0.5	1.25	2.5	5	12.5	62.5
NEtFOSAA	0.2	0.5	1.25	2.5	5	12.5	62.5
Perfluorooctane sulfonamide ethanols							
NMeFOSE	2	5	12.5	25	50	125	625
NEtFOSE	2	5	12.5	25	50	125	625
Per- and polyfluoroether carboxylic acids							
HFPO-DA	0.8	2	5	10	20	50	250
ADONA	0.8	2	5	10	20	50	250
PFMPA	0.4	1	2.5	5	10	25	125
PFMBA	0.4	1	2.5	5	10	25	125
NFDHA	0.4	1	2.5	5	10	25	125
Ether sulfonic acids							
9Cl-PF3ONS	0.8	2	5	10	20	50	250
11Cl-PF3OUdS	0.8	2	5	10	20	50	250
PFEESA	0.4	1	2.5	5	10	25	125

Table 4. Calibration Solutions (ng/mL)

Compound	CS1 (LOQ)	CS2	CS3	CS4 (CV[1])	CS5	CS6	CS7[2]
Fluorotelomer carboxylic acids							
3:3FTCA	1.0	2.5	6.26	12.5	25	62.4	312
5:3FTCA	5.0	12.5	31.3	62.5	125	312	1560
7:3FTCA	5.0	12.5	31.3	62.5	125	312	1560
Extracted Internal Standard (EIS) Analytes							
$^{13}C_4$-PFBA	10	10	10	10	10	10	10
$^{13}C_5$-PFPeA	5	5	5	5	5	5	5
$^{13}C_5$-PFHxA	2.5	2.5	2.5	2.5	2.5	2.5	2.5
$^{13}C_4$-PFHpA	2.5	2.5	2.5	2.5	2.5	2.5	2.5
$^{13}C_8$-PFOA	2.5	2.5	2.5	2.5	2.5	2.5	2.5
$^{13}C_9$-PFNA	1.25	1.25	1.25	1.25	1.25	1.25	1.25
$^{13}C_6$-PFDA	1.25	1.25	1.25	1.25	1.25	1.25	1.25
$^{13}C_7$-PFUnA	1.25	1.25	1.25	1.25	1.25	1.25	1.25
$^{13}C_2$-PFDoA	1.25	1.25	1.25	1.25	1.25	1.25	1.25
$^{13}C_2$-PFTeDA	1.25	1.25	1.25	1.25	1.25	1.25	1.25
$^{13}C_3$-PFBS	2.5	2.5	2.5	2.5	2.5	2.5	2.5
$^{13}C_3$-PFHxS	2.5	2.5	2.5	2.5	2.5	2.5	2.5
$^{13}C_8$-PFOS	2.5	2.5	2.5	2.5	2.5	2.5	2.5
$^{13}C_2$-4:2FTS	5	5	5	5	5	5	5
$^{13}C_2$-6:2FTS	5	5	5	5	5	5	5
$^{13}C_2$-8:2FTS	5	5	5	5	5	5	5
$^{13}C_8$-PFOSA	2.5	2.5	2.5	2.5	2.5	2.5	2.5
D_3-NMeFOSA	2.5	2.5	2.5	2.5	2.5	2.5	2.5
D_5-NEtFOSA	2.5	2.5	2.5	2.5	2.5	2.5	2.5
D_3-NMeFOSAA	5	5	5	5	5	5	5
D_5-NEtFOSAA	5	5	5	5	5	5	5
D_7-NMeFOSE	25	25	25	25	25	25	25
D_9-NEtFOSE	25	25	25	25	25	25	25
$^{13}C_3$-HFPO-DA	10	10	10	10	10	10	10
Non-extracted Internal Standard (NIS) Analytes							
$^{13}C_3$-PFBA	5	5	5	5	5	5	5
$^{13}C_2$-PFHxA	2.5	2.5	2.5	2.5	2.5	2.5	2.5
$^{13}C_4$-PFOA	2.5	2.5	2.5	2.5	2.5	2.5	2.5
$^{13}C_5$-PFNA	1.25	1.25	1.25	1.25	1.25	1.25	1.25
$^{13}C_2$-PFDA	1.25	1.25	1.25	1.25	1.25	1.25	1.25
$^{18}O_2$-PFHxS	2.5	2.5	2.5	2.5	2.5	2.5	2.5
$^{13}C_4$-PFOS	2.5	2.5	2.5	2.5	2.5	2.5	2.5

[1] This calibration point is used as the calibration verification (CV)

[2] A minimum of six contiguous calibrations standards are required for linear models and a minimum of seven calibration standards are required for second-order models.

Table 5. Single-Laboratory Validation Performance Summary for Target Compounds and Extracted Internal Standards

Compounds	Blank (ng/mL)	Aqueous Matrices[1]			Solid Matrices[1]			Tissue Matrices[1]		
		IPR Rec (%)	RSD (%)	OPR Rec (%)	IPR Rec (%)	RSD (%)	OPR Rec (%)	IPR Rec (%)	RSD (%)	OPR Rec (%)
Target Compounds										
PFBA	< 0.4	89 - 107	4.8	89 – 113	95 – 99	1.0	92 – 108	89 – 104	3.9	90 – 110
PFPeA	< 0.2	85 - 106	5.5	89 – 121	92 – 105	3.4	94 – 115	80 – 98	5.0	96 – 114
PFHxA	< 0.1	75 - 109	9.1	89 – 111	93 – 101	2.2	89 – 107	72 – 110	10.2	90 – 111
PFHpA	< 0.1	87 - 102	4.1	90 – 110	94 – 102	2.2	89 – 107	87 – 102	4.0	87 – 118
PFOA	< 0.1	88 - 98	2.8	87 – 112	92 – 100	2.0	90 – 106	78 – 85	2.4	82 – 114
PFNA	< 0.1	88 - 104	4.1	90 – 111	91 – 102	2.7	88 – 112	85 – 110	6.3	87 – 119
PFDA	< 0.1	82 - 115	8.3	92 – 115	97 – 103	1.5	89 – 118	76 – 115	10.2	84 – 112
PFUnA	< 0.1	83 - 98	4.2	89 – 112	91 – 107	4.0	92 – 111	83 – 102	5.1	91 – 117
PFDoA	< 0.1	58 - 111	15.7	84 – 123	73 – 120	12.1	88 – 119	83 – 105	5.7	77 – 141
PFTrDA	< 0.1	80 - 111	8.1	92 – 119	91 – 112	5.2	89 – 125	92 – 114	5.3	106 – 133
PFTeDA	< 0.1	88 - 103	4.1	89 – 116	94 – 104	2.5	92 – 110	76 – 103	7.4	91 – 111
PFBS	< 0.1	85 - 111	6.6	87 – 116	91 – 103	3.2	91 – 111	69 – 105	10.3	89 – 117
PFPeS	< 0.1	87 - 115	6.9	87 – 115	87 – 103	4.3	89 – 112	77 – 96	5.4	89 – 112
PFHxS	< 0.1	90 - 107	4.4	97 – 119	98 – 106	2.0	96 – 113	81 – 101	5.3	91 – 123
PFHpS	< 0.1	84 - 126	10.2	86 – 114	87 – 104	4.4	88 – 104	77 – 108	8.4	86 – 108
PFOS	< 0.1	93 - 122	6.7	91 – 120	95 – 108	3.4	94 – 115	98 – 112	3.2	97 – 124
PFNS	< 0.1	64 - 141	18.8	86 – 123	98 – 111	3.0	76 – 117	65 – 88	7.5	85 – 114
PFDS	< 0.1	75 - 121	11.7	84 – 107	83 – 102	5.2	84 – 107	82 – 94	3.6	78 – 110
PFDoS	< 0.1	74 - 114	10.6	78 – 102	76 – 99	6.5	77 – 100	73 – 96	6.9	29 – 108
4:2FTS	< 0.4	76 - 123	12.0	91 – 119	98 – 100	0.5	87 – 113	66 – 126	15.6	90 – 103
6:2FTS	< 0.4	71 - 148	17.5	81 – 129	94 – 123	6.5	60 – 166	77 – 105	7.8	92 – 119
8:2FTS	< 0.4	85 - 109	6.1	99 – 124	109 – 128	3.8	104 – 127	66 – 148	19.3	102 – 136
PFOSA	< 0.1	90 - 107	4.4	91 – 122	92 – 106	3.4	94 – 114	92 – 116	5.7	96 – 121
NMeFOSA	< 0.1	78 - 90	3.6	84 – 112	87 – 104	4.4	91 – 117	81 – 100	5.5	86 – 117
NEtFOSA	< 0.1	79 - 97	5.0	83 – 108	98 – 102	1.0	96 – 115	74 – 114	10.7	90 – 127
NMeFOSAA	< 0.1	82 - 115	8.2	81 – 120	91 – 107	4.0	90 – 113	89 – 136	10.4	93 – 117
NEtFOSAA	< 0.1	79 - 120	10.3	85 – 124	102 – 108	1.6	87 – 117	53 – 115	18.3	90 – 117
NMeFOSE	< 1	87 - 102	3.9	92 – 115	98 – 103	1.3	94 – 112	71 – 292	30.3	118 – 344
NEtFOSE	< 1	87 - 104	4.7	91 – 118	97 – 104	1.9	96 – 115	97 – 133	8.0	61 – 159
HFPO-DA	< 0.4	88 - 114	6.5	84 – 118	83 – 105	5.9	80 – 120	73 – 100	7.8	86 – 114
ADONA	< 0.4	77 - 106	7.9	77 – 117	85 – 96	3.2	76 – 124	82 – 95	3.8	86 – 132
PFMPA	< 0.2	86 - 106	6.6	83 – 120	91 – 98	1.8	85 – 117	78 – 93	4.2	86 – 109
PFMBA	< 0.2	62 - 122	5.2	81 – 115	88 – 97	2.6	85 – 120	74 – 104	8.4	84 – 117
NFDHA	< 0.2	44 - 149	16.3	56 – 138	53 – 103	16.2	58 – 136	49 – 86	13.8	56 – 115
9Cl-PF3ONS	< 0.4	84 - 101	27.4	80 – 120	84 – 100	4.4	79 – 131	69 – 98	8.7	95 – 126
11Cl-PF3OUdS	< 0.4	80 - 95	4.5	76 – 116	84 – 96	3.3	77 – 127	85 – 100	4.3	94 – 138
PFEESA	< 0.2	80 - 104	4.4	85 – 115	80 – 93	3.8	89 – 109	68 – 99	9.3	88 – 107
3:3FTCA	< 0.5	84 - 103	5.0	66 – 127	86 – 98	3.3	76 – 116	66 – 94	9.0	41 – 126
5:3FTCA	< 2.5	84 - 101	4.6	84 – 113	83 – 94	3.1	80 – 101	95 – 131	7.9	78 – 199
7:3FTCA	< 2.5	78 - 103	7.0	82 – 116	90 – 106	4.1	75 – 104	84 – 111	6.7	99 – 139

Table 5. Single-Laboratory Validation Performance Summary for Target Compounds and Extracted Internal Standards

Compounds	Blank (ng/mL)	Aqueous Matrices[1]			Solid Matrices[1]			Tissue Matrices[1]		
		IPR Rec (%)	RSD (%)	OPR Rec (%)	IPR Rec (%)	RSD (%)	OPR Rec (%)	IPR Rec (%)	RSD (%)	OPR Rec (%)
Extracted Internal Standard (EIS)										
$^{13}C_4$-PFBA	N/A	85 - 91	1.6	88 – 108	92 – 99	1.6	95 – 109	93 – 97	1.0	95 – 105
$^{13}C_5$-PFPeA	N/A	87 - 95	2.4	84 – 111	86 – 106	5.3	80 – 110	85 – 108	6.0	89 – 103
$^{13}C_5$-PFHxA	N/A	85 - 92	1.9	83 – 108	83 – 101	4.8	92 – 106	79 – 111	8.5	88 – 98
$^{13}C_4$-PFHpA	N/A	78 - 100	6.2	83 – 106	87 – 102	4.1	90 – 100	88 – 93	1.3	80 – 102
$^{13}C_8$-PFOA	N/A	77 - 98	6.0	84 – 107	89 – 101	3.2	92 – 104	91 – 98	1.7	86 – 102
$^{13}C_9$-PFNA	N/A	82 - 96	3.8	84 – 107	86 – 101	4.1	90 – 106	91 – 104	3.3	89 – 101
$^{13}C_6$-PFDA	N/A	81 - 98	4.7	84 – 106	79 – 101	6.0	86 – 109	89 – 104	4.0	90 – 104
$^{13}C_7$-PFUnA	N/A	84 - 100	4.4	84 – 109	84 – 104	5.4	91 – 116	84 – 118	8.4	88 – 109
$^{13}C_2$-PFDoA	N/A	61 - 103	12.9	73 – 101	70 – 93	7.1	73 – 106	95 – 125	6.8	70 – 108
$^{13}C_2$-PFTeDA	N/A	72 - 89	5.4	74 – 97	83 – 88	1.5	74 – 107	81 – 114	8.5	10 – 110
$^{13}C_3$-PFBS	N/A	87 - 94	2.0	88 – 110	97 – 105	1.8	96 – 109	87 – 114	6.5	95 – 106
$^{13}C_3$-PFHxS	N/A	83 - 89	1.9	85 – 103	92 – 97	1.4	92 – 106	92 – 97	1.4	91 – 103
$^{13}C_8$-PFOS	N/A	78 - 92	3.9	86 – 110	87 – 107	4.9	95 – 109	87 – 93	1.6	95 – 103
$^{13}C_2$-4:2FTS	N/A	64 - 106	12.1	87 – 137	132 – 135	0.6	123 – 145	106 – 221	17.6	155 – 291
$^{13}C_2$-6:2FTS	N/A	93 - 102	2.2	67 – 149	118 – 129	2.3	104 – 138	87 – 135	10.8	117 – 149
$^{13}C_2$-8:2FTS	N/A	99 - 109	2.5	71 – 137	96 – 122	6.1	93 – 123	179 – 299	12.5	79 – 304
$^{13}C_8$-PFOSA	N/A	60 - 107	14.2	57 – 109	69 – 86	5.4	66 – 100	104 – 153	9.4	88 – 120
D_3-NMeFOSA	N/A	55 - 85	10.8	39 – 84	47 – 59	5.4	25 – 64	20 – 58	24.5	3 – 34
D_5-NEtFOSA	N/A	54 - 91	12.9	43 – 84	43 – 51	4.5	18 – 58	30 – 56	15.2	0 – 56*
D_3-NMeFOSAA	N/A	63 - 117	14.9	66 – 117	98 – 107	2.1	86 – 109	102 – 187	14.7	144 – 196
D_5-NEtFOSAA	N/A	66 - 115	13.7	63 – 115	98 – 104	1.3	85 – 109	178 – 216	4.9	175 – 223
D_7-NMeFOSE	N/A	61 - 106	13.6	42 – 99	50 – 61	5.1	35 – 76	3 – 5	11.6	0 – 8*
D_9-NEtFOSE	N/A	63 - 108	13.2	44 – 90	46 – 57	5.5	32 – 72	8 – 33	30.0	0 – 33*
$^{13}C_3$-HFPO-DA	N/A	89 - 106	4.5	88 – 121	98 – 108	2.4	83 – 125	87 – 106	4.9	81 – 106

[1] The recovery limits are applied to all samples, method blanks, IPR, OPR samples for all matrix types.

* Ranges were determined at ± 2 standard deviations from the mean. Because of the low recoveries for these EIS, the calculated lower limits were negative values. Therefore, the lower limits have been set to 0 for these analytes.

Data for this table are derived from the single-laboratory validation study, and are only provided as examples for this draft method. The data will be updated to reflect the interlaboratory study results in a subsequent revision. Therefore, these criteria will change after interlaboratory validation. Several sections of this method state that Table 5 criteria are required, this is standard language that will be applicable when the method is finalized.

Table 6. Pooled MDLs and ML values from the Single-laboratory Validation Study, by Matrix[1]

	Aqueous (ng/L)		Solid (ng/g)		Tissue (ng/g)	
Compound	**MDLs**	**ML[2]**	**MDLs**	**ML**	**MDLs**	**ML**
PFBA	0.330	6.4	0.401	0.8	0.593	2.0
PFPeA	0.196	3.2	0.021	0.4	0.083	1.0
PFHxA	0.318	1.6	0.020	0.2	0.096	0.5
PFHpA	0.221	1.6	0.029	0.2	0.088	0.5
PFOA	0.302	1.6	0.037	0.2	0.086	0.5
PFNA	0.221	1.6	0.086	0.2	0.160	0.5
PFDA	0.333	1.6	0.031	0.2	0.124	0.5
PFUnA	0.264	1.6	0.033	0.2	0.152	0.5
PFDoA	0.379	1.6	0.059	0.2	0.130	0.5
PFTrDA	0.238	1.6	0.038	0.2	0.086	0.5
PFTeDA	0.264	1.6	0.032	0.2	0.185	0.5
PFBS	0.245	1.6	0.014	0.2	0.070	0.5
PFPeS	0.204	1.6	0.015	0.2	0.032	0.5
PFHxS[1]	0.217	1.6	0.018	0.2	0.083	0.5
PFHpS	0.137	1.6	0.057	0.2	0.043	0.5
PFOS[1]	0.327	1.6	0.067	0.2	0.294	0.5
PFNS	0.303	1.6	0.046	0.2	0.114	0.5
PFDS	0.334	1.6	0.040	0.2	0.101	0.5
PFDoS	0.179	1.6	0.038	0.2	0.177	0.5
4:2FTS	2.281	6.4	0.282	0.8	0.740	2.0
6:2FTS	3.973	6.4	0.116	0.8	1.149	2.0
8:2FTS	1.566	6.4	0.225	0.8	0.373	2.0
PFOSA	0.227	1.6	0.068	0.2	0.094	0.5
NMeFOSA	0.196	1.6	0.049	0.2	0.161	0.5
NEtFOSA	0.585	1.6	0.038	0.2	0.169	0.5
NMeFOSAA[1]	0.586	1.6	0.030	0.2	0.093	0.5
NEtFOSAA[1]	0.324	1.6	0.044	0.2	0.138	0.5
NMeFOSE	1.191	16	0.203	2.0	9.978	5.0
NEtFOSE	1.022	16	0.247	2.0	1.501	5.0
HFPO-DA	0.406	6.4	0.136	0.8	0.161	2.0
ADONA	0.779	6.4	0.057	0.8	0.082	2.0
PFEESA	0.137	3.2	0.018	0.4	0.045	1.0
PFMPA	0.177	3.2	0.033	0.4	0.070	1.0
PFMBA	0.117	3.2	0.029	0.4	0.069	1.0
NFDHA	1.384	3.2	0.084	0.4	0.294	1.0
9CL-PF3ONS	0.871	6.4	0.038	0.8	0.152	2.0
11CL-PF3OUDS	0.819	6.4	0.071	0.8	0.312	2.0
3:3FTCA	0.721	8.0	0.060	1.0	0.247	2.5
5:3FTCA	5.066	40	0.363	5.0	1.537	12.5
7:3FTCA	5.942	40	0.308	5.0	0.845	12.5

[1] A standard containing a mixture of branched and linear isomer of suitable quality to be used for quantitation is currently available and required to be used for all calibration, calibration verifications, and QC samples. If more become commercially available for other target analytes, they must be utilized in the same manner.

[2] The ML values in this table were derived from the concentrations of the lowest calibration standard in Table 4, based on the alternative described in the Glossary, using the nominal sample volume (aqueous) or weight (all other matrices) described in the method.

Data for this table are derived from the single-laboratory validation study, and are only provided as examples for this draft method. The data will be updated with the pooled MDLs from the interlaboratory study results in a subsequent revision.

Table 7. Summary of Quality Control

Method Reference	Requirement	Specification and Frequency
Section 10.1	Mass Calibration	Annually and on as-needed basis
Section 10.1.7	Mass Calibration Verification	After mass calibration
Section 10.3	Initial Calibration (ICAL)	Minimum 6 calibration standards for linear model and 7 calibration standards for non-linear models.
Sections 10.2.2, 14.4	Retention Time (RT) window	After ICAL and at the beginning of analytical sequence
Sections 7.3.1, 9.4	Extracted Internal Standard (EIS) Analytes	All CAL standards, batch QC and field samples
Sections 7.3.2	Non-extracted Internal Standards (NIS)	All CAL standards, batch QC and field samples
Sections 7.3.4, 10.3.1, 13.3	Instrument Sensitivity Check (ISC)	Daily, prior to analysis
Section 14.2	Calibration Verification (CV)	At the beginning and every 10 samples
Section 14.6	Instrument Blank	Daily prior to analysis and after high standards
Sections 9.1.3, 9.5, 14.7	Method Blank (MB)	One per preparation batch
Section 14.5	Ongoing Precision Recovery (OPR)	One per preparation batch
Section 11.0	Limit of Quantitation Verification (LLOPR)	Prior to analyzing samples
Section 11.0	Matrix Spike (MS/MSD)	One per preparation batch (if required)

Table 8. Cross-reference of Abbreviations, Analyte Names, CAS Numbers for the Acid and Anion Forms of the Perfluoroalkyl carboxylates and Perfluoroalkyl sulfonates

Perfluoroalkyl carboxylic acids/anions				
Abbreviation	**Acid Name**	**CASRN**	**Anion Name**	**CASRN**
PFBA	Perfluorobutanoic acid	375-22-4	Perfluorobutanoate	45048-62-2
PFPeA	Perfluoropentanoic acid	2706-90-3	Perfluoropentanoate	45167-47-3
PFHxA	Perfluorohexanoic acid	307-24-4	Perfluorohexanoate	92612-52-7
PFHpA	Perfluoroheptanoic acid	375-85-9	Perflluoroheptanoate	120885-29-2
PFOA	Perfluorooctanoic acid	335-67-1	Pefluorooctanoate	45285-51-6
PFNA	Perfluorononanoic acid	375-95-1	Perfluorononanoate	72007-68-2
PFDA	Perfluorodecanoic acid	335-76-2	Perfluorodecanoate	73829-36-4
PFUnA	Perfluoroundecanoic acid	2058-94-8	Perfluoroundecanoate	196859-54-8
PFDoA	Perfluorododecanoic acid	307-55-1	Perfluorododecanoate	171978-95-3
PFTrDA	Perfluorotridecanoic acid	72629-94-8	Perfluorotridecanoate	862374-87-6
PFTeDA	Perfluorotetradecanoic acid	376-06-7	Perfluorotetradecanoate	365971-87-5
Perfluoroalkyl sulfonic acids/anions				
PFBS	Perfluorobutanesulfonic acid	375-73-5	Perfluorobutane sulfonate	45187-15-3
PFPeS	Perfluoropentansulfonic acid	2706-91-4	Perfluoropentane sulfonate	175905-36-9
PFHxS	Perfluorohexanesulfonic acid	355-46-4	Perfluorohexane sulfonate	108427-53-8
PFHpS	Perfluoroheptanesulfonic acid	375-92-8	Perfluoroheptane sulfonate	146689-46-5
PFOS	Perfluorooctanesulfonic acid	1763-23-1	Perfluorooctane sulfonate	45298-90-6
PFNS	Perfluorononanesulfonic acid	68259-12-1	Perfluorononane sulfonate	474511-07-4
PFDS	Perfluorodecanesulfonic acid	335-77-3	Perfluorodecane sulfonate	126105-34-8
PFDoS	Perfluorododecanesulfonic acid	79780-39-5	Perfluorododecane sulfonate	343629-43-6

Table 9. Range of Recoveries for Extracted Internal Standards (EIS) in the Single-laboratory Validation Study, by Matrix

EIS Compounds	Aqueous			Solid			Tissue		
	% Recovery		RSD (%)	% Recovery		RSD (%)	% Recovery		RSD (%)
	Min	Max		Min	Max		Min	Max	
$^{13}C_4$-PFBA	9	97	15.9	3	113	37.4	84	99	8.0
$^{13}C_5$-PFPeA	39	103	13.3	28	112	17.2	86	107	11.1
$^{13}C5$-PFHxA	73	97	2.7	79	110	5.5	92	95	1.6
$^{13}C_4$-PFHpA	77	95	2.4	73	111	6.0	80	93	8.2
$^{13}C_8$-PFOA	87	95	0.8	86	115	4.4	90	95	2.8
$^{13}C_9$-PFNA	82	95	1.6	87	110	4.2	90	98	4.3
$^{13}C_6$-PFDA	71	93	3.3	87	112	4.9	83	97	7.7
$^{13}C_7$-PFUnA	56	94	6.5	66	124	11.6	71	91	12.9
$^{13}C_2$-PFDoA	34	87	13.7	26	109	24.3	54	96	29.2
$^{13}C_2$-PFTeDA	17	153	26.2	18	110	30.1	31	102	67.8
$^{13}C_3$-PFBS	72	100	4.7	89	120	5.4	89	98	5.1
$^{13}C_3$-PFHxS	79	95	1.6	87	110	4.4	98	99	0.1
$^{13}C_8$-PFOS	67	96	3.6	79	113	5.7	92	103	6.0
$^{13}C_2$-4:2FTS	81	199	14.8	95	248	17.0	192	215	6.2
$^{13}C_2$-6:2FTS	64	183	16.4	76	127	9.4	145	230	27.2
$^{13}C_2$-8:2FTS	65	139	8.4	86	173	15.2	136	220	24.6
$^{13}C_8$-PFOSA	27	93	15.4	61	123	10.0	87	96	4.5
D_3-NMeFOSA	14	74	16.4	28	86	22.7	8	38	61.9
D_5-NEtFOSA	12	70	16.5	21	70	25.5	8	30	57.8
D_3-NMeFOSAA	21	113	7.3	52	142	14.8	106	139	13.1
D_5-NEtFOSAA	12	106	8.2	68	151	16.9	79	151	31.8
D_7-NMeFOSE	11	77	18.6	13	107	27.9	5	30	81.1
D_9-NEtFOSE	8	73	19.6	16	97	30.4	0	29	103.1
$^{13}C_3$-HFPO-DA	92	113	2.0	70	119	10.4	93	102	5.1

Data for this table are derived from the single-laboratory validation study, and are only provided as examples for this draft method. The data will be updated with the interlaboratory study results in a subsequent revision.

Table 10. Range of Recoveries for Non-Extracted Internal Standards in the Single-laboratory Validation Study, by Matrix

	Aqueous			Solid			Tissue		
	% Recovery		RSD	% Recovery		RSD	% Recovery		RSD
NIS Compounds	Min	Max	(%)	Min	Max	(%)	Min	Max	(%)
$^{13}C_3$-PFBA	60	91	10.3	54	89	6.4	51	82	7.0
$^{13}C_2$-PFHxA	43	94	18.6	52	90	7.4	41	80	19.3
$^{13}C_4$-PFOA	59	87	9.7	54	89	6.4	51	82	9.5
$^{13}C_5$-PFNA	64	87	7.5	59	94	7.1	52	88	11.2
$^{13}C_2$-PFDA	57	86	10.0	55	91	8.6	47	85	19.4
$^{18}O_2$-PFHxS	59	87	9.6	53	87	7.1	51	80	8.1
$^{13}C_4$-PFOS	60	82	7.5	58	86	7.0	52	85	10.3

Data for this table are derived from the single-laboratory validation study, and are only provided as examples for this draft method. The data will be updated with the interlaboratory study results in a subsequent revision.

21.0 Glossary

These definitions and purposes are specific to this method, but have been conformed to common usage to the extent possible.

21.1 Units of weight and measure and their abbreviations

21.1.1 Symbols

ºC	degrees Celsius
Da	Dalton (equivalent to "amu" below)
µg	microgram
µL	microliter
µm	micrometer
<	less than
≤	less than or equal
>	greater than
≥	greater than or equal
%	percent
±	plus or minus

21.1.2 Alphabetical abbreviations

amu	atomic mass unit (equivalent to Dalton)
cm	centimeter
g	gram
h	hour
L	liter
M	molar
mg	milligram
min	minute
mL	milliliter
mm	millimeter
cm	centimeter
m/z	mass-to-charge ratio
ng	nanogram
Q1	quantitation ion
Q2	confirmation ion
rpm	revolutions per minute
v/v	percent volume per volume

21.2 Definitions and acronyms (in alphabetical order)

Analyte – A PFAS compound included in this method. The analytes are listed in Table 1.

Calibration standard (CS) – A solution prepared from a secondary standard and/or stock solutions and used to calibrate the response of the LC-MS/MS instrument.

Calibration verification standard (CV) – The mid-point calibration standard (CS-4) that is used to verify calibration. See Table 4.

CFR – Code of Federal Regulations

Compound - One of many variants or configurations of a common chemical structure. Individual compounds are identified by the number of carbon atoms and functional group attached at the end of the chain.

Class A glassware – Volumetric glassware that provides the highest accuracy. Class A volumetric glassware complies with the Class A tolerances defined in ASTM E694, must be permanently labeled as Class A, and is supplied with a serialized certificate of precision.

CWA – Clean Water Act

Extracted internal standard (EIS) quantification – The response of the target compound is compared to the response of the labeled analog of another compound in the same LOC.

LC – Liquid chromatograph or liquid chromatography

Internal standard – A labeled compound used as a reference for quantitation of other labeled compounds and for quantitation of native PFAS compounds other than the compound of which it is a labeled analog. See Internal standard quantitation.

Instrument sensitivity check – solution used to check the sensitivity of the instrument. The solution contains the native compounds at the concentration of the LOQ.

Internal standard quantitation – A means of determining the concentration of (1) a naturally occurring (native) compound by reference to a compound other than its labeled analog and (2) a labeled compound by reference to another labeled compound

IPR – Initial precision and recovery; four aliquots of a reference matrix spiked with the analytes of interest and labeled compounds and analyzed to establish the ability of the laboratory to generate acceptable precision and recovery. An IPR is performed prior to the first time this method is used and any time the method or instrumentation is modified.

Isotope dilution (ID) quantitation – A means of determining a naturally occurring (native) compound by reference to the same compound in which one or more atoms has been isotopically enriched. The labeled PFAS are spiked into each sample and allow identification and correction of the concentration of the native compounds in the analytical process.

Isotopically labeled compound – An analog of a target analyte in the method which has been synthesized with one or more atoms in the structure replaced by a stable (non-radioactive) isotope of that atom. Common stable isotopes used are ^{13}C (Carbon-13) or Deuterium (D or ^{2}H). These labeled compounds do not occur in nature, so they can be used for isotope dilution quantitation or other method-specific purposes.

Limit of Quantitation (LOQ) – The smallest concentration that produces a quantitative result with known and recorded precision and bias. The LOQ shall be set at or above the concentration of the lowest initial calibration standard (the lowest calibration standard must fall within the linear range).

Method blank – An aliquot of reagent water that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and labeled compounds that are used with samples. The method blank is used to determine if analytes or interferences are present in the laboratory environment, the reagents, or the apparatus.

Method Detection Limit (MDL) – The minimum measured concentration of a substance that can be reported with 99% confidence that the measured analyte concentration is distinguishable from method blank results (40 CFR 136, Appendix B).

MESA – Mining Enforcement and Safety Administration

Minimum level of quantitation (ML) – The lowest level at which the entire analytical system must give a recognizable signal and acceptable calibration point for the analyte. The ML represents the lowest concentration at which an analyte can be measured with a known level of confidence. It may be equivalent to the concentration of the lowest calibration standard, assuming that all method-specified sample weights, volumes, and cleanup procedures have been employed. Alternatively, the ML may be established by multiplying the MDL (pooled or unpooled, as appropriate) by 3.18 and rounding the result to the number nearest to 1, 2, or 5 x 10^n, where n is zero or an integer (see 68 FR 11770).

MS – Mass spectrometer or mass spectrometry

Matrix Spike/Matrix Spike Duplicate (MS/MSD) – Aliquots of field samples that have been fortified with a known concentration of target compounds, prior to sample preparation and extraction, and analyzed to measure the effect of matrix interferences. The use of MS/MSD samples is generally not required in isotope dilution methods because the labeled compounds added to every sample provide more performance data than spiking a single sample in each preparation batch.

Multiple reaction monitoring (MRM) – Also known as selected reaction monitoring (SRM). A type of mass spectrometry where a parent mass of the compound is fragmented through MS/MS and then specifically monitored for a single fragment ion.

Must – This action, activity, or procedural step is required.

NIOSH – The National Institute of Occupational Safety and Health

Non-extracted internal standard (NIS) –Labeled PFAS compounds spiked into the concentrated extract immediately prior to injection of an aliquot of the extract into the LC-MS/MS.

OPR – Ongoing precision and recovery standard (OPR); a method blank spiked with known quantities of analytes. The OPR is analyzed exactly like a sample. Its purpose is to assure that the results produced by the laboratory remain within the limits specified in this method for precision and recovery.

Precursor Ion – For the purpose of this method, the precursor ion is the deprotonated molecule ([M-H]-) of the method analyte. In MS/MS, the precursor ion is mass selected and fragmented by collisionally activated dissociation to produce distinctive product ions of smaller *m/z*.

PFAS – Per- and Polyfluoroalkyl substances –A group of man-made fluorinated compounds that are hydrophobic and lipophobic, manufactured and used in a variety of industries globally. These compounds are persistent in the environment as well as in the human body. This method analyzes for the PFAS listed in Table 1.

Reagent water – Water demonstrated to be free from the analytes of interest and potentially interfering substances at the method detection limit for the analyte.

Relative standard deviation (RSD) – The standard deviation multiplied by 100 and divided by the mean. Also termed "coefficient of variation."

Relative Standard Error (RSE) – The standard error of the mean divided by the mean and multiplied by 100.

RF – Response factor. See Section 10.3.3.2.

RR – Relative response. See Section 10.3.3.2.

RT – Retention time**;** the time it takes for an analyte or labeled compound to elute off the HPLC/UPLC column

Should – This action, activity, or procedural step is suggested but not required.

Signal-to-noise ratio (S/N) – The height of the signal as measured from the mean (average) of the noise to the peak maximum divided by the mean height of the noise.

SPE – Solid-phase extraction; a technique in which an analyte is extracted from an aqueous solution or a solid/tissue extract by passage over or through a material capable of reversibly adsorbing the analyte. Also termed liquid-solid extraction.

Stock solution – A solution containing an analyte that is prepared using a reference material traceable to EPA, NIST, or a source that will attest to the purity and authenticity of the reference material.

Appendix A - Sample Pre-screening Instructions

Samples that are known or suspected to contain high levels of analytes may be pre-screened using the following procedure. These are example procedures using smaller sample aliquots spiked with EIS and NIS and no clean up procedures. Other pre-screening procedures may be used.

Aqueous Samples

1. Weight out 10 (±0.1) g of sample into a 50-mL centrifuge tube.
2. Add 50 µL of EIS and NIS to the sample and vortex to mix.
3. Filter 1 mL of the sample through 0.2-µm membrane filter into a microvial. Sample is ready for instrumental analysis.

Solid and Tissue Samples

1. Weigh 1.0 (±0.1) g sample into 50-mL polypropylene centrifuge tubes.
2. Add 20 mL of 0.3% methanolic ammonium hydroxide (Section 7.1.7.1). Vortex and mix on a shaker table (or equivalent) for 10 min. Allow to settle and/or centrifuge to produce a clear extract.
3. Filter using a Single Step® filter vial:
   a. Add 20 µL of EIS to a clean Single Step® filter vial (chamber).
   b. Add 400 µL of clear extract from step 2 (e.g., by adding extract until it reaches the fill line), carefully vortex to mix.
   c. Use filter/plunger part and filter.
4. Transfer 30 µL of filtrate to a ~300-µL polypropylene micro-vial and dilute to 300 µL with 0.3% methanolic ammonium hydroxide (Section 7.1.7.1). Add NIS to the filtrate.
5. The extract is now a 10x dilution.
6. Sample is ready for instrumental analysis.

Calculate results using the equivalent sample weight computed as follows:

$$Equivalent\ Weight = Sample\ weight\ (g) \times \frac{0.4\ mL}{20\ mL}$$

Note that the EIS concentration in the diluted portion is 0.5x the level in the regular analysis of solid samples.

Appendix B - Aqueous Sample Subsampling Instructions

Warning: Because some target analytes may be stratified within the sample (e.g., AFFF-contaminated media, surfactants), or adhere to the walls of the sample container, subsampling may only be done on a project-specific basis. Subsampling has been shown to increase uncertainty in PFAS analysis, especially on foaming samples.

If a reduced sample size is required, transfer a weighed subsample using the following subsampling procedure to a 60-mL HDPE bottle and dilute to approximately 60 mL using reagent water. This container is now considered the "sample bottle."

1. Gently invert sample 3-4 times being careful to avoid foam formation and subsample immediately (do not let stand).

2. If foam forms and more than 5 mL is required – pour sample, avoiding any foam.

3. If foaming forms and a volume less than 5 mL is required – pipette from ½ cm below the foam.

4. If no foam forms – pour or pipette based on volume required.