METHOD 533: DETERMINATION OF PER- AND POLYFLUOROALKYL SUBSTANCES IN DRINKING WATER BY ISOTOPE DILUTION ANION EXCHANGE SOLID PHASE EXTRACTION AND LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY

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Disclaimer

This analytical method may support a variety of monitoring applications, which include the analysis of multiple short-chain per- and polyfluoroalkyl substances (PFAS) that cannot be measured by Method 537.1. This publication meets an agency commitment identified within the 2019 EPA PFAS Action Plan. Publication of the method, in and of itself, does not establish a requirement, although the use of this method may be specified by the EPA or a state through independent actions. Terms such as "must" or "required," as used in this document, refer to procedures that are to be followed to conform with the method. References to specific brands and catalog numbers are included only as examples and do not imply endorsement of the products. Such reference does not preclude the use of equivalent products from other vendors or suppliers.

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1 Scope and Application

This is a solid phase extraction (SPE) liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the determination of select per- and polyfluoroalkyl substances (PFAS) in drinking water. Method 533 requires the use of MS/MS in Multiple Reaction Monitoring (MRM) mode to enhance selectivity. Accuracy and precision data have been generated in reagent water and drinking water for the compounds included in the Analyte List.

This method is intended for use by analysts skilled in the performance of solid phase extractions, the operation of LC-MS/MS instrumentation, and the interpretation of the associated data.

Analyte List

Analyte[a]	Abbreviation	CASRN
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF3OUdS	763051-92-9
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acd	9Cl-PF3ONS	756426-58-1
4,8-Dioxa-3*H*-perfluorononanoic acid	ADONA	919005-14-4
Hexafluoropropylene oxide dimer acid	HFPO-DA	13252-13-6
Nonafluoro-3,6-dioxaheptanoic acid	NFDHA	151772-58-6
Perfluorobutanoic acid	PFBA	375-22-4
Perfluorobutanesulfonic acid	PFBS	375-73-5
1*H*,1*H*, 2*H*, 2*H*-Perfluorodecane sulfonic acid	8:2FTS	39108-34-4
Perfluorodecanoic acid	PFDA	335-76-2
Perfluorododecanoic acid	PFDoA	307-55-1
Perfluoro(2-ethoxyethane)sulfonic acid	PFEESA	113507-82-7
Perfluoroheptanesulfonic acid	PFHpS	375-92-8
Perfluoroheptanoic acid	PFHpA	375-85-9
1*H*,1*H*, 2*H*, 2*H*-Perfluorohexane sulfonic acid	4:2FTS	757124-72-4
Perfluorohexanesulfonic acid	PFHxS	355-46-4
Perfluorohexanoic acid	PFHxA	307-24-4
Perfluoro-3-methoxypropanoic acid	PFMPA	377-73-1
Perfluoro-4-methoxybutanoic acid	PFMBA	863090-89-5
Perfluorononanoic acid	PFNA	375-95-1
1*H*,1*H*, 2*H*, 2*H*-Perfluorooctane sulfonic acid	6:2FTS	27619-97-2
Perfluorooctanesulfonic acid	PFOS	1763-23-1
Perfluorooctanoic acid	PFOA	335-67-1
Perfluoropentanoic acid	PFPeA	2706-90-3
Perfluoropentanesulfonic acid	PFPeS	2706-91-4
Perfluoroundecanoic acid	PFUnA	2058-94-8

a. Some PFAS are commercially available as ammonium, sodium, and potassium salts. This method measures all forms of the analytes as anions while the identity of the counterion is inconsequential. Analytes may be purchased as acids or as any of the corresponding salts.

1.1 Detection of PFAS Isomers

Both branched and linear PFAS isomers may be found in the environment. This method includes procedures for summing the contribution of multiple isomers to the final reported concentration. In those cases where standard materials containing multiple isomers are commercially available, laboratories should obtain such standards for the method analytes.

1.2 Lowest Concentration Minimum Reporting Limits

The lowest concentration minimum reporting level (LCMRL) is the lowest concentration for which the future recovery is predicted to fall between 50 and 150% with high confidence (99%). Single-laboratory LCMRLs determined for the method analytes during method development are reported in **Table 7**. It should be noted that most of the LCMRL values determined during the second laboratory evaluation were lower than the values listed in **Table 7**. The values that a laboratory can obtain are dependent on the design and capability of the instrumentation used. The procedure used to determine the LCMRL is described elsewhere.[1,2] Laboratories using this method are not required to determine LCMRLs, but they must demonstrate that they are able to meet the minimum reporting level (MRL) (Sect. 3.15) for each analyte per the procedure described in Section 9.1.4.

1.3 Method Flexibility

The laboratory may select LC columns, LC conditions, and MS conditions different from those used to develop the method. At a minimum, the isotope dilution standards and the isotope performance standards specified in the method must be used, if available. The laboratory may select the aqueous sample volume within the range of 100–250 mL that meets their objectives. During method development, 250 mL aqueous samples were extracted using a 500 mg solid phase extraction (SPE) sorbent bed volume. The ratio of sorbent mass to aqueous sample volume may not be decreased. If a laboratory uses 100 mL aqueous samples, the sorbent mass must be at least 200 mg. Changes may not be made to sample preservation, the quality control (QC) requirements, or the extraction procedure. The chromatographic separation should minimize the number of compounds eluting within a retention window to obtain a sufficient number of scans across each peak. Instrumental sensitivity (or signal-to-noise) will decrease if too many compounds are permitted to elute within a retention time window. Method modifications should be considered only to improve method performance. In all cases where method modifications are proposed, the analyst must perform the procedures outlined in the Initial Demonstration of Capability (IDC, Sect. 9.1), verify that all QC acceptance criteria in this method (Sect. 9.2) are met, and verify method performance in a representative sample matrix (Sect. 9.3.2).

2 Method Summary

A 100–250 mL sample is fortified with isotopically labeled analogues of the method analytes that function as isotope dilution standards. The sample is passed through an SPE cartridge containing polystyrene divinylbenzene with a positively charged diamino ligand to extract the method analytes and isotope dilution analogues. The cartridge is rinsed with sequential washes of aqueous ammonium acetate followed by methanol, then the compounds are eluted from the solid phase sorbent with methanol containing ammonium hydroxide. The extract is concentrated to dryness with nitrogen in a heated water bath. The extract volume is adjusted to 1.0 mL with 20% water in methanol (v/v), and three isotopically labeled isotope performance standards are added. Extracts are analyzed by LC-MS/MS

in the MRM detection mode. The concentration of each analyte is calculated using the isotope dilution technique. For QC purposes, the percent recoveries of the isotope dilution analogues are calculated using the integrated peak areas of isotope performance standards, which are added to the final extract and function as traditional internal standards, exclusively applied to the isotope dilution analogues.

3 Definitions

3.1 Analysis Batch

A set of samples that are analyzed on the same instrument during a 24-hour period that begins and ends with the analysis of the appropriate Continuing Calibration Check (CCC) standards. Additional CCCs may be required depending on the length of the Analysis Batch and the number of field samples.

3.2 Calibration Standard

A solution of the method analytes, isotope dilution analogues, and isotope performance standards prepared from the Primary Dilution Standards and stock standards. The calibration standards are used to calibrate the instrument response with respect to analyte concentration.

3.3 Continuing Calibration Check (CCC)

A calibration standard that is analyzed periodically to verify the accuracy of the existing calibration.

3.4 Extraction Batch

A set of up to 20 field samples (not including QC samples) extracted together using the same lot of solid phase extraction devices, solvents, and fortifying solutions.

3.5 Field Duplicates (FD)

Separate samples collected at the same time and sampling location, shipped and stored under identical conditions. Method precision, including the contribution from sample collection procedures, is estimated from the analysis of Field Duplicates. Field Duplicates are used to prepare Laboratory Fortified Sample Matrix and Laboratory Fortified Sample Matrix Duplicate QC samples. For the purposes of this method, Field Duplicates are collected to support potential repeat analyses (if the original field sample is lost or if there are QC failures associated with the analysis of the original field sample).

3.6 Field Reagent Blank (FRB)

An aliquot of reagent water that is placed in a sample container in the laboratory and treated as a sample in all respects, including shipment to the sampling site, exposure to sampling site conditions, storage, and all analytical procedures. The purpose of the FRB is to determine if method analytes or other interferences are introduced into the sample from shipping, storage, and the field environment.

3.7 Isotope Dilution Analogues

Isotopically labeled analogues of the method analytes that are added to the sample prior to extraction in a known amount. Note: Not all target PFAS currently have an isotopically labelled analogue. In these cases, an alternate isotopically labelled analogue is used as recommended in **Table 5**.

3.8 Isotope Dilution Technique

An analytical technique for measuring analyte concentration using the ratio of the peak area of the native analyte to that of an isotopically labeled analogue, added to the original sample in a known amount and carried through the entire analytical procedure.

3.9 Isotope Performance Standards

Quality control compounds that are added to all standard solutions and extracts in a known amount and used to measure the relative response of the isotopically labelled analogues that are components of the same solution. For this method, the isotope performance standards are three isotopically labeled analogues of the method analytes. The isotope performance standards are indicators of instrument performance and are used to calculate the recovery of the isotope dilution analogues through the extraction procedure. In this method, the isotope performance standards are not used in the calculation of the recovery of the native analytes.

3.10 Laboratory Fortified Blank (LFB)

An aliquot of reagent water to which known quantities of the method analytes and isotope dilution analogues are added. The results of the LFB verify method performance in the absence of sample matrix.

3.11 Laboratory Fortified Sample Matrix (LFSM)

An aliquot of a field sample to which known quantities of the method analytes and isotope dilution analogues are added. The purpose of the LSFM is to determine whether the sample matrix contributes bias to the analytical results. Separate field samples are required for preparing fortified matrix so that sampling error is included in the accuracy estimate.

3.12 Laboratory Fortified Sample Matrix Duplicate (LFSMD)

A Field Duplicate of the sample used to prepare the LFSM that is fortified and analyzed identically to the LFSM. The LFSMD is used instead of the Field Duplicate to assess method precision when the method analytes are rarely found at concentrations greater than the MRL.

3.13 Laboratory Reagent Blank (LRB)

An aliquot of reagent water fortified with the isotope dilution analogues and processed identically to a field sample. An LRB is included in each Extraction Batch to determine if the method analytes or other interferences are introduced from the laboratory environment, the reagents, glassware, or extraction apparatus.

3.14 Lowest Concentration Minimum Reporting Level (LCMRL)

The single-laboratory LCMRL is the lowest spiking concentration such that the probability of spike recovery in the 50% to 150% range is at least 99%.[1,2]

3.15 Minimum Reporting Level (MRL)

The minimum concentration that may be reported by a laboratory as a quantified value for a method analyte. For each method analyte, the concentration of the lowest calibration standard must be at or

below the MRL and the laboratory must demonstrate its ability to meet the MRL per the criteria defined in Section 9.1.4.

3.16 Precursor Ion

The gas-phase species corresponding to the method analyte that is produced in the electrospray ionization interface. During tandem mass spectrometry, or MS/MS, the precursor ion is mass selected and fragmented by collision-activated dissociation to produce distinctive product ions of smaller mass to charge (*m/z*) ratio. For this method, the precursor ion is usually the deprotonated molecule ($[M - H]^-$) of the method analyte, except for HFPO-DA. For this analyte, the precursor ion is formed by decarboxylation of HFPO-DA.

3.17 Primary Dilution Standard (PDS)

A solution that contains method analytes (or QC analytes) prepared from stock standards. PDS solutions are used to fortify QC samples and diluted to prepare calibration standards.

3.18 Product Ion

One of the fragment ions that is produced in MS/MS by collision-activated dissociation of the precursor ion.

3.19 Quality Control Standard (QCS)

A calibration standard prepared independently from the primary calibration solutions. For this method, the QCS is a repeat of the entire dilution scheme starting with the same stock materials (neat compounds or purchased stock solutions) used to prepare the primary calibration solutions. Independent sources and separate lots of the starting materials are not required, provided the laboratory has obtained the purest form of the starting materials commercially available. The purpose of the QCS is to verify the integrity of the primary calibration standards.

3.20 Quantitative Standard

A quantitative standard of assayed concentration and purity traceable to a Certificate of Analysis.

3.21 Stock Standard Solution

A concentrated standard that is prepared in the laboratory using assayed reference materials or that is purchased from a commercial source with a Certificate of Analysis.

3.22 Technical-Grade Standard

As defined for this method, a technical-grade standard includes a mixture of the branched and linear isomers of a method analyte. For the purposes of this method, technical-grade standards are used to identify retention times of branched and linear isomers of method analytes.

4 Interferences

4.1 Labware, Reagents and Equipment

Method interferences may be caused by contaminants in solvents, reagents (including reagent water), sample bottles and caps, and other sample processing hardware that lead to discrete artifacts or

elevated baselines in the chromatograms. The analytes in this method can also be found in many common laboratory supplies and equipment, such as PTFE (polytetrafluoroethylene) products, LC solvent lines, methanol, aluminum foil, deactivated syringes, SPE sample transfer lines, etc.[3] Laboratories must demonstrate that these items are not contributing to interference by analyzing LRBs as described in Section 9.2.1.

4.2 Sample Contact with Glass

Aqueous samples should not come in contact with any glass containers or pipettes as PFAS analytes can potentially adsorb to glass surfaces. Standards dissolved in organic solvent may be purchased in glass ampoules. These standards in organic solvent are acceptable and subsequent transfers may be performed using glass syringes and pipets. Following extraction, the eluate must be collected in a polypropylene tube prior to concentration to dryness. Concentration to dryness in glass tubes may cause poor recovery.

4.3 Matrix Interferences

Matrix interferences may be caused by contaminants that are co-extracted from the sample. The extent of matrix interferences will vary considerably from source to source, depending upon the nature of the water. Humic and fulvic material may be co-extracted during SPE and high levels may cause enhancement or suppression in the electrospray ionization source.[4] Inorganic salts may cause low recoveries during the anion-exchange SPE procedure.

4.3.1 Co-extracted Organic Material

Under the LC conditions used during method development, matrix effects due to co-extracted organic material enhanced the ionization of 4:2 FTS appreciably. Total organic carbon (TOC) is a good indicator of humic content of the sample.

4.3.2 Inorganic Salts

The authors confirmed acceptable method performance for matrix ion concentrations up to 250 mg/L chloride, 250 mg/L sulfate, and 340 mg/L hardness measured as $CaCO_3$. Acceptable performance was defined as recovery of the isotope dilution analogues between 50–200%.

4.3.3 Ammonium Acetate

Relatively large quantities of ammonium acetate are used as a preservative. The potential exists for trace-level organic contaminants in this reagent. Interferences from this source should be monitored by analysis of LRBs, particularly when new lots of this reagent are acquired.

4.3.4 SPE Cartridges

Solid phase extraction cartridges may be a source of interferences. The analysis of LRBs provides important information regarding the presence or absence of such interferences. Each brand and lot of SPE devices must be monitored to ensure that contamination does not preclude analyte identification and quantitation. SPE cartridges should be sealed while in storage to prevent ambient contamination of the SPE sorbent.

4.4 Bias Caused by Isotopically Labeled Standards

During method development, no isotopically labeled standard solution yielded any signal that gave the same mass and retention time as any native analyte. However, due to isotopic impurity, the $^{13}C_3$-PFBA isotope performance standard contained a small amount of $^{13}C_4$-PFBA, slightly contributing to the signal of the isotope dilution analogue. Further, due to natural abundance of ^{34}S, the native telomer sulfonates produced a small contribution to the $^{13}C_2$ labeled telomer sulfonate isotope dilution analogues. The effects on quantitation are insignificant. However, these cases are described below in Sections 4.4.2 and 4.4.3 to alert the user that these situations could occur.

4.4.1 Method Analytes

At the concentrations used to collect method performance data, the authors could not detect any contribution from the isotope dilution analogues or isotope performance standards to the corresponding native analyte response. However, the user should evaluate each source of isotopically labeled analogues and isotope performance standards to verify that they do not contain any native analyte at concentrations greater than 1/3 of the MRL.

4.4.2 Isotopic purity of $^{13}C_3$-PFBA

In this method, $^{13}C_3$-PFBA is used as an isotope performance standard and $^{13}C_4$-PFBA is used as an isotope dilution analogue. Both share the same product ion, *m/z* 172. Ten nanograms per liter of $^{13}C_4$-PFBA is added to the sample prior to extraction (10 ng/mL extract concentration assuming 100% recovery), and 10 ng/mL of $^{13}C_3$-PFBA is added to the final extract. Because the natural abundance of ^{13}C is 1.1%, there is a 1.1% contribution to the $^{13}C_4$-PFBA area from the lone, unlabeled ^{12}C atom in $^{13}C_3$-PFBA. The authors confirmed this contribution empirically. Users of this method may consider this bias to the area of the PFBA isotope dilution analogue insignificant.

4.4.3 Isotopic purity of $^{13}C_4$-PFBA

A trace amount of $^{13}C_3$-PFBA was detected in the $^{13}C_4$-PFBA. The contribution was no greater than 1%. The contribution of the isotope performance standard to the isotope dilution analogue is insignificant.

4.4.4 Telomer Sulfonates

Each of the three telomer sulfonates in the analyte list (4:2FTS, 6:2FTS, and 8:2FTS) are referenced to their $^{13}C_2$ isotope dilution analogue. The mass difference between the telomer sulfonates and the isotope dilution analogues is 2 mass units. The single sulfur atom in each of the unlabeled molecules has a naturally occurring M+2 isotope (^{34}S) at 4.25%. Thus, the precursor ions of the $^{13}C_2$ isotopically labeled analogues and the naturally occuring ^{34}S analogues present in the native analytes have the same nominal masses. The product ions of the telomer sulfonate isotope dilution analogues listed in **Table 6** would contain a small contribution from the ^{34}S analogue of the native telomer sulfonates. At the concentrations used in this study, the contribution of the ^{34}S analogue to the isotope dilution analogue was not greater than 2.7%. Alternate product ions may be used if there is sufficient abundance.

5 Safety

Each chemical should be treated as a potential health hazard and exposure to these chemicals should be minimized. Each laboratory is responsible for maintaining an awareness of OSHA regulations regarding

safe handling of chemicals used in this method. A reference file of safety data sheets should be made available to all personnel involved in the chemical analysis.

6 Equipment and Supplies

References to specific brands and catalog numbers are included as examples only and do not imply endorsement of the products. Such reference does not preclude the use of equivalent products from other vendors or suppliers. Due to potential adsorption of analytes onto glass, polypropylene containers were used for sample preparation and extraction steps. Other plastic materials (e.g., polyethylene) that meet the QC requirements of Section 9 may be substituted.

6.1 Sample Containers

Polypropylene bottles with polypropylene screw caps (for example, 250 mL bottles, Fisher Scientific, Cat. No. 02-896-D or equivalent).

6.2 Polypropylene Vials

These vials are used to store stock standards and PDS solutions (4 mL, VWR Cat. No. 16066-960 or equivalent).

6.3 Centrifuge Tubes

Conical polypropylene centrifuge tubes (15 mL) with polypropylene screw caps for storing standard solutions and for collection of the eluate during the extraction procedure (Thomas Scientific Cat. No. 2602A10 or equivalent).

6.4 Autosampler Vials

Polypropylene autosampler vials (ThermoFisher, Cat. No. C4000-14) with polypropylene caps (ThermoFisher, Cat. No. C5000-50 or equivalent). Note: Polypropylene vials and caps are necessary to prevent contamination of the sample from PTFE coated septa. However, polypropylene caps do not reseal, creating the potential for evaporation to occur after injection. Multiple injections from the same vial are not permissible unless the cap is replaced immediately after injection.

6.5 Micro Syringes

Suggested sizes include 10, 25, 50, 100, 250, 500 and 1000 µL.

6.6 Pipets

Polypropylene or glass pipets may be used for methanolic solutions.

6.7 Analytical Balance

Capable of weighing to the nearest 0.0001 g.

6.8 Solid Phase Extraction (SPE) Apparatus

6.8.1 SPE Cartridges

SPE cartridges containing weak anion exchange, mixed-mode polymeric sorbent (polymeric backbone and a diamino ligand), particle size approximately 33 µm. The SPE sorbent must have a pKa above 8 so that it remains positively charged during extraction. SPE cartridges containing 500 mg sorbent

(Phenomenex Cat. No. 8B-S038-HCH) were used during method development. Use of 200 mg cartridges is acceptable for the extraction of 100 mL samples.

6.8.2 Vacuum Extraction Manifold

Equipped with flow and vacuum control [Supelco Cat. No. 57030-U, UCT Cat. No. VMF016GL (the latter requires UCT Cat. No. VMF02116 control valves), or equivalent systems]. Automated devices designed for use with SPE cartridges may be used; however, all extraction and elution steps must be the same as in the manual procedure. Care must be taken with automated SPE systems to ensure that Teflon tubing and other PTFE components commonly used in these systems, do not contribute to unacceptable analyte concentrations in LRBs.

6.8.3 Sample Delivery System

Use of large volume sampling lines, constructed with polyethylene tubing, are recommended, but not mandatory. Large volume sample transfer lines, constructed with PTFE tubing, are commercially available for standard extraction manifolds (Supelco Cat. No. 57275 or equivalent). The PTFE tubing can be replaced with 1/8" o.d. x 1/16" i.d. polyethylene tubing [Freelin-Wade (McMinnville, Oregon) LLDPE or equivalent] cut to an appropriate length. This prevents potential contamination from PTFE transfer lines. Other types of non-PTFE tubing may be used provided it meets the LRB and LFB QC requirements. PTFE tubing may be used, but an LRB must be run on each individual transfer line and the QC requirements in Section 9.2.1 must be met. In the case of automated SPE, the removal of PTFE lines may not be feasible; therefore, acceptable performance for the LRB must be met for each port during the IDC (Sect 9.1.1). LRBs must be rotated among the ports during routine analyses thereafter. Plastic reservoirs are difficult to rinse during elution and their use may lead to lower recovery.

6.9 Extract Concentration System

Extracts are concentrated by evaporation with high-purity nitrogen using a water bath set no higher than 60 °C [N-Evap, Model 11155, Organomation Associates (Berlin, MA), Inc., or equivalent].

6.10 Laboratory Vacuum System

Sufficient capacity to maintain a vacuum of approximately 15 to 20 inches of mercury for extraction cartridges.

6.11 pH Meter

Used to verify the pH of the phosphate buffer and to measure the pH of the aqueous sample prior to anion exchange SPE.

6.12 LC-MS/MS System

6.12.1 LC System

The LC system must provide consistent sample injection volumes and be capable of performing binary linear gradients at a constant flow rate. On some LC systems, PFAS may build up in PTFE transfer lines when the system is idle for more than one day. To prevent long delays in purging high levels of PFAS from the LC solvent lines, it may be useful to replace PTFE tubing with PEEK™ tubing and the PTFE solvent frits with stainless steel frits. These modifications were not used on the LC system used for method development. However, a delay column, HLB Direct Connect 2.1 x 30 mm (Waters 186005231),

was placed in the mobile phase flow path immediately before the injection valve. This direct connect column may have reduced the co-elution of PFAS originating from sources prior to the sample loop from the PFAS injected in the sample. It may not be possible to remove all PFAS background contamination.

6.12.2 Analytical Column

C18 liquid chromatography column (2 x 50 mm) packed with 3 µm C18 solid phase particles (Phenomenex Part Number 00B-4439-B0 or equivalent).

6.12.3 Electrospray Ionization Tandem Mass Spectrometer (ESI-MS/MS)

The mass spectrometer must be capable of electrospray ionization in the negative ion mode. The system must be capable of performing MS/MS to produce unique product ions for the method analytes within specified retention time segments. A minimum of 10 scans across the chromatographic peak is needed to ensure adequate precision. Some ESI-MS/MS instruments may not be suitable for PFAS analysis. See the procedures in Section 10.1.2.1 to ensure that the selected MS/MS platform is capable of monitoring all the required MS/MS transitions for the method analytes.

6.12.4 MS/MS Data System

An interfaced data system is required to acquire, store, and output MS data. The computer software must have the capability of processing stored data by recognizing a chromatographic peak within a given retention time window. The software must allow integration of the abundance of any specific ion between specified time or scan number limits. The software must be able to construct a linear regression or quadratic regression calibration curve and calculate analyte concentrations using the internal standard technique.

7 Reagents and Standards

Reagent grade or better chemicals must be used. Unless otherwise indicated, all reagents must conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society (ACS), where such specifications are available. Other grades may be used if the reagent is demonstrated to be free of analytes and interferences and all requirements of the IDC are met when using these reagents.

7.1 Reagent Water

Purified water which does not contain any measurable quantities of any method analytes or interfering compounds greater than one-third of the MRL for each method analyte. It may be necessary to flush the water purification unit to rinse out any build-up of PFAS in the system prior to collection of reagent water.

7.2 Methanol

CH_3OH, CASRN 67-56-1, LC grade (Fisher Scientific, Cat. No. A456 or equivalent).

7.3 Ammonium Acetate

$NH_4C_2H_3O_2$, CASRN 631-61-8, HPLC grade, molecular weight equals 77.08 g/mole.

7.3.1 20 mM Ammonium Acetate

Chromatographic mobile phase. To prepare 1 L, add 1.54 g ammonium acetate to 1 L of reagent water. This solution is volatile and must be replaced at least once per week. More frequent replacement may be necessary if unexplained losses in sensitivity or retention time shifts are encountered.

7.3.2 1 g/L Ammonium Acetate

Used to rinse SPE cartridges after loading the aqueous sample and prior to the methanol rinse. Prepare in reagent water.

7.4 Concentrated Ammonium Hydroxide Reagent

NH_4OH, CASRN 1336-21-6, approximately 56.6% in water as ammonium hydroxide (w/w), approximately 28% in water as ammonia, approximately 14.5 N (Fisher Scientific, Cat. No. A669, Certified ACS Plus grade, or equivalent).

7.5 Solution of Ammonium Hydroxide in Methanol

Used for elution of SPE cartridges. Dilute 2 mL of concentrated ammonium hydroxide (56.6% w/w) in 100 mL methanol. This solution should be made fresh on the day of extraction.

7.6 Sodium Phosphate Dibasic (Na_2HPO_4)

Used for creating the aqueous buffer for conditioning the SPE cartridges. Dibasic sodium phosphate may be purchased in either the anhydrous or any hydrated form. The formula weight will vary based on degree of hydration.

7.7 Sodium Phosphate Monobasic (NaH_2PO_4)

Used for creating the aqueous buffer for conditioning the SPE cartridges. Monobasic sodium phosphate may be purchased in either the anhydrous or any hydrated form. The formula weight will vary based on degree of hydration.

7.8 0.1 M Phosphate Buffer pH 7.0

Mix 500 mL of 0.1 M dibasic sodium phosphate with approximately 275 mL of 0.1 M monobasic sodium phosphate. Verify that the solution pH is approximately 7.0.

7.9 Nitrogen

7.9.1 Nitrogen Nebulizer Gas

Nitrogen used as a nebulizer gas in the ESI interface and as collision gas in some MS/MS platforms should meet or exceed the instrument manufacturer's specifications.

7.9.2 Nitrogen used for Concentrating Extracts

Ultra-high-purity-grade nitrogen should be used to concentrate sample extracts.

7.10 Argon

Used as collision gas in MS/MS instruments. Argon should meet or exceed instrument manufacturer's specifications. Nitrogen may be used as the collision gas if recommended by the instrument manufacturer.

7.11 Sodium Hydroxide

May be purchased as pellets or as aqueous solution of known concentration. Added to methanolic solutions of PFAS to prevent esterification.

7.12 Acetic Acid (glacial)

May be necessary to adjust pH of aqueous samples. The pH of the aqueous sample containing 1 g/L ammonium acetate must be between 6 and 8.

7.13 Standard Solutions

7.13.1 Stability of Methanolic Solutions

Fluorinated carboxylic acids will esterify in anhydrous acidic methanol. To prevent esterification, standards must be stored under basic conditions. If base is not already present, this may be accomplished by the addition of sodium hydroxide (approximately 4 mole equivalents) when standards are diluted in methanol. When calculating molarity for solutions containing multiple PFAS, the molecular weight can be estimated as 250 atomic mass units (amu). It is necessary to include sodium hydroxide in solutions of both isotopically labeled and native analytes. The amount of sodium hydroxide needed may be calculated using the following equation:

$$\frac{Total\ PFAS\ mass\ (g) \times 160(\frac{g}{mol})}{250\ (\frac{g}{mol})} = Mass\ of\ NaOH\ Required\ (g)$$

7.13.2 Preparation of Standards

When a compound purity is assayed to be 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. Sorption of PFAS analytes in methanol solution to glass surfaces after prolonged storage has not been evaluated. PFAS analyte and isotopically labeled analogues commercially purchased in glass ampoules are acceptable; however, all subsequent transfers or dilutions performed by the analyst must be stored in polypropylene containers.
Solution concentrations listed in this section were used to develop this method and are included as examples. Alternate concentrations may be used as necessary depending on instrument sensitivity and the calibration range used. Standards for sample fortification generally should be prepared in the smallest volume that can be accurately measured to minimize the addition of excess organic solvent to aqueous samples. Laboratories should use standard QC practices to determine when standards need to be replaced. The analyte supplier's guidelines may be helpful when making this determination.

7.14 Storage Temperatures for Standards Solutions

Store stock standards at less than 4 °C unless the vendor recommends otherwise. The Primary Dilution Standards may be stored at any temperature, but cold storage is recommended to prevent solvent evaporation. During method development, the PDS was stored at –20 °C and no change in analyte concentrations was observed over a period of 6 months.

7.15 Isotope Performance Standards

This method requires three isotope performance standards listed in the table below. These isotopically labeled compounds were chosen during method development to include the analogues of three method analytes: two carboxylates with different chain lengths and a sulfonate.

Obtain the isotope performance standards as certified standard solutions, if available, or as the neat compounds. During method development, the isotope performance standards were obtained from Wellington Laboratories (Guelph, ON, Canada) as certified stocks in basic methanol. Note that Chemical Abstracts Registry Numbers are not currently available for these compounds. The concentrations of the stocks supplied by Wellington are listed in the table below.

Isotope Performance Standards	Abbreviation	Wellington Stock, µg/mL	PDS, ng/µL
Perfluoro-*n*-[2,3,4-$^{13}C_3$]butanoic acid	$^{13}C_3$-PFBA	50	1.0
Perfluoro-[1,2-$^{13}C_2$]octanoic acid	$^{13}C_2$-PFOA	50	1.0
Sodium perfluoro-1-[1,2,3,4-$^{13}C_4$]octanesulfonate	$^{13}C_4$-PFOS	50[a]	3.0

a. 47.8 µg/mL as the anion.

All the isotope performance standards listed in this section must be used, if available. Additional isotope performance standards may be used provided they are isotopically labeled analytes or labeled analytes with similar functional groups as the method analytes. Linear isomers are recommended to simplify peak integration. Method modification QC requirements must be met (Sect. 9.3) whenever additional isotope performance standards are used.

7.15.1 Isotope Performance Standard PDS

Prepare the isotope performance standard PDS in methanol and add sodium hydroxide if not already present to prevent esterification as described in Section 7.13.1. The PDS concentrations used to develop the method are listed in the table above (Sect. 7.15). During collection of method performance data, the final extracts were fortified with 10 µL of the PDS to yield a concentration of 10 ng/mL for $^{13}C_3$-PFBA and $^{13}C_2$-PFOA, and 30 ng/mL for $^{13}C_4$-PFOS (28.7 ng/mL as the anion).

7.16 Isotope Dilution Analogues

Obtain the isotopically labeled analogues listed in the table in this section as individual certified standard solutions or as certified standard mixes. All listed isotope dilution analogues must be used, if available. Linear isomers are recommended to simplify peak integration. During method development, the isotope dilution analogues were obtained from Wellington Laboratories (Guelph, ON, Canada) as certified stocks in basic methanol. These analogues were chosen during method development because they encompass most of the functional groups, as well as the molecular weight range of the method analytes. Note that Chemical Abstracts Registry Numbers are not currently available for these isotopically labeled analogues.

Isotope Dilution Standards	Abbreviation	PDS, ng/µL[a]
Perfluoro-*n*-[1,2,3,4-$^{13}C_4$]butanoic acid	$^{13}C_4$-PFBA	0.50
Perfluoro-*n*-[1,2,3,4,5-$^{13}C_5$]pentanoic acid	$^{13}C_5$-PFPeA	0.50
Sodium perfluoro-1-[2,3,4-$^{13}C_3$]butanesulfonate	$^{13}C_3$-PFBS	0.50
Sodium 1*H*,1*H*,2*H*,2*H*-perfluoro-1-[1,2-$^{13}C_2$]hexane sulfonate	$^{13}C_2$-4:2FTS	2.0
Perfluoro-*n*-[1,2,3,4,6-$^{13}C_5$]hexanoic acid	$^{13}C_5$-PFHxA	0.50
2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy-$^{13}C_3$-propanoic acid	$^{13}C_3$-HFPO-DA	0.50
Perfluoro-*n*-[1,2,3,4-$^{13}C_4$]heptanoic acid	$^{13}C_4$-PFHpA	0.50
Sodium perfluoro-1-[1,2,3-$^{13}C_3$]hexanesulfonate	$^{13}C_3$-PFHxS	0.50
Sodium 1*H*,1*H*,2*H*,2*H*-perfluoro-1-[1,2-13C_2]-octane sulfonate	$^{13}C_2$-6:2FTS	2.0
Perfluoro-*n*-[$^{13}C_8$]octanoic acid	$^{13}C_8$-PFOA	0.50
Perfluoro-*n*-[$^{13}C_9$]nonanoic acid	$^{13}C_9$-PFNA	0.50
Sodium perfluoro-[$^{13}C_8$]octanesulfonate	$^{13}C_8$-PFOS	0.50
Sodium 1*H*,1*H*,2*H*,2*H*-perfluoro-1-[1,2-$^{13}C_2$]-decane sulfonate	$^{13}C_2$-8:2FTS	2.0
Perfluoro-*n*-[1,2,3,4,5,6-$^{13}C_6$]decanoic acid	$^{13}C_6$-PFDA	0.50
Perfluoro-*n*-[1,2,3,4,5,6,7-$^{13}C_7$]undecanoic acid	$^{13}C_7$-PFUnA	0.50
Perfluoro-*n*-[1,2-$^{13}C_2$]dodecanoic acid	$^{13}C_2$-PFDoA	0.50

a. Concentrations used during method development.

As additional isotopically labelled PFAS analogues become commercially available they may be integrated into the method provided they have similar functional groups as the method analytes or are isotopically labeled analogues of the method analytes. Method modification QC requirements must be met (Sect. 9.3) whenever new analogues are proposed.

7.16.1 Isotope Dilution Analogue PDS

Prepare the isotope dilution analogue PDS in methanol and add sodium hydroxide if not already present to prevent esterification as described in Section 7.13.1. The PDS concentrations used during method development are listed in the table above. Method performance data were collected using 20 µL of this PDS to yield concentrations of 40–160 ng/L in the 250 mL aqueous samples. Note that the concentrations of sulfonates in the isotope dilution analogue PDS is based on the weight of the salt. It is not necessary to account for difference in the formula weight of the salt compared to the free acid for sample quantitation.

7.17 Analyte Standard Materials

Analyte standards may be purchased as certified standard solutions or prepared from neat materials of assayed purity. If available, the method analytes should be purchased as technical-grade (as defined in Sect. 3.22) to ensure that linear and branched isomers are represented. Standards or neat materials that contain only the linear isomer can be substituted if technical-grade analytes are not available as quantitative standards.

During method development, analyte standards were obtained from AccuStandard, Inc. (New Haven, CT), Absolute Standards (Hamden, CT), Wellington Laboratories (Guelph, Ontario, Canada), Santa Cruz Biotechnology (Dallas, TX), and Synquest Laboratories, Inc. (Alachua, FL). Stock standards are made by dilution in methanol containing 4 mole equivalents of sodium hydroxide as described in Section 7.13.1

7.17.1 PFOA

A quantitative standard for PFOA is currently available only for the linear isomer; however, a technical-grade standard (Sect. 3.22) is available for PFOA that contains the linear and branched isomers (Wellington Labs, Cat. No. T-PFOA, or equivalent). This product or a similar technical-grade PFOA standard must be used to identify the retention times of the branched and linear PFOA isomers. However, the linear-only PFOA standard must be used for quantitation until a quantitative PFOA standard containing the branched and linear isomers becomes commercially available.

7.17.2 PFHxS and PFOS

Technical grade, quantitative PFHxS and PFOS standards containing branched and linear isomers must be used when available.

7.17.3 Correction for Analytes Obtained in the Salt Form

This method measures all forms of the analytes as anions while the identity of the counterion is inconsequential. Analytes may be commercially available as neat materials or as certified stock standards as their corresponding ammonium, sodium, or potassium salts. These salts are acceptable standards provided the measured mass, or concentration, is corrected for the salt content. The equation for this correction is provided below.

$$mass(acid\ form) = mass(salt\ form) \times \frac{MWacid}{MWsalt}$$

7.17.4 Analyte PDS

The analyte PDS is used to prepare the calibration standards and to fortify the LFBs, LFSMs and LFSMDs with the method analytes. Prepare the analyte PDS by combining and diluting the analyte stock standards in 100% methanol and add sodium hydroxide if not already present to prevent esterification as described in Section 7.13.1. Select nominal analyte concentrations for the PDS such that between 5 and 100 µL of the PDS is used to fortify samples and prepare standard solutions. More than one PDS concentration may be necessary to meet this requirement. During method development, the analyte PDS was prepared at an identical concentration for all analytes, 0.5 ng/µL. The user may modify the concentrations of the individual analytes based on the confirmed MRLs and the desired monitoring range. If the PDS is stored cold, warm the vials to room temperature and vortex prior to use.

7.17.5 Calibration Standards

Prepare a series of calibration standards of at least five levels by diluting the analyte PDS into methanol containing 20% reagent water. The lowest calibration standard must be at or below the MRL for each analyte. The calibration standards may also be used as Continuing Calibration Checks (CCCs). Using the PDS solutions, add a constant amount of the isotope performance standards and the isotope dilution analogues to each calibration standard. The concentration of the isotope dilution analogues should match the concentration of the analogues in sample extracts, assuming 100% recovery through the extraction process. During method development, the concentrations of the isotope dilution analogues were 40 ng/mL extract concentration (160 ng/L in the aqueous sample) for 4:2FTS, 6:2FTS and 8:2FTS, and 10 ng/mL (40 ng/L) for all others. The analyte calibration ranged from approximately 0.50 ng/mL to 25 ng/mL extract concentration.

8 Sample Collection, Preservation, and Storage

8.1 Sample Bottles

Samples must be collected in plastic bottles: polypropylene bottles fitted with polypropylene screw-caps, or polyethylene bottles with polypropylene screw caps. Discard sample bottles after a single use. The bottle volume should approximate the volume of the sample. Subsampling from a single bottle is not permitted except as described in Section 12.5.

8.2 Sample Preservation

Based on sample volume, add ammonium acetate to each sample bottle as a solid (prior to shipment to the field or immediately prior to sample collection) to achieve a 1g/L concentration of ammonium acetate. Ammonium acetate will sequester free chlorine to form chloramine.

8.3 Sample Collection

8.3.1 Precautions against Contamination

Workers must wash their hands before sampling and wear nitrile gloves while filling and sealing the sample bottles. Users should seek to minimize accidental contamination of the samples.

8.3.2 Collection Procedure

Open the tap and allow the system to flush until the water temperature has stabilized. Collect samples from the flowing system. Samples do not need to be collected headspace free. After collecting the sample, cap the bottle and agitate by hand until the preservative is dissolved. Keep the sample sealed from time of collection until extraction.

8.4 Field Reagent Blanks (FRB)

Each sample set must include an FRB. A sample set is defined as samples collected from the same site and at the same time. The same lot of preservative must be used for the FRBs as for the field samples.

8.4.1 Analysis of Reagent Water used for FRBs

Reagent water used for the FRBs must be analyzed prior to shipment to ensure the water has minimal residual PFAS. Extract an LRB prepared with reagent water using the same lot of sample bottles destined for shipment to the sampling site and ensure that analyte concentrations are less than one-third the MRL, as described in Section 9.2.1. This will ensure that any significant contamination detected in the FRBs originated from exposure in the field.

8.4.2 Field Reagent Blank Procedure

In the laboratory, fill the FRB sample bottle with the analyzed reagent water (Sect. 8.4.1), then seal and ship to the sampling site with the sample bottles. For each FRB shipped, a second FRB sample bottle containing only preservative must also be shipped. At the sampling site, open the FRB bottle and pour the reagent water into the second sample bottle containing preservative; seal and label this bottle as the FRB with the date, time and location of the site.

8.5 Sample Shipment and Storage

Samples must be shipped on ice. Samples are valid if any ice remains in the cooler when it is received at the laboratory or bottles are received within 2 days of collection and below 10 ^{0}C. Once at the laboratory, samples must be stored at or below 6 °C until extraction. Samples must not be frozen.

8.6 Sample and Extract Holding Times

Analyze samples as soon as possible. Samples must be extracted within 28 days of collection. Extracts are generally stored at room temperature and must be analyzed within 28 days after extraction.

9 Quality Control

QC procedures include the IDC and ongoing QC requirements. This section describes each QC parameter, its required frequency, and the performance criteria that must be met in order to satisfy method objectives. The QC criteria discussed in the following sections are summarized in **Table 16** and **Table 17**. These QC requirements are considered the minimum for an acceptable QC program. Laboratories are encouraged to institute additional QC practices to meet their specific needs.

9.1 Initial Demonstration of Capability

The IDC must be successfully performed prior to analyzing field samples. The IDC must be repeated if changes are made to analytical parameters not previously validated during the IDC. This may include, for example, changing the sample volume, selecting alternate quantitation ions, extending the calibration range, adding additional isotope performance standards, or adding additional isotope dilution analogues. Prior to conducting the IDC, the analyst must meet the calibration requirements outlined in Section 10. The same calibration range used during the IDC must be used for the analysis of field samples.

9.1.1 Demonstration of Low System Background

Analyze an LRB immediately after injecting the highest calibration standard in the selected calibration range. Confirm that the blank is free from contamination as defined in Section 9.2.1. If an automated extraction system is used, an LRB must be extracted on each port to fulfil this requirement.

9.1.2 Demonstration of Precision

Prepare, extract, and analyze seven replicate LFBs in a valid Extraction Batch (seven LFBs and an LRB). Fortify the LFBs near the midpoint of the initial calibration curve. The percent relative standard deviation (%RSD) of the concentrations of the replicate analyses must be less than 20% for all method analytes.

9.1.3 Demonstration of Accuracy

Using the same set of replicate data generated for Section 9.1.2, calculate the average percent recovery. The average recovery for each analyte must be within a range of 70–130%.

9.1.4 Minimum Reporting Level (MRL) Confirmation

Establish a target concentration for the MRL (Sect. 3.15) based on the intended use of the method. If there is a programmatic MRL requirement, the laboratory MRL must be set at or below this level. In doing so, one should consider that establishing the MRL concentration too low may cause repeated failure of ongoing QC requirements.

Perform initial calibration following the procedures in Section 10.3. The lowest calibration standard used to establish the initial calibration (as well as the low-level CCC) must be at, or below, the MRL. Confirm the laboratory's ability to meet the MRL following the procedure outlined below.

9.1.4.1 Prepare and Analyze MRL Samples

Fortify, extract, and analyze seven replicate LFBs at, or below, the proposed MRL concentration.

9.1.4.2 Calculate MRL Statistics

Calculate the mean and standard deviation for each analyte in these replicates. Determine the Half Range for the Prediction Interval of Results (HR_{PIR}) using the following equation:

$$HR_{PIR} = 3.963S$$

Where,

S = the standard deviation and 3.963 is a constant value for seven replicates.[1]

Calculate the Upper and Lower Limits for the Prediction Interval of Results (*PIR* = *Mean* ± HR_{PIR}) as shown below. These equations are only defined for seven replicate samples.

$$Upper\ PIR\ Limit = \frac{Mean + HR_{PIR}}{Fortified\ Concentration} \times 100$$

$$Lower\ PIR\ Limit = \frac{Mean - HR_{PIR}}{Fortified\ Concentration} \times 100$$

9.1.4.3 MRL Acceptance Criteria

The laboratory's ability to meet the MRL is confirmed if the *Upper PIR Limit* is less than, or equal to, 150%; and the *Lower PIR Limit* is greater than, or equal to, 50%. If these criteria are not met, the MRL has been set too low and must be confirmed again at a higher concentration.

9.1.5 Calibration Verification

Analyze a QCS (Sect. 9.2.9) to confirm the accuracy of the primary calibration standards.

9.2 Ongoing QC Requirements

This section describes the ongoing QC elements that must be included when processing and analyzing field samples.

9.2.1 Laboratory Reagent Blank (LRB)

Analyze an LRB with each Extraction Batch. Background concentrations of method analytes must be less than one-third the MRL. If method analytes are detected in the LRB at concentrations greater than or equal to this level, then all positive field sample results (i.e., results at or above the MRL) for those analytes are invalid for all samples in the Extraction Batch. Subtracting blank values from sample results is not permitted.

9.2.1.1 Estimating Background Concentrations

Although quantitative data below the MRL may not be accurate enough for data reporting, such data are useful in determining the magnitude of background interference. Therefore, the analyte concentrations in the LRB may be estimated by extrapolation when results are below the MRL.

9.2.1.2 Influence of Background on Selection of MRLs

Because background contamination can be a significant problem, some MRLs may be background limited.

9.2.1.3 Evaluation of Background when Analytes Exceed the Calibration Range

After analysis of a sample in which method analytes exceed the calibration range, one or more LRBs must be analyzed (to detect potential carryover) until the system meets the LRB acceptance criteria. If this occurs during an automated sequence, examine the results of samples analyzed following the sample that exceeded the calibration range. If the analytes that exceeded the calibration range in the previous sample are detected at, or above, the MRL, these samples are invalid. If the affected analytes do not exceed the MRL, these subsequent samples may be reported.

9.2.2 Continuing Calibration Check (CCC)

Analyze CCC standards at the beginning of each Analysis Batch, after every tenth field sample, and at the end of the Analysis Batch. See Section 10.4 for concentration requirements and acceptance criteria for CCCs.

9.2.3 Laboratory Fortified Blank

An LFB is required with each Extraction Batch. The concentration of the LFB must be rotated between low, medium, and high concentrations from batch to batch.

9.2.3.1 LFB Concentration Requirements

Fortify the low concentration LFB near the MRL. The high concentration LFB must be near the high end of the calibration range.

9.2.3.2 Evaluate Analyte Recovery

Results for analytes fortified at concentrations near or at the MRL (within a factor of two times the MRL concentration) must be within 50–150% of the true value. Results for analytes fortified at all other concentrations must be within 70–130% of the true value. If the LFB results do not meet these criteria, then all data for the problem analytes must be considered invalid for all samples in the Extraction Batch.

9.2.4 Isotope Performance Standard Areas

The analyst must monitor the peak areas of the isotope performance standards in all injections of the Analysis Batch. The isotope performance standard responses (as indicated by peak area) in any chromatographic run must be within 50–150% of the average area measured during the initial calibration. Random evaporation losses have been observed with the polypropylene caps causing high-biased isotope performance standard areas. If an isotope performance standard area for a sample does not meet these criteria, reanalyze the extract in a subsequent Analysis Batch. If the isotope performance standard area fails to meet the acceptance criteria in the repeat analysis, extraction of the sample must be repeated, provided the sample is still within holding time.

9.2.5 Isotope Dilution Analogue Recovery

Calculate the concentration of each isotope dilution analogue in field and QC samples using the average area in the initial calibration and the internal standard technique. Calculate the percent recovery (%R) for each analogue as follows:

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

Where,
A = measured concentration of the isotope dilution analogue, and
B = fortification concentration of the isotope dilution analogue.

The percent recovery for each analogue must be within a range of 50–200%.

9.2.5.1 Corrective Action for Failed Analogue Recovery

If an isotope dilution analogue fails to meet the recovery criterion, evaluate the area of the isotope performance standard to which the analogue is referenced and the recovery of the analogues in the CCCs. If necessary, recalibrate and service the LC-MS/MS system. Take corrective action, then analyze the failed extract in a subsequent Analysis Batch. If the repeat analysis meets the 50–200% recovery criterion, report only data for the reanalyzed extract. If the repeat analysis fails the recovery criterion after corrective action, extraction of the sample must be repeated provided a sample is available and still within the holding time.

9.2.6 Laboratory Fortified Sample Matrix (LFSM)

Within each Extraction Batch, analyze a minimum of one LFSM. The native concentrations of the analytes in the sample matrix must be determined in a separate field sample and subtracted from the measured values in the LFSM. If various sample matrices are analyzed regularly, for example, drinking water processed from ground water and surface water sources, collect performance data for each source.

9.2.6.1 Prepare the LFSM

Prepare the LFSM by fortifying a Field Duplicate with an appropriate amount of the analyte PDS (Sect. 7.17.4) and isotope dilution analogue PDS (Sect. 7.16.1). Generally, select a spiking concentration that is greater than or equal to the native concentration for the analytes. Selecting a duplicate aliquot of a sample that has already been analyzed aids in the selection of an appropriate spiking level. If this is not possible, use historical data when selecting a fortifying concentration.

9.2.6.2 Calculate the Percent Recovery

Calculate the percent recovery (%R) using the equation:

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

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

In order to obtain meaningful percent recovery results, correct the measured values in the LFSM and LFSMD for the native levels in the unfortified samples, even if the native values are less than the MRL.

9.2.6.3 Evaluate Analyte Recovery in the LFSM

Results for analytes fortified at concentrations near or at the MRL (within a factor of two times the MRL concentration) must be within 50–150% of the true value. Results for analytes fortified at all other concentrations must be within 70–130% of the true value. If the accuracy for any analyte falls outside the designated range, and the laboratory performance for that analyte is shown to be in control in the CCCs and in the LFB, the recovery is judged matrix biased. Report the result for the corresponding analyte in the unfortified sample as "suspect–matrix".

9.2.7 Laboratory Fortified Sample Matrix Duplicate (LFSMD) or Field Duplicate (FD)

Within each Extraction Batch, analyze a minimum of one Field Duplicate or one Laboratory Fortified Sample Matrix Duplicate. If the method analytes are not routinely observed in field samples, analyze an LFSMD rather than an FD.

9.2.7.1 Calculate the RPD for the LFSM and LFSMD

If an LFSMD is analyzed instead of a Field Duplicate, calculate the RPD using the equation:

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

9.2.7.2 Acceptance Criterion for the RPD of the LFSM and LFSMD

RPDs for duplicate LFSMs must be less than, or equal to, 30% for each analyte. Greater variability may be observed when the matrix is fortified at analyte concentrations near or at the MRL (within a factor of two times the MRL concentration). LFSMs at these concentrations must have RPDs that are less than or equal to 50%. If the RPD of an analyte falls outside the designated range, and the laboratory performance for the analyte is shown to be in control in the CCCs and in the LFB, the precision is judged matrix influenced. Report the result for the corresponding analyte in the unfortified sample as "suspect–matrix".

9.2.7.3 Calculate the RPD for Field Duplicates

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

$$RPD = \frac{|\mathrm{FD}_1 - \mathrm{FD}_2|}{(\mathrm{FD}_1 + \mathrm{FD}_2)/2} \times 100$$

9.2.7.4 Acceptance Criterion for Field Duplicates

RPDs for Field Duplicates must be less than, or equal to, 30% for each analyte. Greater variability may be observed when Field Duplicates have analyte concentrations that are near or at the MRL (within a factor of two times the MRL concentration). At these concentrations, Field Duplicates must have RPDs that are less than or equal to 50%. If the RPD of an analyte falls outside the designated range, and the laboratory performance for the analyte is shown to be in control in the CCC and in the LFB, the precision is judged matrix influenced. Report the result for the corresponding analyte in the unfortified sample as "suspect–matrix"

9.2.8 Field Reagent Blank (FRB)

The purpose of the FRB is to ensure that PFAS measured in the field samples were not inadvertently introduced into the sample during sample collection and handling. The FRB is processed, extracted, and analyzed in exactly the same manner as a field sample. Analysis of the FRB is required only if a field

sample contains a method analyte or analytes at, or above, the MRL. If a method analyte found in the field sample is present in the FRB at a concentration greater than one-third of the MRL, then the results for that analyte are invalid for all samples associated with the failed FRB.

9.2.9 Calibration Verification using QCS

A QCS must be analyzed during the IDC, and then quarterly thereafter. For this method, the laboratory is not required to obtain standards from a source independent of the primary calibration standards. Instead, the laboratory should acquire the best available quantitative standards (Sect. 3.20) and use these to prepare both the primary calibration standards and the QCS. The QCS must be an independent dilution beginning with the common starting materials. Preparation by a second analyst is recommended. The acceptance criterion for the QCS is 70–130% of the true value. If the accuracy for any analyte fails the recovery criterion, prepare fresh standard dilutions and repeat the Calibration Verification.

9.3 Method Modification QC Requirements

The analyst is permitted to modify the chromatographic and MS/MS conditions. Examples of permissible method modifications include alternate LC columns, MRM transitions, and additional QC analytes proposed for use with the method. Any method modifications must be within the scope of the established method flexibility and must retain the basic chromatographic elements of this method (Sect. 2). The following are required after a method modification.

9.3.1 Repeat the IDC

Establish an acceptable initial calibration (Sect. 10.3) using the modified conditions. Repeat the procedures of the IDC (Sect. 9.1).

9.3.2 Document Performance in Representative Sample Matrices

The analyst is also required to evaluate and document method performance for the modifications in real matrices that span the range of waters that the laboratory analyzes. This additional step is required because modifications that perform acceptably in the IDC, which is conducted in reagent water, could fail ongoing method QC requirements in real matrices. This is particularly important for methods subject to matrix effects, such as LC-MS/MS-based methods. For example, a laboratory may routinely analyze finished drinking water from municipal treatment plants that process ground water, surface water, or a blend of surface and ground water. In this case, the method modification requirement could be accomplished by assessing precision (Sect. 9.1.2) and accuracy (Sect. 9.1.3) in finished drinking waters derived from a surface water with moderate to high total organic carbon (e.g., 2 mg/L or greater) and from a hard ground water (e.g., 250 mg/L as calcium carbonate ($CaCO_3$) equivalent, or greater).

10 Calibration and Standardization

Demonstration and documentation of acceptable MS calibration and initial analyte calibration are required before performing the IDC and prior to analyzing field samples. The initial calibration should be repeated each time a major instrument modification or maintenance is performed.

10.1 MS/MS Optimization

10.1.1 Mass Calibration

Calibrate the mass spectrometer with the calibration compounds and procedures specified by the manufacturer.

10.1.2 MS Parameters

During the development of this method, instrumental parameters were optimized for the precursor and product ions listed in **Table 6**. Product ions other than those listed may be selected; however, the analyst should avoid using ions with lower mass or common ions that may not provide sufficient discrimination between the analytes of interest and co-eluting interferences.

10.1.2.1 Requirement for Branched Isomers

There have been reports that not all product ions in the linear PFOS are produced in all branched PFOS isomers.[5] (This phenomenon may exist for many of the PFAS.) For this method, the *m/z* 80 product ion must be used for PFOS and PFHxS to minimize this problem and promote comparability between laboratories. Some MS/MS instruments, may not be able to scan a product ion with such a wide mass difference from the precursor ion. These instruments may not be used for this method if PFOS or PFHxS analysis is to be conducted.

10.1.2.2 Precursor Ion

Optimize the response of the precursor ion ($[M - H]^-$ or $[M - CO_2 - H]^-$) for each analyte following manufacturer's guidance. Analyte concentrations of 1.0 µg/mL were used for this step during method development. Vary the MS parameters (source voltages, source and desolvation temperatures, gas flows, etc.) until optimal analyte responses are determined. The electrospray parameters used during method development are listed in **Table 2**. The analytes may have different optimal parameters, requiring some compromise on the final operating conditions. See **Table 6** for ESI-MS conditions used to collect method performance data.

10.1.2.3 Product Ion

Optimize the product ion for each analyte following the manufacturer's guidance. Typically, the carboxylic acids have similar MS/MS conditions and the sulfonic acids have similar MS/MS conditions. See **Table 6** for MS/MS conditions used to collect method performance data.

10.2 Chromatographic Conditions

Establish LC operating parameters that optimize resolution and peak shape. Suggested LC conditions can be found in **Table 1**. Modifying the solvent composition of the standard or extract by increasing the aqueous content to better focus early eluting compounds on the column is not permitted. A decrease in methanol concentration could lead to lower or imprecise recovery of the more hydrophobic method analytes, while higher methanol concentration could lead to the precipitation of salts in some extracts. The peak shape of the 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.

10.2.1 Minimizing PFAS Background

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 Establishing Branched vs. Linear Isomer Profiles

Prepare and analyze the technical-grade standard of PFOA, discussed in Section 7.17.1, at a mid- to high-level concentration. Identify the retention times of the branched isomers of PFOA present in the technical-grade PFOA standard. When PFOA is chromatographed on a reversed-phase column, the branched isomers elute prior to the linear isomer. Repeat the procedure in this section for PFHxS and PFOS discussed in Section 7.17.2, and any other analytes for which technical-grade standards have been acquired. The branched isomer identification checks must be repeated any time chromatographic changes occur that alter analyte retention times.

10.2.3 Establish LC-MS/MS Retention Times and MRM Segments

Inject a mid- to high-level calibration standard under optimized LC-MS/MS conditions to obtain the retention times of each method analyte. Divide the chromatogram into segments that contain one or more chromatographic peaks. For maximum sensitivity, minimize the number of MRM transitions that are simultaneously monitored within each segment. Ensure that the retention time window used to collect data for each analyte is of sufficient width to detect earlier eluting branched isomers.
The retention times observed during collection of the method performance data are listed in **Table 3**, **Table 4**, and **Table 5**.

10.3 Initial Calibration

This method has three isotope performance standards that are used as reference compounds for the internal standard quantitation of the isotope dilution analogues. The suggested isotope performance standard reference for each isotope dilution analogue is listed in **Table 4**. The sixteen isotope dilution analogues are used as reference compounds to quantitate the native analyte concentrations. The suggested isotope dilution analogue references for the native analytes are listed in **Table 5**.

10.3.1 Calibration Standards

Prepare a set of at least five calibration standards as described in Section 7.17.5. The analyte concentrations in the lowest calibration standard must be at or below the MRL.

10.3.2 Calibration Curves of Native Analytes

Quantitate the native analytes using the internal standard calibration technique. The internal standard technique calculates concentration based on the ratio of the peak area of the native analyte to that of the isotope dilution analogue. Calibrate the LC-MS/MS and fit the calibration points with either a linear or quadratic regression. Weighting may be used. Forcing the calibration curve through the origin is mandatory for this method. Forcing zero allows for a better estimate of the background levels of

method analytes. The MS/MS instrument used during method development was calibrated using weighted (1/x) quadratic regression with forced zero.

10.3.3 Calibration of Isotope Dilution Analogues

The isotope dilution analogues are quantified using the internal standard calibration technique. Because isotope dilution analogues are added at a single concentration level to the calibration standards, calibrate for each of these using an average response factor.

10.3.4 Calibration of Isotope Performance Standards

Because Isotope performance standards are added at a single concentration level to the calibration standards, calibrate for each of these using an average response factor.

10.3.5 Calibration Acceptance Criteria

Evaluate the initial calibration by calculating the concentration of each analyte as an unknown against its regression equation. For calibration levels that are less than or equal to the MRL, the result for each analyte should be within 50–150% of the true value. All other calibration points should be within 70–130% of their true value. If these criteria cannot be met, the analyst could have difficulty meeting ongoing QC criteria. In this case, corrective action is recommended such as reanalyzing the calibration standards, restricting the range of calibration, or performing instrument maintenance. If the cause for failure to meet the criteria is due to contamination or standard degradation, prepare fresh calibration standards and repeat the initial calibration.

10.4 Continuing Calibration

Analyze a CCC to verify the initial calibration at the beginning of each Analysis Batch, after every tenth field sample, and at the end of each Analysis Batch. The beginning CCC for each Analysis Batch must be at, or below, the MRL for each analyte. This CCC verifies instrument sensitivity prior to the analysis of samples. If standards have been prepared such that all low calibration levels are not in the same solution, it may be necessary to analyze two standards to meet this requirement. Alternatively, the nominal analyte concentrations in the analyte PDS may be customized to meet these criteria. Alternate subsequent CCCs between the mid and high calibration levels. Verify that the CCC meets the criteria in the following sections.

10.4.1 CCC Isotope Performance Standard Responses

The absolute area of the quantitation ion for each of the three isotope performance standards must be within 50–150% of the average area measured during the initial calibration. If these limits are exceeded, corrective action is necessary (Sect. 10.5).

10.4.2 CCC Isotope Dilution Analogue Recovery

Using the average response factor determined during the initial calibration and the internal standard calibration technique, calculate the percent recovery of each isotope dilution analogue in the CCC. The recovery for each analogue must be within a range of 70–130%. If these limits are exceeded, corrective action is necessary (Sect. 10.5).

10.4.3 CCC Analyte Responses

Calculate the concentration of each method analyte in the CCC. Each analyte fortified at a level less than or equal to the MRL must be within 50–150% of the true value. The concentration of the analytes in CCCs fortified at all other levels must be within 70–130%. If these limits are exceeded, then all data for the failed analytes must be considered invalid. Any field samples analyzed since the last acceptable CCC that are still within holding time must be reanalyzed after an acceptable calibration has been restored.

10.4.3.1 Exception for High Recovery

If the CCC fails because the calculated concentration is greater than 130% (150% for the low-level CCC) for a method analyte, and field sample extracts show no concentrations above the MRL for that analyte, non-detects may be reported without re-analysis.

10.5 Corrective Action

Failure to meet the CCC QC performance criteria requires corrective action. Following a minor remedial action, such as servicing the autosampler or flushing the column, check the calibration with a mid-level CCC and a CCC at the MRL, or recalibrate according to Section 10.3. If isotope performance standard and calibration failures persist, maintenance may be required, such as servicing the LC-MS/MS system or replacing the LC column. These latter measures constitute major maintenance and the analyst must return to the initial calibration step (Sect. 10.3).

11 Procedure

This procedure may be performed manually or in an automated mode using a robotic or automatic sample preparation device. The data published in this method (Sect. 17) demonstrate acceptable performance using manual extraction. The authors did not evaluate automated extraction systems. If an automated system is used to prepare samples, follow the manufacturer's operating instructions, but all extraction and elution steps must be the same as in the manual procedure. Extraction and elution steps may not be changed or omitted to accommodate the use of an automated system. If an automated system is used, the LRBs should be rotated among the ports to ensure that all the valves and tubing meet the LRB requirements (Sect. 9.2.1).

11.1 Sample Bottle Rinse

Some of the PFAS adsorb to surfaces, including polypropylene. During the elution step of the procedure, sample bottles must be rinsed with the elution solvent whether extractions are performed manually or by automation.

11.2 Reuse of Extraction Cartridges

The SPE cartridges described in this section are designed for a single use. They may not be reconditioned for subsequent analyses.

11.3 Sample Preparation

11.3.1 Sample Volume

Determine sample volume. An indirect measurement may be done in one of two ways: by marking the level of the sample on the bottle or by weighing the sample and bottle to the nearest 1 gram. After

extraction, proceed to Section 11.5 to complete the volume measurement. Some of the PFAS adsorb to surfaces, thus the sample may not be transferred to a graduated cylinder for volume measurement. The LRB, LFB and FRB must have the same volume as that of the field samples and may be prepared by measuring reagent water with a graduated cylinder.

11.3.2 Verifying Sample pH

Verify that the sample containing 1 g/L ammonium acetate has a pH between 6.0 and 8.0. Acetic acid may be added as needed to reduce the pH

11.3.3 Fortify QC Samples

Fortify LFBs, LFSMs, and LFSMDs, with an appropriate volume of Analyte PDS (Sect. 7.17.4). Cap and invert each sample several times to mix.

11.3.4 Addition of Isotope Dilution Analogues

Add an aliquot of the isotope dilution analogue PDS (Sect. 7.16.1) to each sample, then cap and invert to mix. During method development, a 20 µL aliquot of the PDS (0.50–2.0 ng/µL) was added to achieve a final concentration of 40 ng/L of the isotopically labeled carboxylates and perfluorinated sulfonates, and 160 ng/L of the telomer sulfonates.

11.4 Extraction Procedure

11.4.1 Cartridge Cleaning and Conditioning

Do not allow cartridge packing material to go dry during any of the conditioning steps. If the cartridge goes dry during the conditioning phase, the conditioning must be repeated. Rinse each cartridge with 10 mL of methanol. Next, rinse each cartridge with 10 mL of aqueous 0.1 M phosphate buffer (Sect. 7.8) without allowing the water to drop below the top edge of the packing. Close the valve and add 2–3 mL of phosphate buffer to the cartridge reservoir and fill the remaining volume with reagent water.

11.4.2 Cartridge Loading

Attach the sample transfer tubes (Sect. 6.8.3) and adjust the vacuum to approximately 5 inches Hg. Begin adding sample to the cartridge. Adjust the vacuum and control valves so that the approximate flow rate is 5 mL/min. Do not allow the cartridge to go dry before all the sample has passed through. Flow rates above 5 mL/min during loading may cause low analyte recovery.

11.4.3 Sample Bottle Rinse and Cartridge Drying

After the entire sample has passed through the cartridge, rinse the sample bottle with a 10 mL aliquot of 1 g/L ammonium acetate in reagent water. Draw the rinsate through the sample transfer tubes and the cartridges. Add 1 mL of methanol to the sample bottle and draw through the transfer tube and SPE cartridge. This step is designed to remove most of the water from the transfer line and cartridge resulting in the reduction of the salt and water present in the eluate. The methanol rinse may also reduce interferences by removing weakly retained organic material prior to elution. If plastic reservoirs are used instead of transfer lines, the reservoirs must be rinsed with the ammonium acetate solution and the 1 mL aliquot of methanol.

11.4.4 Cartridge Drying

Draw air or nitrogen through the cartridge for 5 min at high vacuum (15–20 in. Hg).

11.4.5 Sample Bottle and Cartridge Elution

After the drying step, release the vacuum on the extraction manifold and place a collection tube under each sample position. Rinse the sample bottles with 5 mL of the elution solvent, methanol with 2% ammonium hydroxide (v/v), then elute the analytes from the cartridges by pulling the elution solvent through the sample transfer tubes and the cartridges. Use a low vacuum such that the solvent exits the cartridge in a dropwise fashion. Repeat sample bottle rinse and cartridge elution with a second 5 mL aliquot of elution solvent. If plastic reservoirs are used instead of transfer lines, attempt to rinse the entire inner surface of the reservoir with the elution solvent.

11.4.6 Extract Concentration

Concentrate the extract to dryness under a gentle stream of nitrogen in a heated water bath (55–60 °C). Reconstitute the extract with 1.0 mL of 20% reagent water in methanol (v/v). Add the isotope performance standards to the extract and vortex.

11.4.7 Extract Transfer and Storage

Transfer the final extract to a polypropylene autosampler vial. Store extracts at room temperature. Recap vials as soon as possible after injection to prevent evaporation losses; the polypropylene caps do not reseal after puncture. Alternatively, extracts can be stored in the 15 mL collection tubes after extraction. A small aliquot can be removed for analysis if the autosampler vial and injection system accommodate small volumes.

11.5 Sample Volume Determination

Use a graduated cylinder to measure the volume of water required to fill the original sample bottle to the mark made prior to extraction. If using weight to determine the volume, weigh the empty bottle to the nearest 1 gram and subtract this value from the weight recorded prior to extraction. Assume a sample density of 1.0 g/mL. Record the sample volumes for use in the final calculations of analyte concentrations.

11.6 Sample Analysis

11.6.1 Establish LC-MS/MS Operating Conditions

Establish MS/MS operating conditions per the procedures in Section 10.1 and chromatographic conditions per Section 10.2. Establish a valid initial calibration following the procedures in Section 10.3 or confirm that the existing calibration is still valid by analyzing a low-level CCC. If establishing an initial calibration for the first time, complete the IDC prior to analyzing field samples. Analyze field and QC samples in a properly sequenced Analysis Batch as described in Section 11.7.

11.6.2 Verify Retention Time Windows

The analyst must ensure that each method analyte elutes entirely within the assigned window during each Analysis Batch. Make this observation by viewing the quantitation ion for each analyte in the CCCs analyzed during an Analysis Batch. If an analyte peak drifts out of the assigned window, then data for

that analyte is invalid in all injections acquired since the last valid CCC. In addition, all peaks representing multiple isomers of an analyte must elute entirely within the same MRM window.

11.7 Analysis Batch Sequence

An Analysis Batch is a sequence of samples, analyzed within a 24-hour period, of no more than 20 field samples and includes all required QC samples (LRB, CCCs, the LFSM and LFSMD (or FD)). The required QC samples are not included in counting the maximum field sample total of 20. LC-MS/MS conditions for the Analysis Batch must be the same as those used during calibration.

11.7.1 Analyze Initial CCC

After a valid calibration is established, begin every Analysis Batch by analyzing an initial low-level CCC at or below the MRL. This initial CCC must be within 50–150% of the true value for each method analyte and must pass both the isotope performance standard area response criterion (Sect. 10.4.1) and the isotope dilution analogue recovery criterion (Sect. 10.4.2). The initial CCC confirms that the calibration is still valid. Failure to meet the QC criteria may indicate that recalibration is required prior to analyzing samples.

11.7.2 Analyze Field and QC Samples

After the initial CCC, continue the Analysis Batch by analyzing an LRB, followed by the field samples and QC samples. Analyze a mid- or high-level CCC after every ten field samples and at the end each Analysis Batch. Do not count QC samples (LRBs, FDs, LFSMs, LFSMDs) when calculating the required frequency of CCCs.

11.7.3 Analyze Final CCC

The last injection of the Analysis Batch must be a mid- or high-level CCC. The acquisition start time of the final CCC must be within 24 hours of the acquisition start time of the low-level CCC at the beginning of the Analysis Batch. More than one Analysis Batch within a 24-hour period is permitted. An Analysis Batch may contain field and QC samples from multiple extraction batches.

11.7.4 Initial Calibration Frequency

A full calibration curve is not required before starting a new Analysis Batch. A previous calibration can be confirmed by running an initial, low-level CCC followed by an LRB. If a new calibration curve is analyzed, an Analysis Batch run immediately thereafter must begin with a low-level CCC and an LRB.

12 Data Analysis and Calculations

Because environmental samples may contain both branched and linear isomers of the method analytes, but quantitative standards that contain branched isomers do not exist for all method analytes, integration and quantitation of the PFAS is dependent on the type of standard materials available.

12.1 Identify Peaks by Retention Times

At the conclusion of data acquisition, use the same software settings established during the calibration procedure to identify analyte peaks in the predetermined retention time windows. Confirm the identity of each analyte by comparison of its retention time with that of the corresponding analyte peak in an

initial calibration standard or CCC. Proceed with quantitation based on the type of standard available for each method analyte.

12.1.1 Method Analytes without Technical-Grade Standards

If standards containing the branched and linear isomers cannot be purchased (i.e., only the linear isomer is available), only the linear isomer can be identified and quantitated in field samples and QC samples because the retention time of the branched isomers cannot be confirmed.

12.1.2 PFHxS, PFOS, and other Analytes with Technical-Grade Standards

During method development, multiple chromatographic peaks, representing branched and linear isomers, were observed for standards of PFHxS and PFOS using the LC conditions in **Table 1**. For PFHxS and PFOS, all the chromatographic peaks observed in the standard must be integrated and the areas summed. Chromatographic peaks in all field samples and QC samples must be integrated in the same way as the calibration standard for analytes with quantitative standards containing the branched and linear isomers.

12.1.3 PFOA

For PFOA, identify the branched and linear isomers by analyzing a technical-grade standard that includes both linear and branched isomers as directed in Section 10.2.2 and ensure that all isomers elute within the same acquisition segment. Quantitate field samples and fortified matrix samples by integrating the total response, accounting for peaks that are identified as linear and branched isomers. Quantitate based on the initial calibration with the quantitative PFOA standard containing just the linear isomer.

12.2 Calculate Analyte Concentrations

Calculate analyte concentrations using the multipoint calibration and the measured sample volume. Report only those values that fall between the MRL and the highest calibration standard.

12.3 Calculate Isotope Dilution Analogue Recovery

Calculate the concentration of each isotope dilution analogue using the multipoint calibration and the measured sample volume. Verify that the percent recovery is within 50–200% of the true value.

12.4 Significant Figures

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

12.5 Exceeding the Calibration Range

The analyst must not extrapolate beyond the established calibration range. If an analyte result exceeds the range of the initial calibration curve, a field duplicate of the sample must be extracted, if available. Dilute an aliquot of the field duplicate with reagent water to a final volume equal to that used for the IDC. Add ammonium acetate to a final concentration of 1 g/L and process the diluted sample. Report all concentrations measured in the original sample that do not exceed the calibration range. Report concentrations of analytes that exceeded the calibration range in the in the original sample based on measurement in a diluted sample. Incorporate the dilution factor into final concentration calculations

and the resulting data must be annotated as a dilution. This is the only circumstance when subsampling is permitted.

13 Method Performance

13.1 Precision, Accuracy, and LCMRL Results

Tables for these data are presented in Section 17. LCMRLs are presented in **Table 7**. Single-laboratory precision and accuracy data are presented for three water matrices: reagent water (**Table 8**), finished ground water (**Table 10**), and a drinking water matrix from a surface water source (**Table 12)**. The mean isotope dilution analogue recoveries measured in the replicate samples used in these studies are presented in **Table 9** for reagent water, **Table 11** for finished groundwater, and **Table 13** for the surface water matrix.

13.2 Analyte Stability Study

Chlorinated (finished) surface water samples were inoculated with microbial-rich water from an impacted surface source and fortified with 40 ng/L of the PFAS method analytes. These samples were stored as required in this method. The percent change from the initial analyzed concentration observed after 7, 14, 21, and 28 days is presented in Section 17, **Table 14**.

13.3 Extract Storage Stability

Extract storage stability studies were conducted on extracts obtained from the analyte stability study (Sect. 13.2). The percent change from the initial analyzed concentration observed after 14, 21, and 27 days storage is presented in Section 17, **Table 15**.

14 Pollution Prevention

For information about pollution prevention applicable to laboratory operations described in this method, consult: Less is Better, Guide to Minimizing Waste in Laboratories, a publication available from the American Chemical Society (accessed April 2019) at www.acs.org.

15 Waste Management

Laboratory waste management practices should be consistent with all applicable rules and regulations, and that laboratories protect the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. In addition, compliance is required with any sewage discharge permits and regulations, particularly the hazardous waste identification rules and land disposal restrictions.

16 References

1. US EPA. *Statistical Protocol for the Determination of the Single-Laboratory Lowest Concentration Minimum Reporting Level (LCMRL) and Validation of Laboratory Performance at or Below the Minimum Reporting Level (MRL)*; EPA 815-R-05-006; Office of Water: Cincinnati, OH, November 2004.
2. US EPA. *Technical Basis for the Lowest Concentration Minimum Reporting Level (LCMRL) Calculator*; EPA 815-R-11-001; Office of Water: Cincinnati, OH, December 2010.

3. Martin, J.W., et al. Analytical Challenges Hamper Perfluoroalkyl Research. *Environ. Sci. Technol*. 2004, Vol. 38, 248A–255A.
4. Cahill, J.D., et al. Determination of Pharmaceutical Compounds in Surface- and Ground-Water Samples by Solid-Phase Extraction and High-Performance Liquid Chromatography Electrospray Ionization Mass Spectrometry. *J. Chromatography A*, 2004, 1041, 171–180.
5. Langlois, I. and Oehme, M. Structural Identification of Isomers Present in Technical Perfluorooctane Sulfonate by Tandem Mass Spectrometry. *Rapid Communication Mass Spectrometry*. 2006, Vol. 20, 844–850.

17 Tables, Figures and Method Performance Data

Table 1. HPLC Method Conditions[a]

Time (min)	% 20 mM ammonium acetate	% Methanol
Initial	95.0	5.0
0.5	95.0	5.0
3.0	60.0	40.0
16.0	20.0	80.0
18.0	20.0	80.0
20.0	5.0	95.0
22.0	5.0	95.0
25.0	95.0	5.0
35.0	95.0	5.0

[a] Phenomenex Gemini® C18, 2 x 50 mm, 3.0 µm silica with TMS end-capping. Flow rate of 0.25 mL/min; run time 35 minutes; 10 µL injection into a 50 µL loop. The chromatogram in **Figure 1** was obtained under these conditions.

Table 2. ESI-MS Method Conditions

ESI Conditions for Waters (Milford, MA) Xevo TQD	
Polarity	Negative ion
Capillary needle voltage	-2.7 kV
Cone gas flow	40 L/hour
Nitrogen desolvation gas	800 L/hour
Desolvation gas temperature	300 °C

Table 3. Isotopically Labeled Isotope Performance Standards and Retention Times

Isotope Performance Standard	Peak # (Figure 1)	RT (min)
$^{13}C_3$-PFBA	1	4.14
$^{13}C_2$-PFOA	26	12.19
$^{13}C_4$-PFOS	32	13.73

Table 4. Isotope Dilution Analogues: RTs and Suggested Isotope Performance Standard References

Isotopically Labeled Analyte	Peak # (Fig. 1)	RT (min)	Suggested Isotope Performance Standard
$^{13}C_4$-PFBA	2	4.14	$^{13}C_3$-PFBA
$^{13}C_5$-PFPeA	5	6.13	$^{13}C_3$-PFBA
$^{13}C_3$-PFBS	7	6.62	$^{13}C_4$-PFOS
$^{13}C_2$-4:2FTS	12	8.12	$^{13}C_4$-PFOS
$^{13}C_5$-PFHxA	14	8.35	$^{13}C_2$-PFOA
$^{13}C_3$-HFPO-DA	17	9.06	$^{13}C_2$-PFOA
$^{13}C_4$-PFHpA	19	10.34	$^{13}C_2$-PFOA
$^{13}C_3$-PFHxS	21	10.61	$^{13}C_4$-PFOS
$^{13}C_2$-6:2FTS	24	12.05	$^{13}C_4$-PFOS
$^{13}C_8$-PFOA	27	12.19	$^{13}C_2$-PFOA
$^{13}C_9$-PFNA	30	13.70	$^{13}C_2$-PFOA
$^{13}C_8$-PFOS	33	13.73	$^{13}C_4$-PFOS
$^{13}C_2$-8:2FTS	36	14.94	$^{13}C_4$-PFOS
$^{13}C_6$-PFDA	38	15.00	$^{13}C_2$-PFOA
$^{13}C_7$-PFUnA	40	16.14	$^{13}C_2$-PFOA
$^{13}C_2$-PFDoA	43	17.13	$^{13}C_2$-PFOA

Table 5. Method Analytes, Retention Times and Suggested Isotope Dilution Analogue References

Analyte	Peak # (Figure 1)	RT (min)	Isotope Dilution Analogue
PFBA	3	4.15	$^{13}C_4$-PFBA
PFMPA	4	4.84	$^{13}C_4$-PFBA
PFPeA	6	6.13	$^{13}C_5$-PFPeA
PFBS	8	6.62	$^{13}C_3$-PFBS
PFMBA	9	6.81	$^{13}C_5$-PFPeA
PFEESA	10	7.53	$^{13}C_3$-PFBS
NFDHA	11	8.01	$^{13}C_5$-PFHxA
4:2FTS	13	8.12	$^{13}C_2$-4:2FTS
PFHxA	15	8.36	$^{13}C_5$-PFHxA
PFPeS	16	8.69	$^{13}C_3$-PFHxS
HFPO-DA	18	9.06	$^{13}C_3$-HFPO-DA
PFHpA	20	10.42	$^{13}C_4$-PFHpA
PFHxS	22	10.62	$^{13}C_3$-PFHxS
ADONA	23	10.73	$^{13}C_4$-PFHpA
6:2FTS	25	12.04	$^{13}C_2$-6:2FTS
PFOA	28	12.19	$^{13}C_8$-PFOA
PFHpS	29	12.28	$^{13}C_8$-PFOS
PFNA	31	13.70	$^{13}C_9$-PFNA
PFOS	34	13.74	$^{13}C_8$-PFOS
9Cl-PF3ONS	35	14.53	$^{13}C_8$-PFOS
8:2 FTS	37	14.94	$^{13}C_2$-8:2FTS
PFDA	39	15.00	$^{13}C_6$-PFDA
PFUnA	41	16.14	$^{13}C_7$-PFUnA
11Cl-PF3OUdS	42	16.70	$^{13}C_8$-PFOS
PFDoA	44	17.13	$^{13}C_2$-PFDoA

Table 6. MS/MS Method Conditions[a]

Segment[b]	Analyte	Precursor Ion[c] (*m/z*)	Product Ion[c,d] (*m/z*)	Cone Voltage (v)	Collision Energy[e] (v)
1	PFBA	213	169	22	10
1	$^{13}C_3$-PFBA	216	172	22	10
1	$^{13}C_4$-PFBA	217	172	22	10
1	PFMPA	229	85	23	10
2	PFPeA	263	219	20	8
2	$^{13}C_5$-PFPeA	268	223	20	8
2	$^{13}C_3$-PFBS	302	80	45	30
2	PFBS	299	80	45	30
2	PFMBA	279	85	22	10
3	PFEESA	315	135	44	20
3	NFDHA	295	201	14	8
3	$^{13}C_2$-4:2FTS	329	309	40	18
3	4:2FTS	327	307	40	18
3	$^{13}C_5$-PFHxA	318	273	20	8
3	PFHxA	313	269	20	8
3	PFPeS	349	80	45	35
3	$^{13}C_3$-HFPO-DA	287[f]	169	15	5
3	HFPO-DA	285[f]	169	15	5
4	$^{13}C_4$-PFHpA	367	322	15	8
4	PFHpA	363	319	15	8
4	$^{13}C_3$-PFHxS[g]	402	80	45	40
4	PFHxS[h]	399	80	45	40
4	ADONA	377	251	15	10
5	$^{13}C_2$-6:2FTS	429	409	47	22
5	6:2FTS	427	407	47	22
5	$^{13}C_2$-PFOA	415	370	18	10
5	$^{13}C_8$-PFOA	421	376	18	10
5	PFOA	413	369	18	10
5	PFHpS	449	80	45	40
6	$^{13}C_9$-PFNA	472	427	17	10
6	PFNA	463	419	17	10
6	$^{13}C_4$-PFOS[g]	503	80	45	45
6	$^{13}C_8$-PFOS[g]	507	80	45	45
6	PFOS[h]	499	80	45	45
7	9Cl-PF3ONS	531	351	55	25
7	$^{13}C_2$-8:2FTS	529	509	53	28
7	8:2FTS	527	507	53	28
7	$^{13}C_6$-PFDA	519	474	22	10
7	PFDA	513	469	22	10

Segment[b]	Analyte	Precursor Ion[c] *(m/z)*	Product Ion[c,d] *(m/z)*	Cone Voltage (v)	Collision Energy[e] (v)
8	$^{13}C_7$-PFUnA	570	525	24	10
8	PFUnA	563	519	24	10
8	11Cl-PF3OUdS	631	451	60	30
8	$^{13}C_2$-PFDoA	615	570	22	10
8	PFDoA	613	569	22	10

a. An LC-MS/MS chromatogram of the analytes obtained using these parameters is shown in **Figure 1**.

b. Segments are time durations in which single or multiple scan events occur.

c. Precursor and product ions listed in this table are nominal masses. During MS and MS/MS optimization, the analyst should determine precursor and product ion masses to one decimal place by locating the apex of the mass spectral peak (e.g., *m/z* 498.9→79.9 for PFOS). These precursor and product ion masses (with at least one decimal place) should be used in the MS/MS method for all analyses.

d. Ions used for quantitation purposes.

e. Argon used as collision gas.

f. HFPO-DA is not stable in the ESI source and the $[M - H]^-$ yields a weak signal under typical ESI conditions. The precursor ion used during method development was $[M - CO_2 - H]^-$.

g. The isotope dilution analogue used during method development was composed of the linear isomer exclusively.

h. Analyte has multiple resolved chromatographic peaks due to linear and branched isomers. All peaks summed for quantitation purposes. To reduce bias regarding detection of branched and linear isomers, the *m/z* 80 product ion must be used for this analyte.

Table 7. LCMRL Results

Analyte	LCMRL Fortification Levels (ng/L)	Calculated LCMRL (ng/L)
PFBA	1.0, 2.0, 4.0, 6.0, 10, 14, 20	13
PFMPA	1.0, 2.0, 4.0, 6.0, 10, 14, 20	3.8
PFPeA	1.0, 2.0, 4.0, 6.0, 10, 14, 20	3.9
PFBS	1.0, 2.0, 4.0, 6.0, 10, 14, 20	3.5
PFMBA	1.0, 2.0, 4.0, 6.0, 10, 14, 20	3.7
PFEESA	1.0, 2.0, 4.0, 6.0, 10, 14, 20	2.6
NFDHA	4.0, 6.0, 10, 14, 20, 41, 82	16
4:2FTS	1.0, 2.0, 4.0, 6.0, 10, 14, 20	4.7
PFHxA	1.0, 2.0, 4.0, 6.0, 10, 14, 20	5.3
PFPeS	1.0, 2.0, 4.0, 6.0, 10, 14, 20	6.3
HFPO-DA	1.0, 2.0, 4.0, 6.0, 10, 14, 20	3.7
PFHpA	1.0, 2.0, 4.0, 6.0, 10, 14, 20	2.6
PFHxS	1.0, 2.0, 4.0, 6.0, 10, 14, 20	3.7
ADONA	1.0, 2.0, 4.0, 6.0, 10, 14, 20	3.4
6:2FTS	1.0, 2.0, 4.0, 6.0, 10, 14, 20	14
PFOA	1.0, 2.0, 4.0, 6.0, 10, 14, 20	3.4
PFHpS	1.0, 2.0, 4.0, 6.0, 10, 14, 20	5.1
PFNA	1.0, 2.0, 4.0, 6.0, 10, 14, 20	4.8
PFOS	1.0, 2.0, 4.0, 6.0, 10, 14, 20	4.4
9Cl-PF3ONS	1.0, 2.0, 4.0, 6.0, 10, 14, 20	1.4
8:2FTS	1.0, 2.0, 4.0, 6.0, 10, 14, 20	9.1
PFDA	1.0, 2.0, 4.0, 6.0, 10, 14, 20	2.3
PFUnA	1.0, 2.0, 4.0, 6.0, 10, 14, 20	2.7
11Cl-PF3OUdS	1.0, 2.0, 4.0, 6.0, 10, 14, 20	1.6
PFDoA	1.0, 2.0, 4.0, 6.0, 10, 14, 20	2.2

Table 8. Precision and Accuracy Data for Reagent Water

Analyte	Low Fortification (ng/L)	Mean %R[a] (*n*=7)	%RSD[a]	High Fortification (ng/L)	Mean %R (*n*=5)	%RSD
PFBA	10	128	8.6	80	98.4	2.4
PFMPA	10	108	4.5	80	98.1	2.2
PFPeA	10	107	4.9	80	99.6	3.6
PFBS	10	102	9.1	80	96.2	2.9
PFMBA	10	111	6.8	80	101	3.4
PFEESA	10	107	10	80	98.8	4.0
NFDHA	10	110	15	80	98.5	5.4
4:2FTS	10	94.4	14	80	100	5.7
PFHxA	10	102	8.0	80	97	7.7
PFPeS	10	99.5	19	80	101	7.8
HFPO-DA	10	102	9.7	80	102	4.7
PFHpA	10	108	7.0	80	104	4.1
PFHxS	10	103	9.0	80	97.7	5.5
ADONA	10	96.3	3.1	80	96.8	5.6
6:2FTS	10	109	15	80	111	11
PFOA	10	108	7.4	80	98.5	6.9
PFHpS	10	98.8	8.9	80	102	7.0
PFNA	10	109	6.2	80	99.6	5.6
PFOS	10	104	8.7	80	98.0	4.3
9Cl-PF3ONS	10	99.7	4.6	80	103	6.8
8:2FTS	10	100	17	80	100	13
PFDA	10	100	4.2	80	100	1.8
PFUnA	10	102	10	80	97.3	8.1
11Cl-PF3OUdS	10	106	5.3	80	102	6.1
PFDoA	10	101	6.2	80	96.3	5.1

[a] %R = percent recovery; %RSD = percent relative standard deviation

Table 9. P&A in Reagent Water: Isotope Dilution Analogue Recovery Data[a]

Analyte	Analogue Fortification (ng/L)	Mean %R[b,c] (*n*=7) P&A Low	%RSD[b,c]	Mean %R (*n*=5) P&A High	%RSD
$^{13}C_4$-PFBA	40	95.6	11	92.5	3.4
$^{13}C_5$-PFPeA	40	93.4	9.3	91.7	4.6
$^{13}C_3$-PFBS	40	98.6	9.6	107	6.6
$^{13}C_2$-4:2FTS	160	102	6.7	108	3.5
$^{13}C_5$-PFHxA	40	92.5	6.4	92.8	11
$^{13}C_3$-HFPO-DA	40	88.6	6.5	88.8	7.4
$^{13}C_4$-PFHpA	40	98.0	4.0	94.0	8.3
$^{13}C_3$-PFHxS	40	101	11	106	8.2
$^{13}C_2$-6:2FTS	160	109	9.5	99.8	4.7
$^{13}C_8$-PFOA	40	98.0	4.1	91.5	8.7
$^{13}C_9$-PFNA	40	97.1	4.9	92.1	8.4
$^{13}C_8$-PFOS	40	98.8	6.5	96.5	5.0
$^{13}C_2$-8:2FTS	160	106	13.9	108	8.7
$^{13}C_6$-PFDA	40	104	7.7	104	6.1
$^{13}C_7$-PFUnA	40	107	6.0	98.8	7.5
$^{13}C_2$-PFDoA	40	100	5.7	94.0	6.7

a. P&A = “precision and accuracy”.

b. %R = percent recovery; %RSD = percent relative standard deviation.

c. Mean and %RSD of the isotope dilution analogue results for the fortified samples in the P&A study; number of replicates given in the header row of the table.

Table 10. Precision and Accuracy Data for Finished Ground Water[a]

Analyte	Low Fortification (ng/L)	Mean %R[b] (*n*=5)	%RSD[b]	High Fortification (ng/L)	Mean %R (*n*=5)	%RSD
PFBA	10	127	15	80	98.0	4.0
PFMPA	10	100	8.3	80	103	9.8
PFPeA	10	105	11	80	105	5.1
PFBS	10	111	12	80	101	10
PFMBA	10	99.0	4.6	80	100	2.3
PFEESA	10	101	3.5	80	107	8.8
NFDHA	10	95.1	17	80	98.5	18
4:2FTS	10	70.5	20	80	116	9.2
PFHxA	10	104	18	80	111	17
PFPeS	10	87.5	5.0	80	106	6.2
HFPO-DA	10	105	7.4	80	103	7.5
PFHpA	10	102	6.8	80	101	6.4
PFHxS	10	86.6	18	80	108	6.8
ADONA	10	97.6	8.1	80	94.2	6.9
6:2FTS	10	99.9	15	80	100	12
PFOA	10	95.8	8.1	80	104	9.8
PFHpS	10	94.0	6.3	80	113	6.0
PFNA	10	95.1	7.2	80	108	3.3
PFOS	10	[c]	[c]	80	109	5.8
9Cl-PF3ONS	10	92.7	7.2	80	111	7.9
8:2FTS	10	108	19	80	102	3.2
PFDA	10	90.8	9.8	80	104	7.1
PFUnA	10	98.3	8.8	80	105	3.0
11Cl-PF3OUdS	10	94.6	8.3	80	110	9.3
PFDoA	10	92.7	7.8	80	102	6.3

a. Finished water from a ground water source. Hardness = 320 mg/L as $CaCO_3$. pH = 7.88 at 17 °C. Free Cl_2 = 0.64 mg/L. Total Cl_2 = 0.74 mg/L.

b. %R = percent recovery, corrected for native concentration; %RSD = percent relative standard deviation.

c. The spike level was below the ambient PFOS concentration of 25 ng/L.

Table 11. P&A in Finished Ground Water: Isotope Dilution Analogue Recovery Data[a]

Analyte	Analogue Fortification (ng/L)	Mean %R[b,c] (*n*=6) P&A Low	%RSD[b,c]	Mean %R (*n*=6) P&A High	%RSD
$^{13}C_4$-PFBA	40	89.5	4.4	81.3	7.8
$^{13}C_5$-PFPeA	40	94.0	4.2	84.6	7.7
$^{13}C_3$-PFBS	40	103	1.7	93.6	8.5
$^{13}C_2$-4:2FTS	160	107	6.1	105	2.6
$^{13}C_5$-PFHxA	40	93.8	9.8	75.8	16
$^{13}C_3$-HFPO-DA	40	77.8	8.5	72.0	9.8
$^{13}C_4$-PFHpA	40	90.5	8.4	83.3	10
$^{13}C_3$-PFHxS	40	101	7.8	94.7	6.4
$^{13}C_2$-6:2FTS	160	101	5.2	101	4.5
$^{13}C_8$-PFOA	40	89.5	5.7	82.8	10
$^{13}C_9$-PFNA	40	103	6.6	78.0	11
$^{13}C_8$-PFOS	40	101	7.6	89.7	4.5
$^{13}C_2$-8:2FTS	160	97.2	7.4	94.0	8.0
$^{13}C_6$-PFDA	40	98.7	6.3	82.3	15
$^{13}C_7$-PFUnA	40	102	4.3	82.6	8.0
$^{13}C_2$-PFDoA	40	98.8	4.6	81.2	10

a. P&A = “precision and accuracy”.

b. %R = percent recovery; %RSD = percent relative standard deviation.

c. Mean and %RSD of the isotope dilution analogue results for the unfortified matrix sample and the fortified samples in the P&A study; number of replicates given in the header row of the table.

Table 12. Precision and Accuracy Data for a Surface Water Matrix[a]

Analyte	Low Fortification (ng/L)	Mean %R[b,c] (*n*=5)	%RSD[b]	High Fortification (ng/L)	Mean %R (*n*=5)	%RSD
PFBA	10	95.4	19	80	106	4.8
PFMPA	10	108	16	80	102	5.9
PFPeA	10	93	13	80	101	6.0
PFBS	10	111	17	80	98.3	2.7
PFMBA	10	93.0	12	80	103	3.0
PFEESA	10	95.6	15	80	99.1	2.4
NFDHA	10	102	14	80	101	2.5
4:2FTS	10	70.9	17	80	91.1	7.8
PFHxA	10	96.9	19	80	103	4.2
PFPeS	10	87.5	14	80	104	4.9
HFPO-DA	10	109	8.7	80	105	7.0
PFHpA	10	95.9	11	80	105	4.8
PFHxS	10	78.5	8.2	80	97.1	5.3
ADONA	10	94.3	7.9	80	95.8	6.0
6:2FTS	10	86.5	6.3	80	101	9.7
PFOA	10	91.9	9.8	80	98.7	4.9
PFHpS	10	88.4	14	80	106	3.4
PFNA	10	89.7	9.5	80	95.9	2.8
PFOS	10	95.1	11	80	105	8.0
9Cl-PF3ONS	10	82.4	5.0	80	94.1	3.9
8:2FTS	10	102	7.6	80	101	4.0
PFDA	10	87.3	12	80	98.5	8.0
PFUnA	10	96.9	5.4	80	95.2	2.7
11Cl-PF3OUdS	10	82.4	8.9	80	93.0	4.4
PFDoA	10	94.6	2.3	80	98.4	4.1

a. Surface water matrix was sampled after the clarifier and prior to granular activated carbon within the drinking water treatment plant and chlorinated in our laboratory. pH = 8.1 at 20 °C. Free Cl_2 = 0.98 mg/L. Total Cl_2 = 1.31 mg/L. Total Organic Carbon (TOC) = 3.8 mg/L C.

b. %R = percent recovery; %RSD = percent relative standard deviation.

c. Corrected for native concentration.

Table 13. P&A in Surface Water Matrix: Isotope Dilution Analogue Recovery Data[a]

Analyte	Analogue Fortification (ng/L)	Mean %R[b,c] (*n*=6) P&A Low	%RSD[b,c]	Mean %R (*n*=6) P&A High	%RSD
$^{13}C_4$-PFBA	40	86.9	18	86.3	6.5
$^{13}C_5$-PFPeA	40	105	15	102	5.7
$^{13}C_3$-PFBS	40	98.6	11	99.8	4.5
$^{13}C_2$-4:2FTS	160	136	13	138	6.3
$^{13}C_5$-PFHxA	40	88.8	16	84.8	4.5
$^{13}C_3$-HFPO-DA	40	78.4	14	75.4	13
$^{13}C_4$-PFHpA	40	91.6	12	89.3	6.0
$^{13}C_3$-PFHxS	40	98.2	6.5	96.0	9.6
$^{13}C_2$-6:2FTS	160	110	9.7	109	8.4
$^{13}C_8$-PFOA	40	90.1	14	86.6	4.5
$^{13}C_9$-PFNA	40	91.0	14	87.2	6.0
$^{13}C_8$-PFOS	40	98.8	15	95.6	5.0
$^{13}C_2$-8:2FTS	160	101	9.8	97.3	11
$^{13}C_6$-PFDA	40	92.0	16	86.6	10
$^{13}C_7$-PFUnA	40	92.2	16	90.0	5.6
$^{13}C_2$-PFDoA	40	91.2	14	90.8	10

a. P&A = “precision and accuracy”.

b. %R = percent recovery; %RSD = percent relative standard deviation.

c. Mean and %RSD of the isotope dilution analogue results for the unfortified matrix sample and the fortified samples in the P&A study; number of replicates given in the header row of the table.

Table 14. Aqueous Sample Holding Time Data[a]

Analyte	Fortified Conc. (ng/L)	Day Zero Mean (ng/L)	Day Zero %RSD	Day 7 %Change[b]	Day 7 %RSD	Day 14 %Change	Day 14 %RSD	Day 21 %Change	Day 21 %RSD	Day 28 %Change	Day 28 %RSD
PFBA	40	42	4.6	9.1	2.3	3.1	7.2	5.1	5.4	4.2	5.0
PFMPA	40	41	5.2	5.5	2.2	-7.8	5.1	1.0	6.3	-10	3.1
PFPeA	40	43	4.1	1.2	1.9	-2.2	6.5	-0.29	2.5	-6.5	5.8
PFBS	40	43	9.7	-1.9	3.6	-6.1	1.8	-4.0	2.5	-7.6	8.9
PFMBA	40	40	3.0	-2.5	3.7	-5.7	4.3	0.20	5.0	-6.6	6.3
PFEESA	40	39	3.2	2.6	5.7	-1.8	6.7	-2.4	4.5	-1.7	2.6
NFDHA	40	39	6.5	-4.0	7.2	-11	6.9	-3.8	5.2	-2.9	8.0
4:2FTS	40	43	9.7	-1.7	3.8	-2.6	9.6	-2.0	6.1	-0.34	5.3
PFHxA	40	42	5.2	-0.37	4.6	-2.61	5.6	-1.7	5.8	-2.3	7.6
PFPeS	40	41	3.2	5.6	7.5	-3.1	2.6	6.0	9.2	-11	9.4
HFPO-DA	40	42	5.1	6.2	4.8	3.2	9.2	2.1	2.1	-3.5	4.2
PFHpA	40	41	4.6	-0.042	2.4	-4.7	1.7	-2.9	3.6	-3.0	5.4
PFHxS	40	41	4.3	1.8	3.0	-1.8	1.8	-1.8	9.0	-0.99	6.8
ADONA	40	39	4.2	-4.3	3.1	-12	5.7	-6.2	5.9	-2.3	3.1
6:2FTS	40	41	7.5	-4.3	4.4	-0.74	9.4	2.5	6.0	-1.5	6.0
PFOA	40	41	5.4	-1.5	6.7	1.6	5.1	-2.0	4.9	-6.5	7.2
PFHpS	40	41	4.7	-2.4	5.4	1.2	3.1	0.30	3.2	2.9	7.2
PFNA	40	42	4.1	2.05	0.57	-6.0	4.9	-6.1	3.4	-9.5	3.4
PFOS	40	41	7.0	-2.1	4.7	-1.8	5.2	1.0	5.8	-1.6	5.3
9Cl-PF3ONS	40	40	3.5	1.6	4.8	-0.34	1.8	4.0	4.8	-2.6	10
8:2FTS	40	44	7.9	-0.36	2.5	-1.4	6.7	0.026	3.8	-3.6	6.9
PFDA	40	41	5.0	0.12	3.1	-2.7	3.8	-1.4	3.8	-2.4	7.0
PFUnA	40	39	3.9	-1.3	4.7	-12	1.2	3.7	3.1	-6.7	3.5
11Cl-PF3OUdS	40	40	4.9	-1.1	4.5	-9.4	5.1	-11.0	4.7	-12	7.3
PFDoA	40	39	4.4	9.5	6.5	-4.8	6.0	-3.4	5.8	-16	6.1

a. Finished water from a surface water source. pH = 8.84 at 18 °C; total organic carbon (TOC) = 0.75 mg/L C (mean of 2019 first quarter plant records); free chlorine = 0.87 mg/L, total chlorine = 1.04 mg/L. Day Zero: *n*=7. All other events: *n*=5.

b. %Change = percent change from Day Zero calculated as follows: (Day X mean concentration – Day Zero mean concentration) / Day Zero mean concentration * 100%, where X is the analysis day.

Table 15. Extract Holding Time Data[a]

Analyte	Fortified Conc. (ng/L)	Day Zero Mean (ng/L)	Day Zero %RSD	Day 14 %Change[b]	Day 14 %RSD	Day 21 %Change	Day 21 %RSD	Day 27 %Change	Day 27 %RSD
PFBA	40	42	4.6	-8.0	4.2	-4.4	0.89	-12	6.4
PFMPA	40	41	5.2	-3.9	4.5	-0.10	5.1	-3.9	12
PFPeA	40	43	4.1	-6.0	6.0	-0.55	4.8	-5.4	1.1
PFBS	40	43	9.7	2.6	2.0	6.6	2.3	2.9	3.6
PFMBA	40	40	3.0	-10	7.1	-4.8	5.3	-8.8	2.7
PFEESA	40	39	3.2	1.3	8.9	-3.6	2.1	-4.9	3.6
NFDHA	40	39	6.5	-10	3.9	-13	6.8	-11	3.1
4:2FTS	40	43	9.7	-4.7	8.5	-6.2	8.8	-7.3	8.5
PFHxA	40	42	5.2	-4.6	6.3	-20	3.0	-14	4.7
PFPeS	40	41	3.2	-6.7	8.6	-11	5.2	-10	4.5
HFPO-DA	40	42	5.1	-4.9	4.9	-4.7	5.1	-4.4	7.7
PFHpA	40	41	4.6	-1.9	1.9	-6.1	4.8	-8.7	7.8
PFHxS	40	41	4.3	-19	9.9	-21	8.4	-22	11
ADONA	40	39	4.2	-1.2	1.9	-7.8	6.4	-7.5	5.0
6:2FTS	40	41	7.5	-5.3	13	-7.6	5.8	-8.4	14
PFOA	40	41	5.4	-5.7	6.3	-2.2	4.2	-2.4	3.3
PFHpS	40	41	4.7	-8.7	7.3	-6.0	5.2	-3.2	4.2
PFNA	40	42	4.1	-5.8	5.6	0.17	3.2	-2.0	6.0
PFOS	40	41	7.0	-3.8	10	-4.2	2.5	-3.7	4.4
9Cl-PF3ONS	40	40	3.5	-5.8	7.7	-9.3	4.0	-8.6	4.7
8:2FTS	40	44	7.9	-4.7	6.3	-1.3	5.8	-6.4	2.9
PFDA	40	41	5.0	-3.7	5.3	-1.8	5.6	-4.8	3.1
PFUnA	40	39	3.9	6.2	4.0	0.63	7.5	-2.8	5.2
11Cl-PF3OUdS	40	40	4.9	-12	5.9	-18	4.6	-10	6.3
PFDoA	40	39	4.4	1.9	5.5	1.0	6.4	-2.6	3.3

a. Finished water from a surface water source. pH = 8.84 at 18 °C; total organic carbon (TOC) = approximately 0.75 mg/L C (2019 first quarter plant records); free chlorine = 0.87 mg/L, total chlorine = 1.04 mg/L. Day Zero: *n*=7. All other events: *n*=7.

b. %Change = percent change from Day Zero calculated as follows: (Day X mean concentration – Day Zero mean concentration) / Day Zero mean concentration * 100%, where X is the analysis day.

Table 16. Initial Demonstration of Capability (IDC) Quality Control Requirements

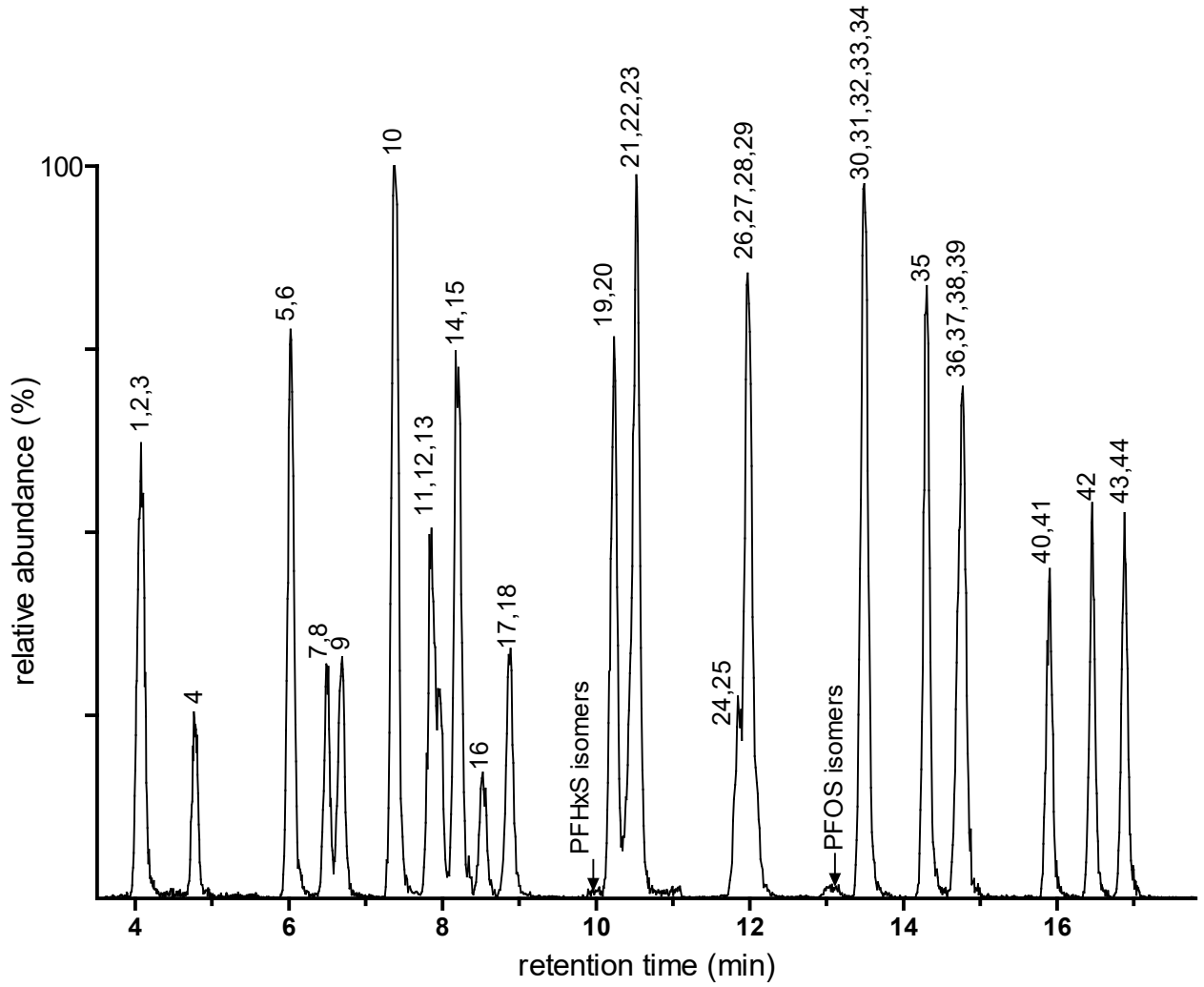
Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Section 10.2.2	Establish retention times for branched isomers	Each time chromatographic conditions change	All isomers of each analyte must elute within the same MRM window.
Section 9.1.1	Demonstration of low system background	Analyze a Laboratory Reagent Blank (LRB) after the highest standard in the calibration range.	Demonstrate that the method analytes are less than one-third of the Minimum Reporting Level (MRL).
Section 9.1.2	Demonstration of precision	Extract and analyze 7 replicate Laboratory Fortified Blanks (LFBs) near the mid-range concentration.	Percent relative standard deviation must be ≤20%.
Section 9.1.3	Demonstration of accuracy	Calculate mean recovery for replicates used in Section 9.1.2.	Mean recovery within 70–130% of the true value.
Section 9.1.4	MRL confirmation	Fortify and analyze 7 replicate LFBs at the proposed MRL concentration. Confirm that the Upper Prediction Interval of Results (PIR) and Lower PIR meet the recovery criteria.	Upper PIR ≤150% Lower PIR ≥50%
Section 9.1.5	Calibration Verification	Analyze mid-level QCS.	Results must be within 70–130% of the true value.

Table 17. Ongoing Quality Control Requirements

Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Section 10.3	Initial calibration	Use the isotope dilution calibration technique to generate a linear or quadratic calibration curve. Use at least 5 standard concentrations. Evaluate the calibration curve as described in Section 10.3.5.	When each calibration standard is calculated as an unknown using the calibration curve, analytes fortified at or below the MRL should be within 50–150% of the true value. Analytes fortified at all other levels should be within 70–130% of the true value.
Section 9.2.1	Laboratory Reagent Blank (LRB)	Include one LRB with each Extraction Batch. Analyze one LRB with each Analysis Batch.	Demonstrate that all method analytes are below one-third the Minimum Reporting Level (MRL), and that possible interference from reagents and glassware do not prevent identification and quantitation of method analytes.

Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Section 9.2.3	Laboratory Fortified Blank	Include one LFB with each Extraction Batch.	For analytes fortified at concentrations ≤2 x the MRL, the result must be within 50–150% of the true value; 70–130% of the true value if fortified at concentrations greater than 2 x the MRL.
Section 10.4	Continuing Calibration Check (CCC)	Verify initial calibration by analyzing a low-level CCC (concentrations at or below the MRL for each analyte) at the beginning of each Analysis Batch. Subsequent CCCs are required after every tenth field sample and to complete the batch.	The lowest level CCC must be within 50–150% of the true value. All other levels must be within 70–130% of the true value.
Section 9.2.4	Isotope performance standards	Isotope performance standards are added to all standards and sample extracts.	Peak area counts for each isotope performance standard must be within 50–150% of the average peak area in the initial calibration.
Section 9.2.5	Isotope dilution analogues	Isotope dilution analogues are added to all samples prior to extraction.	50%–200% recovery for each analogue
Section 9.2.6	Laboratory Fortified Sample Matrix (LFSM)	Include one LFSM per Extraction Batch. Fortify the LFSM with method analytes at a concentration close to but greater than the native concentrations (if known).	For analytes fortified at concentrations ≤2 x the MRL, the result must be within 50–150% of the true value; 70–130% of the true value if fortified at concentrations greater than 2 x the MRL.
Section 9.2.7	Laboratory Fortified Sample Matrix Duplicate (LFSMD) or Field Duplicate (FD)	Include at least one LFSMD or FD with each Extraction Batch.	For LFSMDs or FDs, relative percent differences must be ≤30% (≤50% if analyte concentration ≤2 x the MRL).
Section 9.2.8	Field Reagent Blank (FRB)	Analyze the FRB if any analyte is detected in the associated field samples.	If an analyte detected in the field sample is present in the associated FRB at greater than one-third the MRL, the results for that analyte are invalid.
Section 9.2.9	Calibration Verification using QCS	Perform a Calibration Verification at least quarterly.	Results must be within 70–130% of the true value.

Figure 1. Example Chromatogram for Reagent Water Fortified with Method Analytes at 80 ng/L[a]



a. Numbered peaks are identified in **Table 3**, **Table 4**, and **Table 5**.