Perfluorinated Compounds (PFCs) in Drinking Water by Liquid Chromatography/Tandem Mass Spectrometry- EPA 533

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Laboratory and Environmental Assessment Division
7202 NE Evergreen Pkwy
Suite 150
Hillsboro, OR 97124
Phone: 503-693-5700
Fax: 503-693-4999
www.oregon.gov/DEQ

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Approvals

Prepared By: _____ Date _____

Amy Nomani, Chemist III

Approved By: _____ Date _____

Nicole Marz, Interim Organic Section Manager

Approved By: _____ Date _____

Sara Krepps, Laboratory Quality Assurance Officer

Signed Copy on File

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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.

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. DEQ lab purchases standards containing linear and branched isomers of PFHxS and PFOS.

1.1. Applicable Matrices

This method is applicable to the following matrices:

- Drinking water

1.2. Detection Limits

Limits of Detection (LOD's) for the analytes in Table 1 are determined in reagent water following the requirements of 40CFR part 136, Appendix B, Revision 2 and the Limits of Detection (LOD) and Quantitation (LOQ) SOP (DEQ18-LAB-0053-SOP). LOD's are updated annually or following significant changes in method or instrumentation. Current detection limit studies are found on the lab QA drive: \\deqlab1\QA\MethodValidationPersonnelDOC\Methods

The Limits of Quantitation (LOQ's) are confirmed by following the procedures described in Section 11.2.3.

Table 1. List of target analytes and Limits of Quantitation (LOQ's).

Analyte	Abbreviation	CAS Number	LOQ[1], ng/L
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF3OUdS	763051-92-9	10
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	9Cl-PF3ONS	756426-58-1	10
4,8-Dioxa-3H-perfluorononanoic acid	ADONA	919005-14-4	10
Hexafluoropropylene oxide dimer acid	HFPO-DA	13252-13-6	100
Nonafluoro-3,6-dioxaheptanoic acid	NFDHA	151772-58-6	Not reported
Perfluorobutanoic acid	PFBA	375-22-4	10
Perfluorobutanesulfonic acid	PFBS	375-73-5	40
1H,1H, 2H, 2H-Perfluorodecane sulfonic acid	8:2FTS	39108-34-4	10
Perfluorodecanoic acid	PFDA	335-76-2	10
Perfluorododecanoic acid	PFDoA	307-55-1	10
Perfluoro(2-ethoxyethane)sulfonic acid	PFEESA	113507-82-7	10
Perfluoroheptanesulfonic acid	PFHpS	375-92-8	10
Perfluoroheptanoic acid	PFHpA	375-85-9	10

1H,1H, 2H, 2H-Perfluorohexane sulfonic acid	4:2FTS	757124-72-4	10
Perfluorohexanesulfonic acid	PFHxS	355-46-4	10
Perfluorohexanoic acid	PFHxA	307-24-4	10
Perfluoro-3-methoxypropanoic acid	PFMPA	377-73-1	40
Perfluoro-4-methoxybutanoic acid	PFMBA	863090-89-5	20
Perfluorononanoic acid	PFNA	375-95-1	10
1H,1H, 2H, 2H-Perfluorooctane sulfonic acid	6:2FTS	27619-97-2	10
Perfluorooctanesulfonic acid	PFOS	1763-23-1	10
Perfluorooctanoic acid	PFOA	335-67-1	10
Perfluoropentanoic acid	PFPeA	2706-90-3	100
Perfluoropentanesulfonic acid	PFPeS	2706-91-4	10
Perfluoroundecanoic acid	PFUdA	2058-94-8	10

[1]The LOQ or Limit of Quantitation is also commonly known as the method reporting limit (MRL). In general, the DEQ LEAP policy is to prepare the lowest calibration standard at concentrations less than or equal to the LOQ.

1.3. Method Flexibility

The laboratory may select LC columns, LC conditions, and MS conditions different from those described in the reference method. At a minimum, the isotope dilution standards and the isotope performance standards specified in the method must be used.

An aqueous sample volume within the range of 100–250 mL may be used, depending on the objectives of the project. During DEQ lab 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 the 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 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 (Section 11.2), verify that all QC acceptance criteria in this method are met (Section 11.0), and verify method performance in a representative sample matrix (Section 11.5).

2. 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. Personnel/Qualifications

The analyst should meet the minimum qualifications for a Chemist III position. The extraction can be carried out by a person meeting the minimum qualifications for a Chemist I Position. A chemist who has previously demonstrated proficiency at performing the method should train the analyst. An Initial Demonstration of Capability (IDOC) is to be conducted and passed prior to reporting data as required by the DEQ Laboratory Quality Manual (LQM).

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. Laboratories must demonstrate that these items are not contributing to interference by analyzing Lab Reagent Blanks (LRBs).

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 are purchased in glass ampoules. These standards are acceptable and subsequent transfers may be performed using glass syringes and pipets. Following extraction, the eluate must be collected in a polypropylene or polyethylene 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. Inorganic salts may cause low recoveries during the anion-exchange SPE procedure.

4.3.1 Co-extracted Organic Material

Matrix effects due to co-extracted organic material have the potential to cause enhanced ionization of analytes. The EPA experienced appreciable enhancement of 4:2 FTS during development of the reference method. Total organic carbon (TOC) is a good indicator of humic content of the sample.

4.3.2 Inorganic Salts

During the development of the reference method, the EPA 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 CaCO3. 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 of the reference method, the EPA confirmed 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 EPA noted that 13C3-PFBA isotope performance standard contained a small amount of 13C4-PFBA, slightly contributing to the signal of the isotope dilution analogue. Further, due to natural abundance of 34S, the native telomer sulfonates produced a small contribution to the 13C2 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 EPA reference method 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 EPA reference method 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

During development of the reference method, the EPA observed a trace amount of $^{13}C_3$-PFBA detected in the $^{13}C_4$-PFBA window. The contribution was no greater than 1%. The contribution of the isotope performance standard to the isotope dilution analogue was insignificant. Oregon DEQ did not experience any significant quantifiable results due to isotopic impurity, but standards will be monitored for the effects.

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 (34S) at 4.25%. Thus, the precursor ions of the $^{13}C_2$ isotopically labeled analogues and the naturally occurring 34S analogues present in the native analytes have the same nominal masses. The product ions of the telomer sulfonate isotope dilution analogues listed in Appendix E would contain a small contribution from the 34S analogue of the native telomer sulfonates. At the concentrations used in the EPA's primary study, the contribution of the 34S 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

Analysts working in the LEAD facility must review the laboratory's Chemical Hygiene Plan / Laboratory Safety Plan (DEQ04-LAB-0006-SFTY) and the Emergency Operation Plan (EOP/DEQ04-LAB-0050-SFTY).

Refer to the Job Safety Assessment (JSA) for this procedure and conduct analysis in accordance with the safety precautions specified (Appendix A).

Safety Data Sheets (SDS) are located on Q-Net under workplace safety. https://sps.deq.state.or.us/ws/SitePages/Home.aspx Under quick links, select Online (M)SDS to reach the Oregon DEQ (M)SDS Listing View.

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

Due to potential adsorption of analytes onto glass, polypropylene or polyethylene containers are used for sample preparation and extraction steps. Other plastic materials (e.g., polyethylene) that meet the QC requirements of Section 11 may be substituted.

- Sample Containers: 250mL HDPE Oblong with 43-415 HDPE cap (QEC Cat#: 6212-Q008)
- Polypropylene Vials: These vials are used to store stock standards and PDS solutions (4 mL, VWR Cat. No. 16066-960 or equivalent).
- 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).
- Autosampler Vials: Waters Vials, 12 x 32-mm, 2-mL, polypropylene, screw-neck, with polypropylene, septumless caps/

  - 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.
- Micro Syringes: Suggested sizes include 2, 5, 10, 25, 50, 100, 250, 500 and 1000 µL.
- Pipets: Polypropylene or glass pipets may be used for methanolic solutions.
- Analytical Balance: Capable of weighing to the nearest 0.0001 g.
- Solid Phase Extraction (SPE) Apparatus: Promochrom Technologies SPE-03 with MOD-004 low Teflon option
- SPE Cartridges: Phenomenex Strata™-X-AW 33 µm Polymeric Weak Anion, 500 mg / 6 mL (Cat# 8B-S038-HCH)
- Empty 6mL SPE cartridges
- 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].
- pH strips, range 0-14: Used to verify the pH of the phosphate buffer and to measure the pH of the aqueous sample prior to anion exchange SPE.
- LC-MS/MS System− Waters XEVO TQS
  - 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 have been made to DEQ's Waters XEVO TQS. A delay column, HLB Direct Connect 2.1 x 30 mm (Waters 186005231), was also placed in the mobile phase flow path immediately before the injection valve. This direct connect column reduces 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.
- Analytical Column: Waters Acquity UPLC BEH C18, 1.7 µm, 2.1 mm X 50 mm
- 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.
- MS/MS Data System

  Data Acquisition software − Waters Masslynx
  Data Processing software – Waters Targetlynx

  - An interfaced data system is required to acquire, store, and output MS data. The Waters Masslynx software has the capability of processing stored data by recognizing a chromatographic peak within a given retention time window. It allows integration of the abundance of any specific ion between specified times or scan number limits, construct a linear regression or quadratic regression calibration curve, and calculates the analyte concentration using the internal standard technique.

7. Reagents

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.

- 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.
- Methanol: CH_3OH, CASRN 67-56-1, LC grade (Fisher Scientific, Cat. No. A456 or equivalent).
- Ammonium Acetate: $NH_4CH_3CO_2$, CASRN 631-61-8, HPLC grade, molecular weight equals 77.08 g/mole.
- 20 mM Ammonium Acetate: Used as LC 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.
- 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.
- 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).
- 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 shall be made fresh each day of extraction.
- 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.
- 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.
- 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.
- Nitrogen: Ultra-high-purity-grade nitrogen is used for concentrating extracts and as a nebulizer gas in the MS/MS instrument.
- Argon: Used as collision gas in MS/MS instruments.
- Sodium Hydroxide: May be purchased as pellets or as aqueous solution of known concentration. Added to methanolic solutions of PFAS to prevent esterification.
- 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.

8. Standards

See the Organic section of the shared directory @ \\deqlab1\sp-organics\Standard Preparation\LCMS for full details on preparing all standards. The standard preparation spreadsheet also includes spike volumes

and final extract concentrations. In addition, standards are logged in Element with the Certificates of Analysis attached to the stocks as pdf.

According to the reference method, 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.

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. Solution concentrations listed in Table 2 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. Store stock standards at less than 4°C unless the vendor recommends otherwise. The Primary Dilution Standards (PDS) may be stored at any temperature, but cold storage is recommended to prevent solvent evaporation.

8.1. 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:

$$Mass\ of\ NaOH\ required\ (g) = \frac{\text{Total PFAS mass (g)} \times\ 160\left(\frac{\text{g}}{\text{mole}}\right)}{250\ (\frac{g}{mol})}$$

DEQ lab purchases labeled and native standards that contains 4 mole eq. of NaOH to prevent conversion of the carboxylic acids to their respective methyl esters.

8.2. Isotope Performance Standards

This method requires three isotope performance standards listed in Table 2. These isotopically labeled compounds, also referred to as the labeled injection standard, include two carboxylates with different chain lengths and a sulfonate.

The isotope performance standards are purchased as certified standard solutions, 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.

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. Method modification QC requirements must be met (Sect. 11.5) whenever additional isotope performance standards are used.

The standard concentrations used to develop the method are listed in Table 2. During DEQ method validation, the final extracts were fortified with 10 µL of the labeled injection standard stock to yield a final extract 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).

8.3. Isotope Dilution Analogues

Isotopically labeled analogues, also referred to as the labeled surrogate standard, are listed in Table 2. Purchase 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. Note that Chemical Abstracts Registry Numbers are not currently available for these isotopically labeled analogues.

Additional isotopically labelled PFAS analogues 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 whenever new analogues are proposed.

The standard concentrations used to develop the method are listed in Table 2. During DEQ method validation, samples were fortified using 20 µL of the labeled stock 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 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.

Table 2. List of Isotopically Labeled Analytes

Isotopically Labeled Analyte	Abbreviation	Stock Concentration (ng/mL)
Isotope Performance Standard		
Perfluoro-*n*-[2,3,4-$^{13}C_3$]butanoic acid	^{13}C3-PFBA	1000
Perfluoro-[1,2-$^{13}C_2$]octanoic acid	$^{13}C_2$-PFOA	1000
Sodium perfluoro-1-[1,2,3,4-$^{13}C_4$]octanesulfonate	$^{13}C_4$-PFOS	3000
Isotope Dilution Analogues		
Perfluoro-n-[1,2,3,4-$^{13}C_4$]butanoic acid	$^{13}C_4$-PFBA	500
Perfluoro-n-[1,2,3,4,5-$^{13}C_5$]pentanoic acid	$^{13}C_5$-PFPeA	500
Perfluoro-n-[1,2,3,4,6-$^{13}C_5$]hexanoic acid	$^{13}C_5$-PFHxA	500
Perfluoro-n-[1,2,3,4-$^{13}C_4$]heptanoic acid	$^{13}C_4$-PFHpA	500
Perfluoro-n-[$^{13}C_8$]octanoic acid	$^{13}C_8$-PFOA	500
Perfluoro-n-[$^{13}C_9$]nonanoic acid	$^{13}C_9$-PFNA	500
Perfluoro-n-[1,2,3,4,5,6-$^{13}C_6$]decanoic acid	$^{13}C_6$-PFDA	500
Perfluoro-n-[1,2,3,4,5,6,7-$^{13}C_7$]undecanoic acid	$^{13}C_7$-PFUdA	500
Perfluoro-n-[1,2-$^{13}C_2$]dodecanoic acid	$^{13}C_2$-PFDoA	500
2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy-$^{13}C_3$-propanoic acid	$^{13}C_3$-HFPO-DA	500

Sodium perfluoro-1-[2,3,4-$^{13}C_3$]butanesulfonate	$^{13}C_3$-PFBS	500
Sodium perfluoro-1-[1,2,3-$^{13}C_3$]hexanesulfonate	$^{13}C_3$-PFHxS	500
Sodium perfluoro-[^{13}C8]octanesulfonate	$^{13}C_8$-PFOS	500
Sodium 1H,1H,2H,2H-perfluoro-1-[1,2-$^{13}C_2$]hexane sulfonate	$^{13}C_2$-4:2FTS	2000
Sodium 1H,1H,2H,2H-perfluoro-1-[1,2-$^{13}C_2$]-octane sulfonate	$^{13}C_2$-6:2FTS	2000
Sodium 1H,1H,2H,2H-perfluoro-1-[1,2-$^{13}C_2$]-decane sulfonate	$^{13}C_2$-8:2FTS	2000

8.4. 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 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. Analyte standards were obtained from Wellington Laboratories (Guelph, Ontario, Canada), Stock standards are made by dilution in methanol containing 4 mole equivalents of sodium hydroxide.

8.4.1 PFHxS and PFOS

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

8.4.2 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.

$$mass\ (acid\ form) = mass\ (salt\ form) x\ \frac{MW\ acid}{MW\ salt}$$

8.4.3 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. 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. In this instance, the analyte PDS was a stock prepared by the vendor and used straight 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.

8.4.4 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 (CCVs). 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. 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 320 ng/mL extract concentration.

9. Sample Collection, Preservation, Shipment, and Storage

9.1. Sample Bottles

Samples must be collected in plastic bottles: polypropylene bottles fitted with polypropylene screwcaps, 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 12.2.4.

9.2. 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.

9.3. Sample Collection

Refer to the PFAS Sampling Guidance Document DEQ21-LAB-0035-SOP for complete details about sampling procedures and precautions against contamination.

9.3.1 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.

9.3.2 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.

9.4. 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.

9.4.1 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.

The laboratory will extract a Laboratory Reagent Blank (LRB) 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. This will ensure that any significant contamination detected in the FRBs originated from exposure in the field.

9.4.2 Field Reagent Blank Procedure

The laboratory will provide a sealed FRB sample bottle with analyzed reagent water in the shipment 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.

9.5. 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°C. Once at the laboratory, samples must be stored at or below 6°C until extraction. Samples must not be frozen.

9.6. Sample and Extract Holding Times

Analyze samples as soon as possible. Samples must be extracted within 28 days of collection. Extracts are stored refrigerated and brought to room temperature for instrument analysis. Sample extracts must be analyzed within 28 days after extraction.

10. Calibration and Standardization

Demonstration and documentation of acceptable MS calibration and initial analyte calibration are required before performing the IDOC and prior to analyzing field samples.

10.1. MS/MS Optimization

10.1.1 Mass Calibration

Calibrate the mass spectrometer with the calibration compounds and procedures specified by the manufacturer. Refer to section 11.0 of the LCMSMS Technology SOP for additional information. DEQ11-LAB-0030-SOP.

10.1.2 MS Parameters

Instrumental parameters should be optimized for the precursor and product ions listed in Appendix D. 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.

a. Requirement for Branched Isomers

There have been reports that not all product ions in the linear PFOS are produced in all branched PFOS isomers. (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.

b. Precursor Ion

Optimize the response of the precursor ion ([M – H]– or [M – CO2 – H] –) for each analyte following manufacturer's guidance. Analyte concentrations of 1.0 µg/mL were used for this step during reference method development. Vary the MS parameters (source voltages, source and desolvation temperatures, gas flows, etc.) until optimal analyte responses are determined. The analytes may have different optimal parameters, requiring some compromise on the final operating conditions. See Appendix D for ESI-MS conditions used to collect method performance data.

c. 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 Appendix D for MS/MS conditions used to collect method performance data.

10.2. Chromatographic Conditions

Establish LC operating parameters that optimize resolution and peak shape. Current LC conditions can be found in Appendix D. 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 PFHxS and PFOS, discussed in Section 8.4.1, at a mid- to high level concentration. Identify the retention times of the branched isomers of PFHxS and PFOS present in the technical-grade standards. When PFOS and PFHxS are chromatographed on a reversed-phase column, the branched isomers elute prior to the linear isomer. Repeat the procedure in this section for 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 Appendix E.

10.3. Initial Calibration

If all the above stated criteria are met, analysis may proceed. Prepare a multi-point calibration for the analytes as described in Section 8. The calibration levels will be: 0.5, 1.0, 2.5, 5, 10, 20, 40, 80, 160, 320 ng/L for each native analyte on instrument and contain 10 ng/mL of the Isotope Dilution Analogues and 10 ng/mL of the Isotope Performance Standards.

Table 3. Summary of Initial Calibration Requirements

Number of calibration standards	**5 minimum, 10 currently**
Low calibration standard	0.5 ng/mL on instrument
High calibration standard	320 ng/mL on instrument
Signal response	Peak Area
Calibration Method	Native Compounds: Linear or quadratic regression. Weighting may be used. Forcing the calibration curve through the origin is mandatory. Labeled Compounds: Average Response.
Acceptance Criteria	Native Compounds: $r^2 \geq 0.990$ Cal. Level Concentrations ≤ MRL, within 50-150% of true value. All other points within 70-130% of TV. Labeled Compounds: No criteria
Calibration Frequency	Each time a major instrument modification or maintenance is performed, or as needed.
Calibration Verification	Analyze CCV after initial calibration. See QC summary Table 4 for acceptance limits.

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 Appendix E. 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 Appendix E.

10.3.1 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.

10.3.2 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.

10.3.3 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.

10.3.4 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.

10.3.5 Continuing Calibration

Analyze a CCV to verify the initial calibration at the beginning of each instrument sequence, after every tenth field sample, and at the end of each sequence. The beginning CCV for each sequence must be at, or below, the MRL for each analyte. This CCV verifies instrument sensitivity prior to the analysis of samples. DEQ injects the level 3 and level 6 calibration standards as the low-level CCV (LCV) to account for different analyte MRLs. Alternate subsequent CCVs between the mid and high calibration levels. Verify that the CCV meets the criteria in the following sections.

11. Quality Control

QC procedures include determination of the Limit of Detection (LOD), Demonstration of Capability (DOC) 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 are summarized in Table 4, and discussed in the following sections. These QC requirements are considered the minimum for an acceptable QC program.

All analysts and laboratories using this method are required to operate a formal QC program. The laboratory is required to maintain performance records that define the quality of the data thus generated.

11.1. Data Assessment and QC Acceptance Criteria

Data assessment and QC acceptance criteria are of primary importance in assessing the quality of data resulting from an analytical batch. The following data assessment and QC data are required for all analysis.

Table 4. Summary of Analytical Quality Control Elements, Frequencies, and Acceptance Criteria

QC Element	Frequency	Acceptance Criteria	Comments
Calibration Curve	As needed	See Section 10.3	Must be acceptable before analyzing IDOC, LOD, and samples.
Initial Demonstration of Capability for analyst (IDOC)	At end of method development and prior to reporting data. Each new analyst. With substantial change to method or instrumentation.	Successful completion of four QC components.	Four components: demonstration of low system background, determination of precision and accuracy, MRL confirmation, and QCS.

QC Element	Frequency	Acceptance Criteria	Comments
Initial Demonstration of Capability for extractionist (IDOC)	At end of method development and prior to reporting data. Each new extractionist. With substantial change to extraction procedure.	Successful completion of precision and accuracy determination.	
Continuing Demonstration of Capability (CDOC)	Yearly.	Successful PT or repeat the precision and accuracy component of the IDOC	Follow procedures in Section 6.7 of DEQ20-LAB-0011-SOP
Determination of Precision and Accuracy	With IDOC or with substantial change to method or instrumentation.	%RSD < 20 Avg. recovery 70-130%	1 BLK/LRB and 7 replicate LCS/LRB's and fortified at mid-point cal concentration.
MRL Confirmation study	With analyst IDOC or with substantial change to method or instrumentation.	Seven replicates fortified at or below the proposed MRL. PIR meets criteria.	Upper PIR ≤ 150% Lower PIR ≥ 50%
Low System Background Evaluation	With analyst IDOC or with substantial change to method or instrumentation. Analyze a CCB/ICB immediately after high CAL point. Each port of SPE equipment.	< 1/3 MRL	If using SPE, extract a LRB/BLK on each port. The DEQ SPE instrument has 8 ports.
Initial LOD	Substantial change to method or instrumentation. Extract and analyze over three days per 40 CFR Appendix B to Part 136, Revision 2.	Seven replicates fortified at or below the proposed MRL to calculate LODs. Minimum of seven method blanks to calculate LODb.	Use MRL confirmation study to calculate LODs. Use the 8 blanks from the low system background evaluation to calculate LODb.
Ongoing LOD	Analyze at least one spiked LRB fortified at the LOQ concentration each quarter per instrument. Alternatively, perform an initial LOD, annually.	If the verified LOD is within 0.5 to 2.0 times the existing LOD and < 3% of the method blank results have results above the existing LOD, then the existing LOD may be left unchanged.	Quarterly spikes must be at the same concentration as the MRL confirmation/Initial LOD. Use blanks analyzed with samples to calculate LODb, additional blanks may not be necessary.

QC Element	Frequency	Acceptance Criteria	Comments
Quality Control Sample (QCS)	With analyst IDOC or with substantial change to method or instrumentation. Quarterly thereafter.	Recovery 70-130% of TV	QCS is an independent dilution beginning with common starting materials as ICAL. Prep by second analyst is recommended.
Continuous Calibration Verification standard (CCV)	Immediately following Initial Calibration. At the beginning of each analytical run, after every 10th sample, and at the end of each run.	Isotope Performance Standards: Area ±50% of Avg. ICAL areas Isotope Dilution Standards: Recovery 70-130% of TV Native analytes: LLCCV recovery 50-150% of TV. All other CCV levels 70-130% of TV.	Opening CCV conc. ≤ MRL, for each analyte. Use Element QC type "LCV" Alternate CCV concentration levels, thereafter using the QC type "CCV"
Continuous Calibration Blank (CCB)	Immediately following Initial Calibration, and after each CCV.	< 1/3 MRL	
Laboratory Control Sample (LCS) / Laboratory Fortified Blank (LFB)	At least once per analytical batch. Rotate concentration; low, medium and high from batch to batch.	Low LCS (within 2x MRL): recovery 50-150% of TV. Medium & High LCS: 70-130% of TV	Low level: Use Element QC type "MRL" Med & High: Use Element QC type "LCS"
Method Blank (MB) or Laboratory Reagent Blank (LRB)	At least once per extraction batch. Rotate blanks among the 8 SPE ports during routine analysis.	< 1/3 MRL	Record SPE port # for the MB on the Element bench sheet.
Isotope Performance Standard/Labeled Internal Standard	In all injections of the analytical sequence.	Area ±50% of Avg. ICAL areas	
Isotope Dilution Analogue Recovery/Labeled Surrogate Standard	In all field and QC samples.	Recovery 50-200% of TV	
Matrix Spike (MS) or Laboratory Fortified Sample Matrix (LFM)	At least once per analytical batch. Collect MS for each type of source water.	Same as LCS/LFB criteria.	

QC Element	Frequency	Acceptance Criteria	Comments
Duplicate Samples (DUP or MSD)	At least one Field Duplicate (FD) or Matrix Spike Duplicate (MSD) per analytical batch	RPD ≤ 30% for concentrations > 2 times MRL. RPD ≤ 50% for concentrations < 2x MRL.	If the method analytes are not routinely observed in field samples, analyze a MSD rather than a FD.
Field Blank (FB)	Each sample set	< 1/3 MRL	

11.2. Initial Demonstration of Capability

The IDOC must be successfully performed prior to analyzing field samples. Prior to conducting the IDOC, the analyst must meet the calibration requirements outlined in Section 10. The same calibration range used during the IDOC must be used for the analysis of field samples. The IDOC includes four determinations: demonstration of precision and accuracy, demonstration of acceptable system background, MRL confirmation, and QCS. All of these components may be completed in the same analysis batch.

11.2.1 Demonstration of Low System Background

Analyze a system blank (CCB, ICB) immediately after injecting the highest calibration standard in the selected calibration range. Confirm that the blank is free from contamination as defined in Section 9.4 and the QC summary table.

If an automated extraction system is used, an LRB must be extracted on each port to fulfil this requirement.

11.2.2 Demonstration of Precision and Accuracy

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. See QC summary table for acceptance criteria.

11.2.3 Minimum Reporting Level (MRL) Confirmation

Establish a target MRL based on the intended use of the method. 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 CCV) must be at, or below, the MRL. Fortify, extract, and analyze seven replicate LFBs at, or below, the proposed MRL concentration. Calculate the mean and standard deviation for each analyte in these replicates. Determine the Half Range for the Prediction Interval of Results (HRPIR) the upper and lower PIR limits as shown in Section 13.3. The MRL is confirmed if the Upper PIR Limit is ≤150%; and the Lower PIR Limit is ≥50%. If these criteria are not met, the MRL has been set too low and must be confirmed again at a higher concentration. DEQ MRLs were established at 4 different concentrations depending on the analyte; 10 ng/L, 20 ng/L, 40 ng/L, and 100 ng/L, as shown in Table 1.

11.2.4 Calibration Verification using Quality Control Sample

Analyze a QCS during the IDOC, and then quarterly thereafter. The purpose is to confirm the accuracy of the primary calibration standards. 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 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. See QC summary table for acceptance criteria.

11.3. Ongoing QC Requirements

This section describes the ongoing QC elements that must be included in a sample batch when processing and analyzing field samples. Standard preparation and spike information can be found in the Element data system and the shared network drive: \\deqlab1\sp-organics\Standard Preparation\LCMS

11.3.1 Laboratory Reagent Blank (LRB/BLK)

Analyze an LRB with each extraction batch. Background concentrations of method analytes must be less than one-third the MRL. Subtracting blank values from sample results is not permitted.

a. 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.

b. Influence of Background on Selection of MRLs

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

c. 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.

11.3.2 Laboratory Fortified Blank (LFB/LCS)

An LFB is required with each batch. The concentration of the LFB must be rotated between low, medium, and high concentrations from batch to batch. Fortify the low concentration LFB near the MRL. The high concentration LFB must be near the high end of the calibration range. See QC summary table for acceptance criteria.

11.3.3 Isotope Performance Standard Areas

The peak areas of the isotope performance standards are monitored in all injections of the analytical sequence. See QC summary table for acceptance criteria.

11.3.4 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. See QC summary table for acceptance criteria.

11.3.5 Laboratory Fortified Sample Matrix (LFSM/MS)

Analyze a minimum of one LCS each batch. 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. See QC summary table for acceptance criteria

11.3.6 Laboratory Fortified Sample Matrix Duplicate (LFSMD/MSD) 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. See QC summary table for acceptance criteria.

11.3.7 Field Reagent Blank (FRB/FBK)

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.

11.3.8 Ongoing LOD evaluation

Analyze LOQ spikes and method blanks following the Procedure Section of the LOD and LOQ SOP DEQ18-LAB-0053-SOP.

11.4. Method Modification QC Requirements

The analyst is permitted to modify the chromatographic and MS/MS conditions as described in Section 1.3. The following are required after a method modification.

Repeat the IDOC

Establish an acceptable initial calibration using the modified conditions. Repeat the procedures of the IDOC.

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 IDOC, 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.

To date, ODEQ lab has not made method modifications to warrant this study. This SOP will be updated as applicable to include precision and accuracy or LCMRL studies in representative sample matrices.

11.5. Corrective Actions for QC Failures

Table 5. List of Corrective Actions for QC failures

Quality Control Element	Corrective Action
Initial Calibration	Re-analyze the calibration standards, restrict the range of calibration, or perform 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.
Demonstration of Capability (DOC)	Determine source of problem, correct and reanalyze. Repeat if changes are made to analytical parameters not previously validated during the IDOC.

Quality Control Element	Corrective Action
System Blank	Reduce background contamination and re-analyze.
QCS	Identify and correct the source of the problem. Prepare fresh standard dilutions and repeat the Calibration Verification.
Continuing Calibration Verification (CCV)	Re-inject CCV. If CCV fails a second time, a new initial calibration is required. Instrument maintenance may be required before re-calibrating. Service may include maintenance of the autosampler, flushing or replacing the column.
Lab Reagent Blank	Reduce background contamination and re-analyze. 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.
Field Blank	Analyze lab blank to determine the source of the problem.
Laboratory Control Sample (LCS)	Determine source of problem, correct and reanalyze entire batch. Re-extraction may be warranted.
Matrix Spike (MS)	Reanalyze. Check makeup of Matrix Spike solution. If CCV and LCS passes, indicates problem is matrix related, not system related.
Matrix Spike Duplicate or Sample Duplicate (MSD, Dup, Field Dup)	Reanalyze. If CCV and LCS passes, indicates problem is matrix related, not system related.
Isotope Performance Standard Areas	Random evaporation losses have been observed with the polypropylene caps causing high biased isotope performance standard areas. Re-analyze. 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.
Isotope Dilution Analogue Recovery	Evaluate the area of the isotope performance standard to which the analogue is referenced and the recovery of the analogues in the CCVs. If necessary, recalibrate and service the LC-MS/MS system. Re-analyze. If the repeat analysis meets the 50–200% recovery criterion, report results from the re-analysis. If the repeat analysis fails, extraction of the sample must be repeated provided a sample is available and still within the holding time.

11.6. Contingencies for Out-of-Control Data

If the samples cannot be reanalyzed or the analysis otherwise brought under-control:

a) Identify if there is any guidance in the relevant QAPP or SAP and follow that guidance.

b) Contact the end user and verify the importance of the data and to see if the analyte can be reported as estimated.

- If so, report the affected analyte(s) with a data qualifier and set the DQL to B.
- If not, void the analysis and set the DQL to C.

c) Determine with the end user if an alternate sample can be used for his/her evaluation purposes.

d) If it seems appropriate in your best professional judgment, speak with the lab sample tracker or sample collector about obtaining an alternate sample that may provide the required information.

e) If the end user cannot be reached, consult with the section manager or QA officer to determine if the data should be reported as an estimate (DQL B), rejected (DQL C) or voided (DQL D).

f) In every case, the problem(s) and attempted corrections should be noted in the laboratory notebook (when instrumental problems have occurred), with the analytical batch data, and in the LIMS system for final reporting.

12. Procedure

12.1. Extraction Procedure

12.1.1 Pre-Run Instrument Cleaning

Cleaning of the SPE-03 instrument is required before and after every run, see Appendix B for cleaning procedure.

12.1.2 Sample Preparation

1. Batch the samples in Element, print out the bench sheet, and export the bench sheet as an XLS file to open in Excel.

2. Open the exported bench sheet in Excel on the computer in extractions. Weigh each sample bottle and enter this weight into the spreadsheet under the “Bottle Weight Full” column.

   Note: While not routine, over filled sample bottles (>270mLs / 295grams), require volume to be poured off. Remove cap and pour off excess volume to the 250mL line on the bottle, replace cap and follow step 2.

3. Verify that the sample containing 1 g/L ammonium acetate has a pH between 6.0 and 8.0. Remove the sample cap, dip the end of a Pasteur pipette into the sample to transfer a few drops onto pH paper, range 0-14. Use a new pipette and pH strip for each sample.
   Acetic acid may be added as needed to reduce the pH. When needed, remove cap and add 1-2 drops using a pipette, then cap and invert to mix. Check pH and repeat if necessary.

4. Prep QC by filling new sample bottles with DIW and adding appropriate preservation. For a 250mL sample container, add 0.25 grams of ammonium acetate to the empty bottle, fill with 250mL of DIW, then cap and invert sample to mix.

5. Add labeled surrogate to each sample, then cap and invert to mix. (See note)

6. Fortify BS, MS, and MSDs, with an appropriate volume of spike. Cap and invert each sample several times to mix. (See note)

Note: Current instructions for spike concentrations and spike volumes are found in the Standard Preparation spreadsheet saved on the shared organics drive (\\deqlab1\sp-organics\Standard Preparation\LCMS\) or by referring to the Element data system.

12.1.3 Running samples on the SPE-03

1. Load SPE cartridges by fitting onto cartridge adapters and placing in cartridge rack. Ensure the cartridges are pressed firmly against the SPE adapter.

2. Load Samples onto SPE by screwing the bottle onto the cap (DO NOT ROTATE CAP, rotate the bottle) and inverting onto sample holders. Note the SPE position number on the bench sheet.

3. Puncture Hole in bottom of inverted bottle.

4. Remove fraction tray by tilting at a 45-degree angle and removing from the SPE-03. Load Fraction tray with labeled 15 mL centrifuge tubes into corresponding sample positions.

5. Load method "*EPA 533"* and hit the play icon to begin, see Appendix C for method steps, parameters, and correct solvent positions.

   Note: Before starting method, check solvent levels to ensure adequate volumes, empty waste containers if needed, and turn on N2.

6. Once the method is complete and the instrument is idle, remove extracts from fraction tray and discard used SPE cartridges. Please used sample bottles and caps in the hood to dry overnight.

7. The following day, weigh the empty bottles with caps and record weights in the "bottle weight empty" column of the excel sheet. Save and print a copy of the excel sheet, and attach to bench sheet. Upload the excel sheet to Element to transfer the sample weights.

12.1.4 Extract Concentration and Final Volume

1. Concentrate the extract to dryness under a gentle stream of nitrogen in a heated water bath (55–60 °C).

2. Reconstitute the extract with 1.0 mL of 20% reagent water in methanol (v/v).

3. Add the isotope performance standards to the extract and vortex. Store extracts in refrigerator.

12.2. Sample Analysis

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, CCVs, LCS, MS/MSD (or FD)). The required QC samples are not included in counting the maximum field sample total of 20. LC-MS/MS conditions for the sequence must be the same as those used during calibration.

12.2.1 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 a 533-30 initial calibration standard or CCV. Proceed with quantitation based on the type of standard available for each method analyte.

12.2.2 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. Currently we only have branched isomers available and included in our calibration standards for PFOS and PFHxS.

12.2.3 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 Appendix D. 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.

12.2.4 Exceeding the Calibration Range

The analyst must not report concentration that exceed the 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 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. If historical data for a specific site indicates an analytes' results may exceed the calibration range, the sample may be diluted prior to extraction as described above. In the event that no sample duplicate is available, the sample extract may be diluted with 20% water in methanol and reanalyzed. Care should be taken to make the smallest dilution necessary to bring analyte concentrations into calibration range, while not diluting the isotope dilution analogue and isotope performance standards to an unquantifiable level. Report concentrations of analytes that exceeded the calibration range in the original sample based on measurement in the dilute extract. The dilution factor must be incorporated into the Waters software SPL prior to processing the data to ensure isotopic dilution calculations are made correctly. The resulting data must be annotated as a dilution.

13. 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.

For a complete explanation of the calculations performed on the raw data by the data system, please refer to the MassLynx 4.1 Peak Integration and Quantitation Algorithm Guide, a copy of which is included in the LCMS method binder.

Please refer to the DW- PFAS by LCMSMS 533 Calculation Algorithm Validation Worksheet produced by Element for the calculations used by the LIMS system to obtain final analytical results from initial values entered by the analyst.

13.1. Precision and Accuracy

$$Average\ \%\ Recovery = \frac{Average\ Measured\ Concentration}{Spiked\ Concentration} \text{ X } 100$$

$$\%RSD = \frac{Standard\ Deviation\ of\ Measured\ Concentrations}{Average\ Measured\ Concentration} \text{X } 100$$

13.2. MRL Confirmation

Calculate the mean and standard deviation of the seven MRL replicates. Determine the Half Range for the prediction interval of results (HR_{PIR}) using the following equation:

$$HR_{PIR} = 3.963s$$

Where s is the standard deviation and 3.963 is a constant value for seven replicates. Note: This value is *only* defined for seven replicates.

$$Upper\ PIR\ Limit = \frac{Mean + HR_{PIR}}{Spiked\ Concentration} \text{X } 100$$

$$Lower\ PIR\ Limit = \frac{Mean - HR_{PIR}}{Spiked\ Concentration} \text{X } 100$$

The MRL is confirmed if the Upper PIR Limit is ≤150%; and the Lower PIR Limit is ≥50%.

14. Records Management

Data files on the LC workstation must be backed up to the Organic server monthly.

Paper printouts of chromatograms are collated based on run order and combined with the following documentation:

- Copy of the run schedule, listing the analysis method used for each sample and ideally with the datafile identified for each injection.
- Printout of the calibration tables showing curve type, origin information, weighting, calculation method, peak area selected, and RT windows.
- Printouts of the sample chromatograms in run order.
- Peer review checklist.

The Masslynx/Targetlynx software makes it difficult to print the chromatograms without printing a large quantity of extraneous information. Consequently, the dataset hardcopies are not complete records of the data and they are not intended to replace the dataset QLD files produced by TargetLynx. Both records must be preserved. The lab maintains data packages on site for a minimum of 2 years and then for the period as required by DEQ and State record retention policies, (at least 10 years).

15. Method Performance

Please refer to the QA directory @ \\deqlab1\QA\MethodValidationPersonnelDOC\Methods for the most current method validation studies including Limits of Quantitation and Verifications.

16. Maintenance

Periodic maintenance and troubleshooting need to be performed to avoid or correct problems. Peak shape, system pressure, and check standards are used to evaluate LC/MS/MS performance before each analytical run. If peak shape deteriorates (diminished response and peak tailing), the columns may need to be cleaned or replaced. If the pressure reading is high (overpressures), there may be a clog in the mobile-phase flow path.

16.1. Periodic Maintenance

Table 6. Periodic Maintenance Schedule

Instrument	Maintenance Activity	Frequency
MS	Gas-ballast rotary pump	Weekly
MS	Change rotary pump oil	Every 3000 hours of pump operation
MS	Clean the source assembly	When sensitivity decreases to unacceptable levels
MS	Clean the ESI probe tip	When sensitivity decreases to unacceptable levels
MS	Clean the ion block assembly	When sensitivity decreases to unacceptable levels
MS	Clean all source components	When sensitivity decreases to unacceptable levels
MS	Replace the ESI probe capillary	When sensitivity decreases to unacceptable levels
HPLC	Replace solvent manager head seals	During scheduled maintenance or as needed
HPLC	Replace solvent manager plungers	During scheduled maintenance or as needed

16.2. Troubleshooting

Waters routinely updates instrument maintenance and troubleshooting procedures. To ensure that the most up-to-date procedures are used, refer to the most recent revision of the relevant instrument manual, available from www.waters.com.

17. Pollution Prevention

Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

Where feasible, solvent waste generated during sample preparation and analysis is to be collected for appropriate disposal. Other chemicals used in this method include the materials used in preparing standards, sample preservatives, and sample extracts. Dispose of these as necessary (i.e., expired standards, sample extracts) in the hazardous waste containers in the lab

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.

18. Waste Management

All standards and sample extracts which are no longer in use are considered hazardous waste and must be disposed of following pertinent procedures. The Agency requires that 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.

19. Definitions

Standard Definitions applicable to laboratory quality systems can be found in Appendix A of the LEAD Quality Manual DEQ91-LAB-0006-LQM

Specific definitions for EPA 533 are explained below.

Isotope Dilution Analogues

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

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.

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.

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.

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%.

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.

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.

Product Ion

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

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.

20. Deviations from Referenced Methods

None

21. References

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, November 2019, EPA Document No. 815-B-19-020

22. Revision History

Revision	Date	Changes	Editor
1.0	11/22/2021	New Method	ASN

Appendix A: Job Safety Assessment.

Hazard Analysis		
	Activity:	Chromatographic Analysis
	Program/Location	DEQ Laboratory
	Position # (s):	0160, 0167, 0193, 2603, 2645, 3010, 3012, 3276, 3277
	Analysis by:	Health & Safety
	Date:	2/21/2019

Required PPE:

Gloves - Nitrile	Safety Glasses	Lab Coat	Gloves - Thermal	Gas Cylinder Hand Truck	Blast Shield	Safety Goggles
						

Required/Recommended Trainings:

1. Chemical Hygiene Plan
2. Review of Chemical Hygiene Plan
3. SIM-plicity Training
4. Review of relevant lab SOPs
5. Compressed Gas Safety Training
6. PPE Policy Review

TASK	HAZARDS	SEVERITY	CONTROLS
1. Computer Use/Data Entry	Repetitive motion injuries	Low	Follow ergonomic recommendations
2. Sample Preparation/Dilutions	(CW) – Glass shards from broken sample vials, glass pipettes or needles on microsyringes (E) – Solvent, acid or heavy metal exposure, unknown sample contaminant exposure	Medium High	Inspect glassware and extract vials prior to handling. Store syringes in a safe manner to prevent accidental punctures Always work in approved hood wearing appropriate PPE: lab coat, safety glasses/goggles, gloves suitable for chemicals in use. Some procedures may require using a blast shield
3. Extract removal from refrigerators	(E) – Wet and cold temperatures	Medium	Gloves

	(CW) – Glass shards from broken sample vials; unknown samples and sample preservatives	Medium	Inspect extracts for hazards prior to removing contents
4. Instrumental Analysis	(E) – Compressed gases and cryogenics;	High	Proper storage/use of gas cylinder & use proper technique when transporting cylinders. Use only compatible regulators with proper fittings. Test all connections for leaks. Wear thermal gloves when handling liquid nitrogen.
	(CW) - burns from heated instrument zones.	Medium	Cool heated zones prior to performing instrument maintenance.
	(E) – Solvents, hazardous waste, used pump oil	Medium	Always work in approved appropriate PPE: lab coat, safety glasses, gloves suitable for chemicals in use.
	(CBT) – Pinch points on autosampler when moving	Low	Ensure areas are clear of body parts and/or obstructions in swing radius of autosampler arm.
	(CW) – shock hazard from electrical components	Low	Ensure instruments are turned off and unplugged when performing any work which may result in a shock hazard, such as board replacement or repair.

* Codes for Potential Hazards

(BIO) Biological		(CO) Caught On		(FS) Fall – Same Level
(CB) Contacted By		(CW) Contact With		(OE) Overexertion
(CBT) Caught Between		(E) Exposure		(SA) Struck Against
(CI) Caught In		(FB) Fall To Below		(SB) Struck By

Risk Severity Level Key	Low	Medium	High	Very High

Appendix B: SPE-03 Instrument Cleaning Procedure

Pre/Post Instrument Cleaning

1. Load the empty cleaning sample bottles onto all 8 bottle lines of the SPE, making sure to turn the bottle not the cap when screwing them together.

2. Puncture holes (if not already punctured) in the bottom of the inverted 250 mL polypropylene sampling bottles.

3. Wipe each SPE cartridge adapter down with a kimwipe and methanol.

4. Insert SPE cartridge adapters into an empty and clean SPE cartridge and place on the SPE instrument.

5. Check solvent levels (Methanol in Solvent 1 position) to ensure adequate volumes, empty waste containers if needed, and turn on N2.

6. Bring up the "*Clean_Sys*" method, and run.

7. After the cleaning run (roughly 10 minutes), remove the cleaning bottles and put aside for reuse in instrument cleaning.

"Clean_Sys" method steps.

Action	Inlet 1	Inlet 2 (ratio)	Flow (mL/min)	Volume (mL)
Clean	Solvent 1		20	6.2
Clean	Sample		20	5.2
Clean	Sample		20	5.2
Add Samp W2	Sample		10	9.0
Clean	Solvent 1		20	6.2
Clean	Sample		20	5.2
Clean	Sample		20	5.2
Add Samp W2	Sample		10	9.0
Air Purge	Air		10	0.1

Appendix C: SPE-03 533 Method Parameters

"EPA 533" Method Steps

Action	Inlet 1	Inlet 2 (ratio)	Flow (mL/min)	Volume (mL)
Elute W2	Solvent 1		10	10
Elute W1	Solvent 3		10	10
Elute W1	Solvent 3		10	3
Elute W1	Solvent 2		10	3
Add Sample W1	Sample		5	270
Rinse W1	Solvent 4		5	10
Clean	Solvent 1		5	1
Add Sample W2	Sample		5	9
Blow N2	-			Timed - 5min
Rinse 1	Solvent 5		2	5
Rinse 1	Solvent 5		2	5
Collect 1	Sample		2	4.5

Note: W1/W2 are referring to Waste 1 (Aqueous Waste) and Waste 2 (Solvent Waste) lines.

"*EPA 533*" solvent list

Solvent Position	Solvent Name
Solvent 1	Methanol
Solvent 2	DIW
Solvent 3	Aqueous 0.1M Phosphate Buffer
Solvent 4	1g/L Ammonium Acetate in DIW
Solvent 5	Methanol with 2% Ammonium Hydroxide

Appendix D: XEVO TQS Method Parameters

Inlet Parameters		
Mobile phase A:	20mM Ammonium Acetate	
Mobile phase B:	Methanol	
Column:	Waters Acquity UPLC BEH C18, 1.7 µm, 2.1 mm X 50 mm	
Column Temp:	40°C	
Run Time:	30 minutes	
HPLC Gradient		
Time (min)	Flow Rate (mL/min)	%B
initial	0.25	5
0.50	0.25	5
3.0	0.25	40
16	0.25	80
18	0.25	80
20	0.25	95
22	0.25	95
25	0.25	5
35	0.25	5
Autosampler Parameters		
Sample Loop:	50 µL	
Sample Syringe:	100 µL	
Injection Volume:	10 µL	
Injection Mode:	Loop Offline unchecked	
Autosampler Temp:	15 °C	
Post-Injection Wash:	6 sec	
MS Conditions		
Ionization:	ESI-	
Mode:	MRM	
Capillary Voltage:	1.5 kV	
Source Temp:	150 °C	
Desolvation Temp:	350 °C	

Cone Gas Flow:		150 L/Hr		
Desolvation Gas Flow:		650 L/Hr		
Collision Gas Flow:		0.25 mL/Min		
Quadrupole 1 parameters:		LM 1 resolution:	2.9	
		HM 1 resolution:	15.0	
		Ion energy:	0.5	
Quadrupole 2 parameters:		LM 2 resolution:	2.9	
		HM 2 resolution:	14.6	
		Ion energy:	1.1	
Collision Cell parameters:		Entrance:	1	
		Exit:	1	
MRM Transitions				
All Functions: Span (Da):		0.00		
All Functions: Delay (secs):		Auto		
All Functions: Method Events[v] (options menu):		0.00 Flow State	LC	
		0.00 Flow State	Waste	
Function 1: Start and End Time (min):		2.75 to 4.5		
Compound	Reaction	Dwell(s)	Cone V	Collision Energy
PFBA	212.9 > 169	0.025	22	10
13C3-PFBA	216 > 171.9	0.025	22	10
13C4-PFBA	217 > 172	0.025	22	10
Function 2: Start and End Time (min):		3.5 to 5.0		
Compound	Reaction	Dwell(s)	Cone V	Collision Energy
PFMPA	228.97 > 84.76	0.025	10	12
Function 3: Start and End Time (min):		4.75 to 6.5		
Compound	Reaction	Dwell(s)	Cone V	Collision Energy
PFPeA	263 > 218.8	0.025	20	8
13C5-PFPeA	334 > 156	0.025	20	8
Function 4: Start and End Time (min):		5.5 to 6.75		
Compound	Reaction	Dwell(s)	Cone V	Collision Energy
PFMBA	278.97 > 84.76	0.025	10	16
PFBS	298.9 > 79.73	0.025	8	30
13C3-PFBS	301.9 > 79.70	0.025	20	28

Function 5: Start and End Time (min):		7.0 to 9.0		
Compound	Reaction	Dwell(s)	Cone V	Collision Energy
HFPO-DA	285.10 > 168.8	0.025	15	5
13C3-HFPO-DA	287 > 168.9	0.025	15	5
PFPeS	348.9 > 79.74	0.025	10	30
Function 6: Start and End Time (min):		6.5 to 8.5		
Compound	Reaction	Dwell(s)	Cone V	Collision Energy
NFDHA	294.84 > 201	0.025	14	8
4:2 FTS	326.97 > 307.00	0.025	40	18
13C2-4:2FTS	328.97 > 80.40	0.025	8	26
Function 7: Start and End Time (min):		6.5 to 8.5		
Compound	Reaction	Dwell(s)	Cone V	Collision Energy
PFHxA	313.0 > 268.8	0.025	20	8
13C5-PFHxA	317.97 > 273.00	0.025	20	8
Function 8: Start and End Time (min):		5.5 to 8.0		
Compound	Reaction	Dwell(s)	Cone V	Collision Energy
PFEESA	314.9 > 134.8	0.025	44	22
Function 9: Start and End Time (min):		9.0 to 10.5		
Compound	Reaction	Dwell(s)	Cone V	Collision Energy
PFHpA	362.97 > 319.00	0.025	15	8
13C4-PFHpA	366.97 > 321.90	0.025	15	8
Function 10: Start and End Time (min):		8.75 to 11.0		
Compound	Reaction	Dwell(s)	Cone V	Collision Energy
ADONA	376.97 > 250.91	0.025	14	12
PFHxS	398.9 > 79.73	0.025	34	32
13C3-PFHxS	401.90 > 79.74	0.025	36	34
Function 11: Start and End Time (min):		11.0 to 12.5		
Compound	Reaction	Dwell(s)	Cone V	Collision Energy
PFOA	412.97 > 369.00	0.025	18	10
13C2-PFOA	414.97 > 370.00	0.025	18	10
13C8-PFOA	420.97 > 376.00	0.025	18	10
Function 12: Start and End Time (min):		11.0 to 12.5		
Compound	Reaction	Dwell(s)	Cone V	Collision Energy

6:2FTS	426.97 > 406.95	0.025	42	22
13C2-6:2FTS	428.97 > 408.95	0.025	46	22
Function 13: Start and End Time (min):		11.0 to 12.5		
Compound	Reaction	Dwell(s)	Cone V	Collision Energy
PFHpS	448.9 > 79.74	0.025	24	40
Function 14: Start and End Time (min):		12.5 to 14.0		
Compound	Reaction	Dwell(s)	Cone V	Collision Energy
PFNA	462.97 > 419.00	0.025	17	10
13C9-PFNA	471.97 > 427.00	0.025	17	10
Function 15: Start and End Time (min):		12.0 to 14.0		
Compound	Reaction	Dwell(s)	Cone V	Collision Energy
PFOS	498.90 > 79.73	0.025	45	44
13C4-PFOS	502.90 > 79.70	0.025	34	46
13C8-PFOS	506.90 > 79.76	0.025	45	46
Function 16: Start and End Time (min):		13.5 to 15.0		
Compound	Reaction	Dwell(s)	Cone V	Collision Energy
PFDA	512.97 > 469.00	0.025	22	10
13C6-PFDA	471.97 > 427.00	0.025	22	10
Function 17: Start and End Time (min):		13.5 to 15.0		
Compound	Reaction	Dwell(s)	Cone V	Collision Energy
8:2FTS	526.97 > 506.99	0.025	28	26
13C2-8:2FTS	528.97 > 508.99	0.025	46	26
Function 18: Start and End Time (min):		13.25 to 14.5		
Compound	Reaction	Dwell(s)	Cone V	Collision Energy
9Cl-PF3ONS	530.9 > 350.93	0.025	10	24
Function 19: Start and End Time (min):		15.0 to 16.25		
Compound	Reaction	Dwell(s)	Cone V	Collision Energy
PFUdA	562.97 > 519.00	0.025	24	10
13C7-PFUdA	569.97 > 525.00	0.025	24	10
Function 20: Start and End Time (min):		16.0 to 17.25		
Compound	Reaction	Dwell(s)	Cone V	Collision Energy
PFDoA	612.97 > 569.00	0.025	22	10
13C2-PFDoA	614.97 > 570.00	0.025	22	10

Function 21: Start and End Time (min):		15.5 to 17.0		
Compound	Reaction	Dwell(s)	Cone V	Collision Energy
11Cl-PF3OUdS	530.9 > 350.93	0.025	10	24

Appendix E: Method Analytes, Retention Times and Isotope Dilution Analogue References.

Analyte Name	RT (minutes)	Labeled Reference
Isotope Performance Standard		
13C3-PFBA	3.9	N/A
13C2-PFOA	11.7	N/A
13C4-PFOS	13.3	N/A
Isotopically Labeled Analyte		
13C4-PFBA	3.9	13C3-PFBA
13C5-PFPeA	5.8	13C3-PFBA
13C3-PFBS	6.3	13C4-PFOS
13C2-4:2FTS	7.7	13C4-PFOS
13C5-PFHxA	7.9	13C2-PFOA
13C3-HFPO-DA	8.5	13C2-PFOA
13C4-PFHpA	9.9	13C2-PFOA
13C3-PFHxS	10.2	13C4-PFOS
13C2-6:2FTS	11.6	13C4-PFOS
13C8-PFOA	11.7	13C2-PFOA
13C9-PFNA	13.2	13C2-PFOA
13C8-PFOS	13.3	13C4-PFOS
13C2-8:2FTS	14.5	13C4-PFOS
13C6-PFDA	14.5	13C2-PFOA
13C7-PFUnA	15.7	13C2-PFOA
13C2-PFDoA	16.7	13C2-PFOA
Native Analyte		
PFBA	3.9	13C4-PFBA
PFMPA	4.6	13C4-PFBA
PFPeA	5.8	13C5-PFPeA
PFBS	6.3	13C3-PFBS
PFMBA	6.3	13C5-PFPeA
PFEESA	6.9	13C3-PFBS

NFDHA	7.4	13C5-PFHxA
4:2FTS	7.6	13C2-4:2FTS
PFHxA	7.9	13C5-PFHxA
PFPeS	8.1	13C3-PFHxS
HFPO-DA	8.5	13C3-HFPO-DA
PFHpA	9.9	13C4-PFHpA
PFHxS (branched)	9.4-10.1	13C3-PFHxS
ADONA	10.0	13C4-PFHpA
6:2FTS	11.6	13C2-6:2FTS
PFOA	11.7	13C8-PFOA
PFHpS	11.7	13C8-PFOS
PFNA	13.2	13C9-PFNA
PFOS (branched)	12.5-13.3	13C8-PFOS
9Cl-PF3ONS	13.9	13C8-PFOS
8:2 FTS	14.5	13C2-8:2FTS
PFDA	14.5	13C6-PFDA
PFUnA	15.7	13C7-PFUnA
11Cl-PF3OUdS	16.1	13C8-PFOS
PFDoA	16.7	13C2-PFDoA