Title: Per- and Polyfluorinated Alkyl Substances (PFAS) in Water, Soils, Sediments and Tissue

[Method 537 (Modified), Method PFAS by LCMSMS Compliant with QSM Table B-15, Revision 5.3 and higher]

Approvals (Signature/Date):

01/27/2021	01/27/2021
Robert Hrabak Date	Joe Schairer Date
Technical Manager	Health & Safety Manager / Coordinator
01/27/2021	01/27/2021
Lisa Stafford Date	Chris Williams Date
Quality Assurance Manager	Laboratory Manager

Facility Distribution No. Uncontrolled **Distributed To:** Sacramento QA

1. SCOPE AND APPLICATION

1.1. This procedure describes the analysis of water, soil, sediment, and tissue samples for the following compounds using liquid chromatography / tandem mass spectrometry (LC/MS/MS).

Table 1.1
PFAS Supported

Compound Name	Abbreviations	CAS #
Perfluoroalkylcarboxylic acids (PFCAs)		
Perfluoro-n-butanoic acid	PFBA	375-22-4
Perfluoro-n-pentanoic acid	PFPeA	2706-90-3
Perfluoro-n-hexanoic acid	PFHxA	307-24-4
Perfluoro-n-heptanoic acid	PFHpA	375-85-9
Perfluoro-n-octanoic acid	PFOA	335-67-1
Perfluoro-n-nonanoic acid	PFNA	375-95-1
Perfluoro-n-decanoic acid	PFDA	335-76-2
Perfluoro-n-undecanoic acid	PFUdA, PFUnA	2058-94-8
Perfluoro-n-dodecanoic acid	PFDoA	307-55-1
Perfluoro-n-tridecanoic acid	PFTrDA	72629-94-8
Perfluoro-n-tetradecanoic acid	PFTeDA, PFTA	376-06-7
Perfluorinated sulfonic acids (PFSAs)		
Perfluoro-1-butanesulfonic acid	PFBS	375-73-5
Perfluoro-1-pentanesulfonic acid	PFPeS	2706-91-4
Perfluoro-1-hexanesulfonic acid	PFHxS	355-46-4
Perfluoro-1-heptanesulfonic acid	PFHpS	375-92-8
Perfluoro-1-octanesulfonic acid	PFOS	1763-23-1
Perfluoro-nonanesulfonic acid	PFNS	68259-12-1
Perfluoro-1-decanesulfonic acid	PFDS	335-77-3
Perfluoro-1-dodecansulfonic acid	PFDoS	79780-39-5
Perfluorinated sulfonamides (FOSA)		
Perfluoro-1-octanesulfonamide	PFOSA, FOSA	754-91-6
N-ethylperfluoro-1-octanesulfonamide	Et-FOSA, N-Et-FOSA	4151-50-2
N-methylperfluoro-1-octanesulfonamide	Me-FOSA, N-Me-FOSA	31506-32-8
Perfluorinated sulfonamide ethanols (FOSE)		
2-(N-ethylperfluoro-1-octanesulfonamido) ethanol	Et-FOSE, N-Et-FOSE	1691-99-2
2-(N-methylperfluoro-1-octanesulfonamido) ethanol	Me-FOSE, N-Me-FOSE	24448-09-7
Perfluorinated sulfonamidoacetic acids (FOSAA)		
N-ethylperfluoro-1-octanesulfonamidoacetic acid	EtFOSAA	2991-50-6

Table 1.1 PFAS Supported		
Compound Name	**Abbreviations**	**CAS #**
	N-EtFOSAA	
N-methylperfluoro-1-octanesulfonamidoacetic acid	MeFOSAA, N-MeFOSAA	2355-31-9
Fluorotelomer sulfonates (FTS)		
1H,1H,2H,2H-perfluorohexane sulfonic acid *(4:2)*	4:2 FTS	757124-72-4
1H,1H,2H,2H-perfluorooctane sulfonic acid *(6:2)*	6:2 FTS	27619-97-2
1H,1H,2H,2H-perfluorodecane sulfonic acid *(8:2)*	8:2 FTS	39108-34-4
1H,1H,2H,2H-perfluorododecane sulfonic acid *(10:2)*	10:2 FTS	120226-60-0
Fluorinated Replacement Chemicals		
4,8-dioxa-3H-perfluorononanoic acid	DONA, ADONA(1)	919005-14-4
Perfluoro(2-propoxypropanoic) acid or Hexafluoropropylene oxide dimer acid	HFPO-DA, GenX	13252-13-6
F53B (reported as the summation of the following)	F53B	NA
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	F53B major, 9Cl-PF3ONS	756426-58-1
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	F5B minor, 11Cl-PF3OUdS	763051-92-9

***Note**: Abbreviations in parenthesis are the abbreviations listed in Method 537/537.1, where they differ from the abbreviation used by the laboratory's LIMS.*

(1) In some literature, the acronym ADONA refers to the ammonium salt, CAS 958445-44-8, and DONA refers to the parent acid. In Method 537.1, ADONA refers to the parent acid. DONA is the acronym present on the laboratory raw data.

1.2. Additional analytes supported by this method: The following analytes can be supported by this method under special request.

Table 1.2 Additional Compounds		
Compound Name	**Abbreviation**	**CAS #**
Perfluoroalkylcarboxylic acids (PFCAs)		
Perfluoro-n-hexadecanoic acid	PFHxDA	67905-19-5
Perfluoro-n-octadecanoic acid	PFODA	16517-11-6
Perfluorinated sulfonic acids (PFSAs)		
Perfluoro-4-ethylcyclohexanesulfonic acid	PFECHS	133201-07-7
Perfluoropropanesulfonic acid	PFPrS	423-41-6
Fluorotelomer carboxylic acids (FTCA)		
3-Perfluoropropylpropanoic acid	3:3 FTCA	356-02-5
3-Perfluoropentylpropanoic acid	5:3 FTCA	914637-49-3
3-Perfluoroheptylpropanoic acid	7:3 FTCA	812-70-4
2-Perfluorohexylethanoic acid	6:2 FTCA	53826-12-3
2-Perfluorooctylethanoic acid	8:2 FTCA, FOEA	27854-31-5

Table 1.2 Additional Compounds		
Compound Name	**Abbreviation**	**CAS #**
2-Perfluorodecylethanoic acid	10:2 FTCA	53826-13-4
Fluorotelomer unsaturated carboxylic acids (FTUCA)		
2H-Perfluoro-2-octenoic acid	6:2 FTUCA	70887-88-6
2H-Perfluoro-2-decenoic acid	8:2 FTUCA	70887-84-2
2H-Perfluoro-2-dodecenoic acid	10:2 FTUCA	70887-94-4
Short Chain		
Perfluoropropionic acid (PPF Acid)	PFPrA, PPF Acid	422-64-0
Perfluoro-3-methoxypropanoic acid (PFMPA)	PFECA F, PFMPA, PFMOPrA	377-73-1
Perfluoro-4-methoxybutanoic acid (PFMBA)	PFECA A, PFMBA, PFMOBA	863090-89-5
Nonafluoro-3,6-dioxaheptanoic acid (NFDHA)	PFECA B, NFDHA	151772-58-6
Perfluoro(2-ethoxyethane) sulfonic acid (PFEESA)	PES, PFEESA	113507-82-7
Difluoro(perfluoromethoxy)acetic acid	PFMOAA	674-13-5
Perfluoro-4-isopropoxybutanoic acid	PFECA G, PFPE-1	801212-59-9
Perfluoro-3,5,7,9-butaoxadecanoic acid	PFO4DA	39492-90-5
Perfluoro-3,5,7-trioxaoctanoic acid	PFO3OA	39492-89-2
Perfluoro-3,5-dioxahexanoic acid	PFO2HxA	39492-88-1
Perfluoro-3,6-dioxa-4-methyl-7-octene-1-sulfonic acid	PFESA BP 1 PS Acid	29311-67-9
Perfluoro-2-{[perfluoro-3-(perfluoroethoxy)-2-propanyl]oxy}ethanesulfonic acid	PFESA BP 2 Hydro-PS Acid	749836-20-2
Perfluoro-3,5,7,9,11-pentaoxadodecanoic acid	PFO5DA, PFO5DoA, TAF	39492-91-6
Perfluoro-2-(perfluoromethoxy)propanoic acid	PMPA	13140-29-9
2,3,3,3-Tetrafluoro-2-(pentafluoroethoxy)propanoic acid	PEPA	267239-61-2
3-(Methoxy)tetrafluoropropionic acid	MTP	93449-21-9
4-(2-carboxy-1,1,2,2-tetrafluoroethoxy)-2,2,3,3,4,5,5,5-octafluoro-pentanoic acid	R-EVE	2416366-22-6
2,2,3,3-Tetrafluoro-3-{[1,1,1,2,3,3-hexafluoro-3-(1,2,2-trifluoroethenoxy)propan-2-yl]oxy}propanoic acid	EVE Acid	69087-46-3
1,1,2,2,4,5,5,5-heptafluoro-3-oxapentanesulfonic acid	NVHOS	1132933-86-8(2)
2,2,3,3-Tetrafluoro-3-{[1,1,1,2,3,3-hexafluoro-3-(1,2,2,2-tetrafluoroethoxy)propan-2-yl]oxy}propanoic acid	Hydro-EVE Acid	773804-62-9
Ethanesulfonic acid, 1,1,2,2-tetrafluoro-2-[1,2,2,3,3-pentafluoro-1-(trifluoromethyl)propoxy]-	Byproduct 6, R-PDSCA	2416366-21-5

(2) The CAS Number listed for NVHOS is for the sodium salt. As of this writing, there isn't a CAS number for the parent acid. The laboratory performs analysis for the sulfonic acid.

1.3. The working range of the method is listed below. The linear range can be extended by diluting the extracts.

Table 1.3
Reporting Limits and Working Range

Matrix	Nominal Sample Size	Reporting Limit	Working Range
Water	250 mL	2.0 ng/L – 20 ng/L	2.0 ng/L - 800 ng/L
Soil/Sediment	5 g	0.2 µg/kg – 1.0 µg/kg	0.2 µg/kg - 50 µg/kg
Tissue	1 g	0.4 µg/kg – 2.0 µg/kg	0.4 µg/kg – 100 µg/kg

1.4. This procedure also includes direction for preparing samples to determine "Total Oxidizable Precursors", which may assist in improving understanding of potential PFAS environmental risk.

1.5. When undertaking projects for the Department of Defense (DoD) and/or the Department of Energy (DOE) the relevant criteria in QA Policy WS-PQA-021, "Federal Program Requirements" must be checked and incorporated.

1.6. Many jurisdictions have additional requirements for PFAS analysis, such as different holding times, preservation, or calibration criteria. These are detailed in the document WS-WI-0066, "Agency Specific Criteria for PFAS in Matrices Other Than Drinking Water".

2. SUMMARY OF METHOD

2.1. Water samples are extracted using a solid phase extraction (SPE) cartridge. PFAS are eluted from the cartridge with an [redacted] solution.

2.2. Soil/sediment/tissue samples are extracted with a KOH/methanol solution using sonication for 1 hour. The mixture is centrifuged and the solvent filtered.

2.3. The final 80:20 methanol:water extracts are analyzed by LC/MS/MS. PFAS are separated from other components on a C18 column with a solvent gradient program using [redacted] The mass spectrometer detector is operated in the electrospray (ESI) negative ion mode for the analysis of PFAS.

2.4. An isotope dilution technique is employed with this method for the compounds of interest. The isotope dilution analytes (IDA) consist of carbon-13 labeled analogs, oxygen-18 labeled analogs, or deuterated analogs of the compounds of interest, and they are fortified into the samples at the time of extraction. This technique allows for the correction for analytical bias encountered when analyzing more chemically complex environmental samples. The isotopically labeled compounds are chemically similar to the compounds of concern and are therefore affected by sample-related

interferences to the same extent as the compounds of concern. Compounds that do not have an identically labeled analog are quantitated by the IDA method using a closely related labeled analog.

2.5. Quantitation by the internal standard method is employed for the IDA analytes/recoveries. Peak response is measured as the area of the peak.

2.6. Samples for the "Total Oxidizable Precursor" assay (TOP) are analyzed in two phases – an aliquot is prepared and analyzed as a normal sample, and a second aliquot is subjected to [redacted] prior to solid phase extraction and analysis. The total perfluorocarboxylic acid value is determined for each aliquot, and the difference calculated.

3. DEFINITIONS

3.1. PFCAs: Perfluorocarboxylic acids

3.2. PFSAs: Perfluorinated sulfonic acids

3.3. FOSA: Perfluorinated sulfonamide

3.4. PFOA: Perfluorooctanoic acid

3.5. PFOS: Perfluorooctane sulfonic acid

3.6. PTFE: Polytetrafluoroethylene (e.g. Teflon®)

3.7. SPE: Solid phase extraction

3.8. PP: Polypropylene

3.9. PE: Polyethylene

3.10. HDPE: High density polyethylene

3.11. AFFF: Aqueous Film Forming Foam

3.12. IDA: Isotope dilution analyte

3.13. Further definitions of terms used in this SOP may be found in the glossary of the Laboratory Quality Assurance Manual (QAM).

4. INTERFERENCES

4.1. PFAS have been used in a wide variety of manufacturing processes, and laboratory supplies should be considered potentially contaminated until they have been tested and shown to be otherwise. The materials and supplies used during the method validation process have been tested and shown to be clean. These items are listed below in Section 6.

4.2. To avoid contamination of samples, standards are prepared in a ventilation hood in an area separate from where samples are extracted.

4.3. PTFE products can be a source of PFOA contamination. The use of PTFE in the procedure should be avoided or at least thoroughly tested before use. Polypropylene (PP) or polyethylene (PE, HDPE) products may be used in place of PTFE products to minimize PFOA contamination.

4.3.1. Standards and samples are injected from polypropylene autosampler vials with polypropylene screw caps once. Multiple injections may be performed on Primers when conditioning the instrument for analysis.

4.3.2. Random evaporation losses have been observed with the polypropylene caps causing high IDA recovery after the vial was punctured and sample re-injected. For this reason, it is best to inject standards and samples once in the analytical sequence.

4.3.3. Teflon-lined screw caps have detected PFAS at low concentrations. Repeated injection from the same Teflon-lined screw cap have detected PFNA at increasing concentration as each repeated injection was performed, therefore, it is best to use polypropylene screw caps.

4.4. Volumetric glassware and syringes are difficult to clean after being used for solutions containing high levels of PFOA. These items should be labeled for use only with similarly concentrated solutions or verified clean prior to re-use. To the extent possible, disposable labware is used.

4.5. Both branched and linear PFAS isomers can potentially be found in the environment. Linear and branched isomers are known to exist for PFOS, PFOA, PFHxS, PFBS, Et-FOSAA, and Me-FOSAA based upon the scientific literature. If multiple isomers are present for one of these PFAS they might be adjacent peaks that completely resolve or not, but usually with a deflection point resolved during peak integration. The later of these peaks matches the retention time of its labeled linear analog. In general, earlier peaks are the branched isomers and are not the result of peak splitting.

As of this writing, only PFOS, PFOA, PFHxS, Et-FOSAA and Me-FOSAA are commercially available as technical mixtures. These reference standards of the technical mixtures for these specific PFAS are used to ensure that all appropriate peaks are included during peak integration.

4.6. In an attempt to reduce PFOS bias, it is required that m/z 499>80 transition be used as the quantitation transition.

4.7. Per the Certificate of Analysis for labeled perfluorohexadecanoic acid ($13C_2$-PFHxDA) produced by Wellington Laboratories, the stock standard contains roughly 0.3% of native perfluorohexadecanoic acid. This equates to roughly 0.15 ng/L or 0.0075 ug/kg of perfluorohexadecanoic acid expected in all samples and blanks.

4.8. Aluminum foil should not be used for this analysis due to the potential interferences from the PFAS used as release agents.

5. SAFETY

Employees must abide by the policies and procedures in the Corporate Safety Manual, Sacramento Supplement to the CSM, and this document. All work must be stopped in the event of a known or potential compromise to the health or safety of an associate. The situation must be reported **immediately** to a supervisor, the EH&S Staff, or a senior manager.

5.1. Specific Safety Concerns

5.1.1. Preliminary toxicity studies indicate that PFAS could have significant toxic effects. In the interest of keeping exposure levels as low as reasonably achievable, PFAS and PFAS samples must be handled in the laboratory as hazardous and toxic chemicals.

5.1.2. Exercise caution when using syringes with attached filter disc assemblies. Application of excessive force has, upon occasion, caused a filter disc to burst during the process.

5.1.3. Laboratory procedures such as repetitive use of pipets, repetitive transferring of extracts and manipulation of filled separatory funnels and other glassware represent a significant potential for repetitive motion or other ergonomic injuries. Laboratory associates performing these procedures are in the best position to realize when they are at risk for these types of injuries. Whenever a situation is found in which an employee is performing the same repetitive motion, the employee shall immediately bring this to the attention of their supervisor, manager, or the EH&S staff. The task will be analyzed to determine a better means of accomplishing it.

5.1.4. Eye protection that satisfies ANSI Z87.1 (as per the Eurofins TestAmerica Safety Manual), laboratory coat, and nitrile gloves must be worn while handling samples, standards, solvents, and reagents. Disposable gloves that have been contaminated will be removed and discarded; other gloves will be cleaned immediately.

5.1.5. Perfluorocarboxylic acids are acids and are not compatible with strong bases.

5.1.6. The use of vacuum systems presents the risk of imploding glassware. All glassware used during vacuum operations must be thoroughly inspected prior to each use. Glass that is chipped, scratched, cracked, rubbed, or marred in any manner must not be used under vacuum. It must be removed from service and replaced.

5.1.7. Glass containers are not to be used for "tumbling" soil samples.

5.2. Primary Materials Used

The following is a list of the materials used in this method, which have a serious or significant hazard rating. NOTE: This list does not include all materials used in the method. The table contains a summary of the primary hazards listed in the SDS for each of the materials listed in the table. A complete list of materials used in the method can be found in the reagents and materials section. Employees must review the information in the SDS for each material before using it for the first time or when there are major changes to the SDS.

Material[1]	**Hazards**	**Exposure Limit[2]**	**Signs and Symptoms of Exposure**
Acetic Acid (3-2-1)	Corrosive Poison Flammable	10 ppm-TWA 15 ppm-STEL	Contact with concentrated solution may cause serious damage to the skin and eyes. Inhalation of concentrated vapors may cause serious damage to the lining of the nose, throat, and lungs. Breathing difficulties may occur.
[redacted] (3-1-0)	Corrosive Poison	50 ppm-TWA	Severe irritant. Effects from inhalation of dust or mist vary from mild irritation to serious damage to the upper respiratory tract. Symptoms may include sneezing, sore throat or runny nose. Contact with skin can cause irritation or severe burns and scarring with greater exposures. Causes irritation of eyes, and with greater exposures it can cause burns that may result in permanent damage, including blindness. Brief exposure to 5000 PPM can be fatal.
Hexane (3-3-1)	Flammable Irritant	50 ppm PEL	Inhalation of vapors irritates the respiratory tract. Overexposure may cause lightheadedness, nausea, headache, and blurred vision. Vapors may cause irritation to the skin and eyes.

Material[1]	Hazards	Exposure Limit[2]	Signs and Symptoms of Exposure
Hydrochloric Acid (3-0-1)	Corrosive Poison	5 ppm (Ceiling)	Can cause pain and severe burns upon inhalation, ingestion, eye or skin contact. Exposure to concentrated solutions may cause deep ulcerations to skin, permanent eye damage, circulatory failure and swallowing may be fatal.
Methanol (2-3-0)	Flammable Poison Irritant	200 ppm PEL 250 ppm STEL	A slight irritant to the mucous membranes. Toxic effects exerted upon nervous system, particularly the optic nerve. Symptoms of overexposure may include headache, drowsiness and dizziness. Methyl alcohol is a defatting agent and may cause skin to become dry and cracked. Skin absorption can occur; symptoms may parallel inhalation exposure. Irritant to the eyes.
Potassium Hydroxide (3-0-1)	Corrosive Poison	2 mg/m^3 (Ceiling)	Severe irritant. Can cause severe burns upon inhalation, ingestion, eye or skin contact. Exposure to concentrated solutions may cause severe scarring of tissue, blindness, and may be fatal if swallowed.
[redacted] (2-0-1-OX)	Oxidizer	None	Causes irritation to the respiratory tract. Symptoms may include coughing, shortness of breath. Causes irritation to skin and eyes. Symptoms include redness, itching, and pain. May cause dermatitis, burns, and moderate skin necrosis.
Sodium Hydroxide (3-0-1)	Corrosive Poison	2 mg/m^3 (Ceiling)	Severe irritant. Can cause severe burns upon inhalation, ingestion, eye or skin contact. Exposure to concentrated solutions may cause severe scarring of tissue, blindness, and may be fatal if swallowed.

(1) Always add acid to water to prevent violent reactions.
(2) Exposure limit refers to the OSHA regulatory exposure limit.

6. EQUIPMENT AND SUPPLIES

Due to the ubiquitous nature of PFAS, all disposable equipment (including, but not limited to vials, pipet tips, and SPE manifold parts) that directly contacts a sample or extract is subject to QC checks on a by-lot basis prior to use. At a minimum, the QC checks include either a rinse with DI water or an extraction with basic methanol to mimic the usage encountered during sample preparation. QC check data is kept on file for reference as needed

6.1. 15 mL polypropylene test tubes with polypropylene screw caps.

6.2. 50 mL graduated plastic centrifuge tubes.

6.3. 125 mL HDPE bottles with HDPE screw caps.

6.4. 250 mL HDPE bottles with HDPE screw caps. The average weight of the HDPE bottles with HDPE screw caps are calibrated once per year. The calibration is

performed by weighing 10 bottles with caps and dividing by 10 to get the average weight. The average weight is used in section (11.3.5.1 Step 4).

6.5. Analytical balance capable of accurately weighing to the nearest 0.0001g, and checked for accuracy each day it is used in accordance with WS-QA-0041.

6.6. Extract concentrator or nitrogen manifold with water bath heating to 65°C.

6.7. Syringe filter, Millipore Millex-HV 0.45 um, or equivalent. Do not use PTFE type filters.

6.8. 300 µL autosampler vials, polypropylene, with polypropylene screw caps, Waters PN 1860004112, or equivalent.

6.9. SPE columns

6.9.1. [redacted] or equivalent for the TOP assay.

6.9.2. [redacted] or equivalent. This cartridge incorporates a graphitized carbon.

6.10. Graphitized carbon (Envi-Carb™ or equivalent).

6.11. Vacuum manifold for Solid Phase Extraction (SPE).

6.12. Miscellaneous laboratory apparatus (beakers, test tubes, volumetric flasks, pipettes, etc.). These should be disposable where possible, or marked and segregated for high-level versus low-level use.

6.13. Water bath: Heated with concentric ring cover capable of temperature control (±5°C) up to 95°C. The bath must be used in a fume hood.

6.14. Plastic tub for an ice bath, AKRO-N.S.T. part No. 35-180 or equivalent.

6.15. pH indicator paper, wide range.

6.16. Bottle rotating apparatus for soil extractions.

6.17. Glass fiber filter, Whatman GF/F, catalog number 1825 090 or equivalent. Filters, if used, are QC checked by lot by extraction with basic methanol. The filters must be clean to less than ½ RL before they can be used, and the data kept on file.

6.18. Liquid Chromatography/Tandem Mass Spectrometer (LC/MS/MS) –The instrument described below, or equivalent, may be used for this method. The HPLC is equipped

with a refrigerated autosampler, an injection valve, and a pump capable of variable flow rate. The use of a column heater is required to maintain a stable temperature throughout the analytical run. Data is processed using Chrom Peak Review, version 2.3 or equivalent. The MS/MS is capable of running in the NI-ESI mode at the recommended flow rate with a minimum of 10 scans per peak.

6.18.1. [redacted] LC/MS/MS

This system consists of a Shimadzu HPLC interfaced with a [redacted] Triple Quad MS, or equivalent. The instrument control and data acquisition software is [redacted] version 1.6.3 or equivalent.

6.18.1.1. [redacted] HPLC equipped with [redacted] pumps and one DGU-20 degassing unit or equivalent.

6.18.1.2. [redacted]

6.18.1.3. PFAS Isolator column, [redacted] This is plumbed between the UPLC pumps and autosampler valve to minimize PFAS background from the UPLC solvent lines and filters.

6.19. Preventive and routine maintenance is described in the table below

Table 6.19
HPLC/MS/MS Preventative Maintenance

As Needed:	**Daily (When in use)**
Change pump seals. Change in-line filters in autosampler (HPLC). Check/replace in-line frit if excessive pressure or poor performance. Replace column if no change following in-line frit change. Clean corona needle. Replace sample inlet tube in APCI (10.1 cm). Replace fused silica tube in ESI interface. Clean lenses. Clean skimmer. Ballast rough pump 30 minutes. Create all eluents in Reagent module, label eluent containers with TALS label and place 2nd label into maintenance log when put into use.	Check solvent reservoirs for sufficient level of solvent. Verify that pump is primed, operating pulse free. Check needle wash reservoir for sufficient solvent. Verify capillary heater temperature functioning. Verify vaporizer heater temperature. Verify rough pump oil levels. Verify turbo-pump functioning. Verify nitrogen pressure for auxiliary and sheath gasses. Verify that corona and multiplier are functioning.
Semi-Annually	**Annually**

Table 6.19 HPLC/MS/MS Preventative Maintenance	
Replace rough-pump oil (4-6 months). Replace oil mist and odor elements. Replace activated alumina filter if applicable	Vacuum system components including fans and fan covers. Clean/replace fan filters, if applicable.

7. REAGENTS AND STANDARDS

7.1. Reagent grade chemicals shall be used in all tests whenever available. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on the Analytical Reagents of the American Chemical Society, where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

7.1.1. Acetic acid, glacial

7.1.2. [redacted] This solution has volatile components, thus it should be replaced every 7 days or sooner.

7.1.3. Ammonium hydroxide (NH4OH), 0.3% in methanol: Prepared by diluting 12 mL of ammonium hydroxide into 4L of methanol.

7.1.4. Hexane

7.1.5. Hydrochloric acid (HCl), 2.0 M solution in water

7.1.6. Hydrochloric acid (HCl), concentrated, reagent grade

7.1.7. Methanol

7.1.8. [redacted]

7.1.9. [redacted], reagent grade

7.1.10. Ottawa Sand (blank matrix for solid samples)

7.1.11. Vegetable Oil, Crisco® brand (blank matrix for tissue samples) – replace within one year of opening.

7.1.12. Sodium hydroxide (NaOH), 0.1 N, in water: Prepared by diluting 400 mL of 1N NaOH into 3.6L of water for a total volume of 4 L.

7.1.13. Sodium hydroxide (NaOH), 10 N, reagent grade

7.1.14. Water, Nanopure or Millipore, must be free of interference and target analytes.

7.1.15. Nitrogen, Ultra High Purity, used for the ESI interface, collision cell, and concentration of extracts.

7.1.16. Air, Ultra-Pure, used for vacuum and source gas.

7.1.17. 30:70 methanol:water (v/v), prepared by diluting 30 mL methanol with 70 mL HPLC reagent water or equivalent volume in respect to the ratio.

7.2. Standards

7.2.1. PFAS are purchased as high purity solids (96% or greater) or as certified solutions. Standard materials are verified compared to a second source material at the time of initial calibration. The solid stock material is stored at room temperature or as specified by the manufacturer or vendor.

7.2.1.1. Per the Certificate of Analysis for labeled perfluorohexadecanoic acid ($13C_2$-PFHxDA) produced by Wellington Laboratories, the stock standard contains roughly 0.3% of native perfluorohexadecanoic acid. This equates to roughly 0.15 ng/L or 0.0075 µg/kg of perfluorohexadecanoic acid expected in all samples and blanks.

7.2.2. As of this writing, only PFOS, PFOA, PFHxS, Et-FOSAA and Me-FOSAA are commercially available as technical mixtures. These reference standards of the technical mixtures for these specific PFAS are used to ensure that all appropriate peaks are included during peak integration.

7.2.3. If solid material is used for preparing a standard, stock standard solutions are prepared from the solids and are stored at 0 - 6°C. Stock standard solutions should be brought to room temperature before using. Standards are monitored for signs of degradation or evaporation. Standard solutions must be replaced at least annually from the date of preparation.

7.2.4. PFBS, PFHxS, PFHpS, PFOS, PFDS, and many other PFAS are not available in the acid form, but rather as their corresponding salts, such as sodium or potassium. The standards are prepared and corrected for their salt content according to the equation below.

$$Mass_{acid} = Measured\ Mass_{salt} \times MW_{acid} / MW_{salt}$$

Where: MW_{acid} is the molecular weight of PFAA

MW_{salt} is the molecular weight of the purchased salt.

For example, the molecular weight of PFOS is 500.1295 and the molecular weight of NaPFOS is 523.1193. Therefore, the amount of NaPFOS used must be adjusted by a factor of 0.956.

7.2.5. For the primary source calibration solutions, individual solutions for each PFAS (both native and isotopically labelled) are purchased from Wellington Laboratories, or other reputable vendors, and are predominantly at a concentration of 50 ug/mL in basic methanol. In the case of the sulfonic compounds, the concentration is 50ug/mL of the alkali (potassium or sodium) salt. The laboratory uses the concentration of the acid form when determining the concentration of individual sulfonic acids in solution (See Section 7.2.4 above).

7.2.6. While PFAS standards commercially purchased are supplied in glass ampoules, all subsequent transfers or dilutions performed by the analyst must be prepared and stored in polypropylene or HDPE containers. Vortex all standard solutions prior to removing aliquots.

7.3. PFC IM/LCS (LCS/Matrix PFC Spike Solution), 20 ng/mL

250 ml of a mixed stock solution at a nominal concentration of 0.02 ug/mL in methanol (see note above) is prepared from the individual solutions, using 100 uL of each individual solution. This mixed stock is used as the spiking solution during sample preparation, as well an intermediate for the calibration curve, using the recipe below:

Table 7.3
PFC IM/LCS Solution Recipe
The solutions below are combined and diluted to 250 mL in methanol

Analyte	Stock Conc. (µg/mL)	Aliquot (mL)	PFC IM/LCS Conc. (µg/mL)	Analyte	Stock Conc. (µg/mL)	Aliquot (mL)	PFC IM/LCS Conc. (µg/mL)
PFBA	50	0.1	0.02	EtFOSAA	50	0.1	0.02
PFPeA	50	0.1	0.02	MeFOSAA	50	0.1	0.02
PFHxA	50	0.1	0.02	4:2 FTS	46.7	0.1	0.01868
PFHpA	50	0.1	0.02	6:2 FTS	47.4	0.1	0.01896
PFOA	50	0.1	0.02	8:2 FTS	47.9	0.1	0.01916
PFNA	50	0.1	0.02	10:2 FTS	48.2	0.1	0.01928
PFDA	50	0.1	0.02	HFPO-DA	50	0.1	0.02
PFUdA	50	0.1	0.02	9CI-PF3ONS	46.6	0.1	0.01864
PFDoA	50	0.1	0.02	11CI-PF3OUdS	47.1	0.1	0.01884
PFTrDA	50	0.1	0.02	4,8-dioxa-3H-PFNA (DONA)	47.1	0.1	0.01884

Table 7.3
PFC IM/LCS Solution Recipe
The solutions below are combined and diluted to 250 mL in methanol

Analyte	Stock Conc. (µg/mL)	Aliquot (mL)	PFC IM/LCS Conc. (µg/mL)	Analyte	Stock Conc. (µg/mL)	Aliquot (mL)	PFC IM/LCS Conc. (µg/mL)
PFTeDA	50	0.1	0.02	3:3 FTCA	50	0.1	0.02
PFHxDA	50	0.1	0.02	5:3 FTCA	50	0.1	0.02
PFODA	50	0.1	0.02	7:3 FTCA	50	0.1	0.02
PFBS	44.2	0.1	0.01768	6:2 FTCA	50	0.1	0.02
PFPeS	46.9	0.1	0.01876	8:2 FTCA	50	0.1	0.02
PFHxS	45.5	0.1	0.0182	10:2 FTCA	50	0.1	0.02
PFHpS	47.6	0.1	0.01904	6:2 FTUCA	50	0.1	0.02
PFOS	46.2	0.1	0.01856	8:2 FTUCA	50	0.1	0.02
PFNS	48	0.1	0.0192	10:2 FTUCA	50	0.1	0.02
PFDS	48.2	0.1	0.01928	PFECHS	46.1	0.1	0.01844
PFDoS	48.4	0.1	0.01936	PFPrA	48.5	0.1	0.0194
FOSA	50	0.1	0.02	PFPrS	45.8	0.1	0.01832
Et-FOSA	50	0.1	0.02	PFECA F	50	0.1	0.02
Me-FOSA	50	0.1	0.02	PFECA A	50	0.1	0.02
Et-FOSE	50	0.1	0.02	PFECA B	50	0.1	0.02
Me-FOSE	50	0.1	0.02	PES	44.5	0.1	0.0178

7.4. PFC Expanded Analyte Intermediate (PFC3SP IM)

250 ml of a mixed stock solution at a nominal concentration of 0.5 ug/mL in methanol (see note above) is prepared from the individual solutions, using 50 uL of each individual solution, as denoted in the recipe below. This mixed stock is used as an intermediate for the calibration curve and for the PFC Expanded Analyte LCS (Section 7.4.1).

Table 7.4
PFC Expanded Analyte IM Solution Recipe
The solutions below are combined and diluted to 250 mL in methanol

Analyte	Stock Conc. (µg/mL)	Aliquot (mL)	PFC3SP IM Conc. (µg/mL)	Analyte	Stock Conc. (µg/mL)	Aliquot (mL)	PFC3SP IM Conc. (µg/mL)
Hydro-EVE Acid	1000	0.05	0.5	PFO3OA	1000	0.05	0.5
Hydro-PS Acid	1000	0.05	0.5	PFO4DA	1000	0.05	0.5
MTP	1000	0.05	0.5	PMPA	1000	0.05	0.5
NVHOS	1000	0.05	0.5	PS Acid	1000	0.05	0.5
PEPA	1000	0.05	0.5	R-EVE	1000	0.05	0.5
PFECA G	1000	0.05	0.5	R-PSDCA	1000	0.05	0.5

Table 7.4
PFC Expanded Analyte IM Solution Recipe
The solutions below are combined and diluted to 250 mL in methanol

Analyte	Stock Conc. (µg/mL)	Aliquot (mL)	PFC3SP IM Conc. (µg/mL)	Analyte	Stock Conc. (µg/mL)	Aliquot (mL)	PFC3SP IM Conc. (µg/mL)
PFMOAA	1000	0.05	0.5	TAF (PFO5DoA)	1000	0.05	0.5
PFO2HxA	1000	0.05	0.5	EVE Acid	1000	0.05	0.5

7.4.1. PFC Expanded Analyte IM/LCS Solution (PFC3SP)

The expanded analyte spike solution is made by diluting 10 mL of the Expanded Analyte Intermediate (Section 7.4, above) to 250 mL in methanol:

Table 7.4.1
PFC Expanded Analyte Spiking Solution Composition
(following dilution of 10 mL IM to 250 mL MeOH)

Analyte	PFC3SP (ug/mL)	Analyte	PFC3SP (ug/mL)
Hydro-EVE Acid	0.02	PFO3OA	0.02
Hydro-PS Acid	0.02	PFO4DA	0.02
MTP	0.02	PMPA	0.02
NVHOS	0.02	PS Acid	0.02
PEPA	0.02	R-EVE	0.02
PFECA G	0.02	R-PSDCA	0.02
PFMOAA	0.02	TAF (PFO5DoA)	0.02
PFO2HxA	0.02	EVE Acid	0.02

7.5. PFC Isotope Dilution Analyte Solution (Extracted Internal Standards), 25 ng/mL

The PFC-IDA solution is added to all samples prior to extraction and used as an intermediate solution for preparation of the instrument calibration standards. 200 mL of the solution at a nominal concentration of 0.025 ug/mL (25 ng/mL) is prepared from the individual solutions described in Section 7.2.5. using the recipe below:

Table 7.5
PFC-IDA Recipe
The solutions below are combined and diluted to 200 mL with Methanol.

IDA	Stock Conc. (µg/mL)	Aliquot (mL)	IDA Mix Conc. (µg/mL)	IDA	Stock Conc. (µg/mL)	Aliquot (mL)	IDA Mix Conc. (µg/mL)
13C4-PFBA	50	0.10	0.025	d3-MeFOSAA	50	0.10	0.025
13C5-PFPeA	50	0.10	0.025	M2-4:2FTS	46.7	0.10	0.02335
13C2-PFHxA	50	0.10	0.025	M2-6:2FTS	47.5	0.10	0.02375
13C4-PFHpA	50	0.10	0.025	M2-8:2FTS	47.9	0.10	0.02395

Table 7.5
PFC-IDA Recipe
The solutions below are combined and diluted to 200 mL with Methanol.

IDA	Stock Conc. (µg/mL)	Aliquot (mL)	IDA Mix Conc. (µg/mL)	IDA	Stock Conc. (µg/mL)	Aliquot (mL)	IDA Mix Conc. (µg/mL)
13C4-PFOA	50	0.10	0.025	M2 10:2 FTS	47.36	0.10	0.02368
13C5-PFNA	50	0.10	0.025	d5-EtFOSA	50	0.10	0.025
13C2-PFDA	50	0.10	0.025	d3-MeFOSA	50	0.10	0.025
13C2-PFUdA	50	0.10	0.025	d9-Et-FOSE	50	0.10	0.025
13C2-PFDoA	50	0.10	0.025	d7-Me-FOSE	50	0.10	0.025
18O2-PFHxS	47.3	0.10	0.02365	13C3-HFPO-DA	50	0.10	0.025
13C4-PFOS	47.8	0.10	0.0239	13C-6:2 FTCA	50	0.10	0.025
13C3-PFBS	46.5	0.10	0.02325	13C-8:2 FTCA	50	0.10	0.025
13C2-PFTeDA	50	0.10	0.025	13C-10:2 FTCA	50	0.10	0.025
13C2-PFHxDA	50	0.10	0.025	13C-6:2 FTUCA	50	0.10	0.025
13C8-FOSA	50	0.10	0.025	13C-8:2 FTUCA	50	0.10	0.025
d5-EtFOSAA	50	0.10	0.025	13C-10:2 FTUCA	50	0.10	0.025

7.6. Internal Standard Solution, 25 ng/mL

The PFC_IS solution is added to all extracts prior to analysis and used as an intermediate solution for preparation of the instrument calibration standards. This solution is prepared by diluting 100 uL of a 50 ug/mL solution containing 13C2-PFOA to 200 mL in methanol, for a final concentration of 0.025 ug/L (25 ng/mL).

7.7. Calibration Standards

Calibration solutions are prepared from the standards described in Sections 7.3, 7.4.1, 7.5, and 7.6, above. For each level, a 100 mL volumetric flask is filled with 20 mL of water, and methanol added. The appropriate amount (see table below) of the solutions are added, and then the flask is filled to the mark with methanol to achieve the ratio of 80% methanol to 20% water, v/v.

Table 7.7
Calibration Solution Recipe

PFAS Standards	Volume (mL) to add in 100 mL FV						
	CS-1	CS-2	CS-3	CS-4	CS-5	CS-6	CS-7
PFC IM/LCS (0.02 µg/mL)	0.125	0.25	1.25	5	12.5	25	50
PFC3SP (0.02 ug/mL)	0.125	0.25	1.25	5	12.5	25	50
IDA Mix (0.025µg/mL)	5.0	5.0	5.0	5.0	5.0	5.0	5.0
IS Mix (0.025 µg/mL)	5.0	5.0	5.0	5.0	5.0	5.0	5.0

7.7.1. Initial Calibration (ICAL) Levels (ng/mL)

Table 7.7.1
Initial Calibration Solution Concentrations (ng/mL)

Compound	CS-1	CS-2	CS-3	CS-4	CS-5	CS-6	CS-7
PFBA	0.025	0.05	0.25	1	2.5	5	10
PFPeA	0.025	0.05	0.25	1	2.5	5	10
PFHxA	0.025	0.05	0.25	1	2.5	5	10
PFHpA	0.025	0.05	0.25	1	2.5	5	10
PFOA	0.025	0.05	0.25	1	2.5	5	10
PFNA	0.025	0.05	0.25	1	2.5	5	10
PFDA	0.025	0.05	0.25	1	2.5	5	10
PFUdA	0.025	0.05	0.25	1	2.5	5	10
PFDoA	0.025	0.05	0.25	1	2.5	5	10
PFTrDA	0.025	0.05	0.25	1	2.5	5	10
PFTeDA	0.025	0.05	0.25	1	2.5	5	10
PFHxDA	0.025	0.05	0.25	1	2.5	5	10
PFODA	0.025	0.05	0.25	1	2.5	5	10
PFBS	0.0221	0.0442	0.221	0.0884	2.21	4.42	0.884
PFPeS	0.02345	0.0469	0.2345	0.0938	2.345	4.69	0.938
PFHxS*	0.02275	0.0455	0.2275	0.91	2.275	4.55	9.1
PFHpS	0.0238	0.0476	0.238	0.952	2.38	4.76	9.52
PFOS*	0.0232	0.0464	0.232	0.928	2.32	4.64	9.28
PFNS	0.024	0.048	0.24	0.96	2.4	4.8	9.6
PFDS	0.0241	0.0482	0.241	0.0964	2.41	4.82	0.964
PFDoS	0.0242	0.0484	0.242	0.0968	2.42	4.84	0.968
FOSA	0.025	0.05	0.25	1	2.5	5	10
Et-FOSA	0.025	0.05	0.25	1	2.5	5	10
Me-FOSA	0.025	0.05	0.25	1	2.5	5	10
Et-FOSE	0.025	0.05	0.25	1	2.5	5	10
Me-FOSE	0.025	0.05	0.25	1	2.5	5	10
EtFOSAA*	0.025	0.05	0.25	1	2.5	5	10
MeFOSAA*	0.025	0.05	0.25	1	2.5	5	10
4:2 FTS	0.02335	0.0467	0.2335	0.934	2.335	4.67	9.34
6:2 FTS	0.0237	0.0474	0.237	0.948	2.37	4.74	9.48
8:2 FTS	0.02395	0.0479	0.2395	0.958	2.395	4.79	9.58
10:2 FTS	0.0241	0.0482	0.241	0.964	2.41	4.82	9.64
HFPO-DA	0.025	0.05	0.25	1	2.5	5	10
9Cl-PF3ONS	0.0233	0.0466	0.233	0.932	2.33	4.66	9.32
11Cl-PF3OUdS	0.02355	0.0471	0.2355	0.0942	2.355	4.71	9.42
DONA	0.02355	0.0471	0.2355	0.0942	2.355	4.71	0.942
Hydro-EVE Acid	0.025	0.05	0.25	1	2.5	5	10
Hydro-PS Acid	0.025	0.05	0.25	1	2.5	5	10
MTP	0.025	0.05	0.25	1	2.5	5	10
NVHOS	0.025	0.05	0.25	1	2.5	5	10
PEPA	0.025	0.05	0.25	1	2.5	5	10
PFECA G	0.025	0.05	0.25	1	2.5	5	10
PFMOAA	0.025	0.05	0.25	1	2.5	5	10
PFO2HxA	0.025	0.05	0.25	1	2.5	5	10
PFO3OA	0.025	0.05	0.25	1	2.5	5	10
PFO4DA	0.025	0.05	0.25	1	2.5	5	10
PMPA	0.025	0.05	0.25	1	2.5	5	10

Table 7.7.1
Initial Calibration Solution Concentrations (ng/mL)

Compound	CS-1	CS-2	CS-3	CS-4	CS-5	CS-6	CS-7
PS Acid	0.025	0.05	0.25	1	2.5	5	10
EVE Acid	0.025	0.05	0.25	1	2.5	5	10
R-EVE	0.025	0.05	0.25	1	2.5	5	10
R-PSDCA	0.025	0.05	0.25	1	2.5	5	10
TAF	0.025	0.05	0.25	1	2.5	5	10
3:3 FTCA	0.025	0.05	0.25	1	2.5	5	10
5:3 FTCA	0.025	0.05	0.25	1	2.5	5	10
7:3 FTCA	0.025	0.05	0.25	1	2.5	5	10
6:2 FTCA	0.025	0.05	0.25	1	2.5	5	10
8:2 FTCA	0.025	0.05	0.25	1	2.5	5	10
10:2 FTCA	0.025	0.05	0.25	1	2.5	5	10
6:2 FTUCA	0.025	0.05	0.25	1	2.5	5	10
8:2 FTUCA	0.025	0.05	0.25	1	2.5	5	10
10:2 FTUCA	0.025	0.05	0.25	1	2.5	5	10
PFECHS	0.02305	0.0461	0.2305	0.922	2.305	4.61	9.22
PFPrA	0.02425	0.0485	0.2425	0.97	2.425	4.85	9.7
PFPrS	0.0229	0.0458	0.229	0.916	2.29	4.58	9.16
PFECA F	0.025	0.05	0.25	1	2.5	5	10
PFECA A	0.025	0.05	0.25	1	2.5	5	10
PFECA B	0.025	0.05	0.25	1	2.5	5	10
PES	0.0215	0.043	0.215	0.86	2.15	4.3	8.9
Labeled Isotope Dilution Analytes (IDA)							
13C4-PFBA	1.25	1.25	1.25	1.25	1.25	1.25	1.25
13C5-PFPeA	1.25	1.25	1.25	1.25	1.25	1.25	1.25
13C2-PFHxA	1.25	1.25	1.25	1.25	1.25	1.25	1.25
13C4-PFHpA	1.25	1.25	1.25	1.25	1.25	1.25	1.25
13C4-PFOA	1.25	1.25	1.25	1.25	1.25	1.25	1.25
13C5-PFNA	1.25	1.25	1.25	1.25	1.25	1.25	1.25
13C2-PFDA	1.25	1.25	1.25	1.25	1.25	1.25	1.25
13C2-PFUdA	1.25	1.25	1.25	1.25	1.25	1.25	1.25
13C2-PFDoA	1.25	1.25	1.25	1.25	1.25	1.25	1.25
18O2-PFHxS	1.1825	1.1825	1.1825	1.1825	1.1825	1.1825	1.1825
13C4-PFOS	1.195	1.195	1.195	1.195	1.195	1.195	1.195
13C3-PFBS	1.1625	1.1625	1.1625	1.1625	1.1625	1.1625	1.1625
13C2-PFTeDA	1.25	1.25	1.25	1.25	1.25	1.25	1.25
13C2-PFHxDA	1.25	1.25	1.25	1.25	1.25	1.25	1.25
13C8-FOSA	1.25	1.25	1.25	1.25	1.25	1.25	1.25
d5-EtFOSAA	1.25	1.25	1.25	1.25	1.25	1.25	1.25
d3-MeFOSAA	1.25	1.25	1.25	1.25	1.25	1.25	1.25
M2-4:2FTS ǂ	1.1675	1.1675	1.1675	1.1675	1.1675	1.1675	1.1675
M2-6:2FTS	1.1875	1.1875	1.1875	1.1875	1.1875	1.1875	1.1875
M2-8:2FTS	1.1975	1.1975	1.1975	1.1975	1.1975	1.1975	1.1975
M2 10:2 FTS	1.184	1.184	1.184	1.184	1.184	1.184	1.184
d5-EtFOSA	1.25	1.25	1.25	1.25	1.25	1.25	1.25
d3-MeFOSA	1.25	1.25	1.25	1.25	1.25	1.25	1.25
d9-Et-FOSE	1.25	1.25	1.25	1.25	1.25	1.25	1.25

Table 7.7.1
Initial Calibration Solution Concentrations (ng/mL)

Compound	CS-1	CS-2	CS-3	CS-4	CS-5	CS-6	CS-7
d7-Me-FOSE	1.25	1.25	1.25	1.25	1.25	1.25	1.25
13C3-HFPO-DA	1.25	1.25	1.25	1.25	1.25	1.25	1.25
13C-6:2 FTCA	1.25	1.25	1.25	1.25	1.25	1.25	1.25
13C-8:2 FTCA	1.25	1.25	1.25	1.25	1.25	1.25	1.25
13C-10:2 FTCA	1.25	1.25	1.25	1.25	1.25	1.25	1.25
13C-6:2 FTUCA	1.25	1.25	1.25	1.25	1.25	1.25	1.25
13C-8:2 FTUCA	1.25	1.25	1.25	1.25	1.25	1.25	1.25
13C-10:2 FTUCA	1.25	1.25	1.25	1.25	1.25	1.25	1.25
Internal Standard (IS)							
13C2-PFOA	1.25	1.25	1.25	1.25	1.25	1.25	1.25

** Both branched and linear isomers are used.*

ǂ - This compound is used as a reverse surrogate for the TOP analysis.

***Note**: Sample extracts are in 80% MeOH/H_2O.*

***Note**: The above calibration limits are provided only as an example. The actual ICAL level used for each analytical batch will depend upon the LOQ requirements of the program.*

7.7.2. A technical (qualitative) grade PFOA standard which contains both linear and branched isomers is used as a retention time (RT) marker. This is used to integrate the total response for both linear and branched isomers of PFOA in environmental samples while relying on the initial calibration with the linear isomer quantitative standard. This technical (qualitative) grade PFOA standard is analyzed initially, after every initial calibration or when significant changes are made to the HPLC parameters.

7.7.2.1. Attach this document to the ICV from the associated ICAL by scanning the document and associating it to the file as a document type of High Res MS Tune in TALS. Use the following naming convention: "_TFOA_Instrument_Date." Example: _TFOA_A10_15Mar2019.

7.8. Initial Calibration Verification Standard (ICV)

7.8.1. The ICV is prepared from commercially available mixed solutions (the PFC-MXB mixture from Wellington) augmented by individual stock solutions for those components not present in the commercial mixture. When available, individual stock solutions are purchased from a vendor other than Wellington laboratories. If not available, a second lot from Wellington is sourced, and if that is not available, a second laboratory chemist will prepare the intermediate mixed solution for the ICV. Currently, the commercially available mixture contains the following

compounds at the listed concentrations in methanol:

Table 7.8.1
PFC-MXB composition

Analyte	Stock Conc. (µg/mL)	Analyte	Stock Conc. (µg/mL)
PFHxA	2	PFBS	2
PFHpA	2	PFHxS	2
PFOA	2	PFOS	2
PFNA	2	EtFOSAA	2
PFDA	2	MeFOSAA	2
PFUdA	2	HFPO-DA	2
PFDoA	2	9CI-PF3ONS	2
PFTrDA	2	11CI-PF3OUdS	2
PFTeDA	2	4,8-dioxa-3H- PFNA (DONA)	2

7.8.2. ICV-IM: 10 mL of a combined stock for the analytes listed below is created, using the recipe below, and methanol as the final solvent:

Table 7.8.2
ICV-IM Recipe

Analyte	Stock Conc. (µg/mL)	Aliquot (mL)	ICV-IM Conc. (µg/mL)	Analyte	Stock Conc. (µg/mL)	Aliquot (mL)	ICV-IM Conc. (µg/mL)
PFBA	50	0.1	0.5	FOSA	50	0.1	0.5
PFPeA	50	0.1	0.5	Et-FOSA	50	0.1	0.5
PFHxDA	50	0.1	0.5	Me-FOSA	50	0.1	0.5
PFODA	50	0.1	0.5	Et-FOSE	50	0.1	0.5
PFPeS	46.9	0.1	0.469	Me-FOSE	50	0.1	0.5
PFHpS	47.6	0.1	0.476	4:2 FTS	46.7	0.1	0.467
PFNS	48	0.1	0.480	6:2 FTS	47.4	0.1	0.474
PFDS	48.2	0.1	0.482	8:2 FTS	47.9	0.1	0.479
PFDoS	48.4	0.1	0.484	10:2 FTS	48.2	0.1	0.482

7.8.3. ICV-IM2: 10 mL of a combined stock for the analytes listed below is created, using the recipe below, and methanol as the final solvent:

Table 7.8.3
ICV-IM2 Recipe

Analyte	Stock Conc. (µg/mL)	Aliquot (mL)	ICV-IM Conc. (µg/mL)	Analyte	Stock Conc. (µg/mL)	Aliquot (mL)	ICV-IM Conc. (µg/mL)
3:3 FTCA	50	0.1	0.5	10:2 FTUCA	50	0.1	0.5
5:3 FTCA	50	0.1	0.5	PES	44.5	0.1	0.445
7:3 FTCA	50	0.1	0.5	PFECA F	50	0.1	0.5
6:2 FTCA	50	0.1	0.5	PFECA A	50	0.1	0.5
8:2 FTCA	50	0.1	0.5	PFECA B	50	0.1	0.5
10:2 FTCA	50	0.1	0.5	PFECHS	46.1	0.1	0.461
6:2 FTUCA	50	0.1	0.5	PFPrS	45.8	0.1	0.458
8:2 FTUCA	50	0.1	0.5	PFPrA (PPF Acid)	97	0.05	0.458

7.8.4. ICV-IM3: 10 mL of a combined stock for the analytes listed below is created, using the recipe below, and methanol as the final solvent:

Table 7.8.4
ICV-IM3 Recipe

Analyte	Stock Conc. (µg/mL)	Aliquot (mL)	ICV-IM Conc. (µg/mL)	Analyte	Stock Conc. (µg/mL)	Aliquot (mL)	ICV-IM Conc. (µg/mL)
DFSA	1000	0.025	0.05	PFMOAA	1000	0.025	0.05
EVE Acid	1000	0.025	0.05	PFO2HxA	1000	0.025	0.05
Hydro-EVE Acid	1000	0.025	0.05	PFO3OA	1000	0.025	0.05
Hydrolyzed PSDA	1000	0.025	0.05	PFO4DA	1000	0.025	0.05
Hydro-PS Acid	1000	0.025	0.05	PMPA	1000	0.025	0.05
MMF	1000	0.025	0.05	PS Acid	1000	0.025	0.05
MTP	1000	0.025	0.05	R-EVE	1000	0.025	0.05
NVHOS	1000	0.025	0.05	R-PSDA	1000	0.025	0.05
PEPA	1000	0.025	0.05	R-PSDCA	1000	0.025	0.05
PFECA G	1000	0.025	0.05	TAF	1000	0.025	0.05

7.8.5. Finally, the ICV solution is created, at a nominal concentration of 2.5 ng/mL for target analytes (sulfonic acids slightly less), and the same concentrations as the calibration solutions for IS and IDA, by filling a 100 mL flask with 20 mL of water, then adding methanol. After adding the solutions below, the contents are diluted to the mark with methanol:

Table 7.8.5
ICV Recipe

PFAS Standards	Volume (mL) to add in 100 mL FV
Commercial PFAS Mix	0.125
Combined ICV IM Mix (0.5 ug/mL)	0.50
Combined ICV IM2 Mix (0.5 ug/mL)	0.50
Combined ICV IM3 Mix (0.5 ug/mL)	0.50
IDA Mix (0.05 µg/mL)	5.0
IS Mix (0.05 µg/mL)	5.0

7.9. TOP-Surr, 1000 ng/mL: The reverse surrogate solution used for samples subjected to the oxidation process to monitor the efficiency of the oxidation process. This solution is prepared by diluting 2 mL of a 50 ug/mL solution containing M2-4:2 FTS to 100 mL in water, for a final concentration of 1000 ng/mL.

7.10. TOP-IDA, 25ng/mL

The TOP-IDA solution is used for those samples subjected to the oxidation process. It omits M2-4:2 FTS, as that compound is used as a reverse surrogate for demonstrating the efficiency of the oxidation step. 200 mL of the solution at a nominal concentration of 0.025 ug/mL (25 ng/mL) is prepared from the individual solutions described in Section 7.2.5 using the recipe below:

Table 7.10
TOP-IDA Recipe
All solutions below are diluted to 200 mL with Methanol.

IDA	Stock Conc. (µg/mL)	Aliquot (mL) to	IDA Mix Conc. (µg/mL)	IDA	Stock Conc. (µg/mL)	Aliquot (mL) to	IDA Mix Conc. (µg/mL)
13C4-PFBA	50	0.10	0.025	d3-MeFOSAA	50	0.1	0.025
13C5-PFPeA	50	0.10	0.025	M2-6:2FTS	47.5	0.1	0.02375
13C2-PFHxA	50	0.10	0.025	M2-8:2FTS	47.9	0.1	0.02395
13C4-PFHpA	50	0.10	0.025	M2 10:2 FTS	47.36	0.1	0.02368
13C4-PFOA	50	0.10	0.025	d5-EtFOSA	50	0.1	0.025
13C5-PFNA	50	0.10	0.025	d3-MeFOSA	50	0.1	0.025
13C2-PFDA	50	0.10	0.025	d9-Et-FOSE	50	0.1	0.025
13C2-PFUdA	50	0.10	0.025	d7-Me-FOSE	50	0.1	0.025
13C2-PFDoA	50	0.10	0.025	13C3-HFPO-DA	50	0.1	0.025
18O2-PFHxS	47.3	0.10	0.02365	13C-6:2 FTCA	50	0.1	0.025
13C4-PFOS	47.8	0.10	0.0239	13C-8:2 FTCA	50	0.1	0.025
13C3-PFBS	46.5	0.10	0.02325	13C-10:2 FTCA	50	0.1	0.025

13C2-PFTeDA	50	0.10	0.025	13C-6:2 FTUCA	50	0.1	0.025
13C2-PFHxDA	50	0.10	0.025	13C-8:2 FTUCA	50	0.1	0.025
13C8-FOSA	50	0.10	0.025	13C-10:2 FTUCA	50	0.1	0.025
d5-EtFOSAA	50	0.10	0.025				

8. SAMPLE COLLECTION, PRESERVATION, AND STORAGE

Laboratory default requirements for sample containers, sample size, preservation and holding time are detailed in the table below.

Table 8
Sample Collection, Preservation, and Storage Requirements

Matrix	Sample Container	Minimum Sample Size	Preservation	Holding Time[1]
Water	250 mL HDPE Bottle	250 mL	0-6°C *(if from a known chlorinated source add Trizma (5g/L))*	14 days
Soil/Sediment	4 oz. HDPE wide-mouth container	100 g	0-6°C	14 days
Tissue	4 oz. HDPE wide-mouth container	50 g	≤ -10 °C	1 year (365 days)

Extraction holding time is calculated from date of collection. Analytical holding time is determined from date of extraction.

8.1. Extracts are stored at 0 - 6°C and must be analyzed within 40 days of extraction.

8.2. Many jurisdictions have additional requirements for PFAS analysis, including different holding times and preservation requirements. These are detailed in the document WS-WI-0066, "Agency Specific Criteria for PFAS in Matrices Other Than Drinking Water".

8.3. Unless otherwise specified by client or regulatory program, after analysis, samples and extracts are retained for a minimum of 30 days after provision of the project report and then disposed of in accordance with applicable regulations.

Note: *As of this writing, Method 537 provides for a 14 day holding time for water samples preserved with Trizma buffer. The scientific literature indicates that perfluorinated substances are highly persistent in the environment. Eurofins TestAmerica Sacramento has conducted time stability studies that support a 14 day holding time for aqueous samples with and without Trizma preservation. TestAmerica Denver has conducted stability studies indicating that medium- and low-level solutions of PFOA are stable for at least three months in polystyrene and polypropylene plastics at 0-6°C. The 14/40 day holding times given above are based on the stability study and general EPA convention for the holding time of extractable organic compounds in water and soil.*

8.4. Biphasic samples

8.4.1. Samples denoted as aqueous (groundwaters, surface waters, and waste waters) are prepared and handled as a liquid sample (Section 11.2) regardless of solids content unless otherwise instructed or agreed upon with the client. Detailed descriptions of such deviations from the procedure must be documented in the LIMS NCM program.

8.4.2. Samples considered solids (including biosolids, sediments, and soils) are prepared and handled as solid samples following appropriate homogenization as per Section 11.7. Correction for moisture content is provided through the LIMS when required by the client.

8.4.3. In the event that results are required individually for the solid and aqueous phases of a sample, the phases are separated via centrifugation, and extracted separately using the appropriate preparation (Section 11.2 for the aqueous phase and Section 11.7 for the solid phase). The extracts are analyzed, and results reported for each phase separately.

9. QUALITY CONTROL

Please note: Many states and regulatory programs have their own specifications which differ from the laboratory's default program for QC, calibration, sample preparation and data evaluation. Please refer to WS-WI-0066 for state/client specific programs that have differing criteria from those listed in Sections 9 through 12.

9.1. Initial Demonstration of Capability (IDOC)

The initial demonstration and method detection limit (MDL) studies described in Section 13 must be acceptable before analysis of samples may begin.

9.2. Batches are defined at the sample preparation step. Batches should be kept together through the whole analytical process as far as possible, but it is not mandatory to analyze prepared extracts on the same instrument or in the same sequence. Refer to the QC program document (WS-PQA-003) for further details of the batch definition.

9.2.1. The quality control batch is a set of up to 20 samples of the same matrix processed using the same procedure and reagents within the same time period. The quality control batch must contain a matrix spike/matrix spike duplicate (MS/MSD), a laboratory control sample (LCS) and a method blank. Laboratory generated QC samples (Blank, LCS, MS/MSD) do not count toward the maximum 20 samples in a batch. Field QC samples are included in the batch count. In some cases, at client request, the MS/MSD may be replaced with a matrix spike and sample duplicate. If insufficient sample is available for an MS/MSD, an LCSD may be substituted if batch

precision is required by the program or client. In the event that multiple MS/MSDs are run with a batch due to client requirements, the additional MS/MSDs do not count toward the maximum 20 samples in a batch.

9.3. One method blank (MB, laboratory reagent blank) must be extracted with every process batch of similar matrix, not to exceed twenty (20) samples. For aqueous samples, the method blank is an aliquot of laboratory reagent water. For solid samples, the method blank is an aliquot of Ottawa sand. The method blank is processed in the same manner and at the same time as the associated samples. Corrective actions must be documented on a Non-Conformance memo, and then implemented when target analytes are detected in the method blank above the reporting limit or when IDA recoveries are outside of the control limits. Re-extraction of the blank, other batch QC and the affected samples are required when the method blank is deemed unacceptable. See policy WS-PQA-003 for specific acceptance criteria.

9.3.1. If the MB produces a peak within the retention time window of any of the analytes, determine the source of the contamination and eliminate the interference before processing samples.

9.3.2. The method blank must not contain any analyte at or above the reporting limit, or at or above 10% of the measured concentration of that analyte in the associated samples, whichever is higher.

9.3.3. If there is no target analyte greater than the RL in the samples associated with an unacceptable method blank, the data may be reported with qualifiers. Such action should be taken in consultation with the client.

9.3.4. Re-extraction and reanalysis of samples associated with an unacceptable method blank is required when reportable concentrations are determined in the samples.

9.3.5. Refer to WS-PQA-003 for further details of the corrective actions.

9.3.6. Projects performed under the auspices of the DOD/DOE must meet QSM specific criteria for method blanks. Results are acceptable if the blank contamination is less than ½ of the reporting limit/LOQ for each analyte, or less than 1/10 of the regulatory limit, or less than 1/10 of the sample result for the same analyte, whichever is greater. If the method blank does not meet the acceptance criteria, the source of contamination must be investigated and measures taken to correct, minimize or eliminate the problem. Reprepare and reanalyze all field and QC samples associated with the contaminated method blank.

9.3.7. The position of the method blank in the SPE manifold during SPE extraction

is rotated across batches.

9.4. A laboratory control sample (LCS) must be extracted with every process batch of similar matrix, not to exceed twenty (20) samples. The LCS is an aliquot of laboratory matrix (e.g. water for aqueous samples and Ottawa sand for solids) spiked with analytes of known identity and concentration. The LCS must be processed in the same manner and at the same time as the associated samples. Corrective actions must be documented on a Non-Conformance memo, then implemented when recoveries of any spiked analyte is outside of the control limits. Re-extraction of the blank, other batch QC, and all associated samples are required if the LCS is deemed unacceptable. See WS-PQA-0003 for specific acceptance criteria. The control limits for the LCS are stored in TALS.

9.5. A matrix spike/matrix spike duplicate (MS/MSD or MS/SD) pair must be extracted with every process batch of similar matrix, not to exceed twenty (20) samples. An MS/MSD pair is aliquots of a selected field sample spiked with analytes of known identity and concentration. The MS/MSD pair must be processed in the same manner and at the same time as the associated samples. Spiked analytes with recoveries or precision outside of the control limits must be within the control limits in the LCS. Corrective actions must be documented on a nonconformance memo, and then implemented when recoveries of any spiked analyte are outside of the control limits provided by TALS or by the client.

9.6. A duplicate control sample (LCSD or DCS) may be added when insufficient sample volume is provided to process an MS/MSD pair, or is requested by the client. The LCSD is evaluated in the same manner as the LCS. See WS-PQA-003 for specific acceptance criteria.

9.7. Initial calibration verification (ICV) –A second source standard is analyzed with the initial calibration curve. The concentration should be at the mid-range of the curve.

Corrective actions for the ICV include:

- Rerun the ICV.
- Remake or acquire a new ICV.
- Evaluate the instrument conditions.
- Evaluate the initial calibration standards.
- Rerun the initial calibration.

9.8. Isotope Dilution Analytes

9.8.1. The IDA solution is added to each field and QC sample at the time of extraction, as described in Section 11. As described in Section 7, this solution consists of isotopically labeled analogs of the analytes of interest.

9.8.2. IDA recoveries are flagged if they are outside of the acceptance limits (25–150%). Quantitation by isotope dilution generally precludes any adverse effect on data quality due to IDA recoveries being outside of the acceptance limits as long as the signal-to-nose ratio is greater than 10:1.

9.8.2.1. Evaluate data quality for usability, flag and submit a non-conformance memo for any analytes outside of the recovery criteria, and report if data is deemed not adversely effected.

9.8.2.2. If IDA recovery is >150%, check for laboratory error and correct if identified. If no laboratory error is identified proceed as follows:

Condition:	**Corrective Action:**
Field samples are ND for associated native target analytes.	Report the data with appropriate qualifiers and narrative comments.
Field samples are positive for the associated native target analytes and IDA recovery is <200%	Report the data with appropriate qualifiers and narrative comments.
Field samples are positive for the associated native analytes and IDA recovery >200%	RI at an appropriate dilution then report both sets of data with appropriate qualifiers and narrative comments.

9.8.2.3. If IDA recovery is <25% (10% for Me/Et-FOSE), check laboratory error and correct if identified, if no laboratory error is identified, proceed as follows:

<table>
<tr><th>Condition:</th><th colspan="2">Corrective Action:</th></tr>
<tr><td>Field samples are positive for the associated native target analyte and IDA recovery is >10% (>5% for Me/Et-FOSE)</td><td colspan="2">Report the data with appropriate qualifiers and narrative comments</td></tr>
<tr><td rowspan="3">Field samples are ND for associated native target analytes and IDA recovery is >10% (>%5 for Me/Et-FOSE)</td><td colspan="2">Evaluate the S/N of the associated Native analytes in the most recent RL (CCVL) standard or L1 if an ICAL is ran that day:</td></tr>
<tr><td>S/N X the IDA recovery is >10</td><td>Report the data with appropriate qualifiers and narrative comments</td></tr>
<tr><td>S/N X the IDA recovery is <10</td><td>Report an elevated RL if project DQO allows. Qualify and narrate, otherwise RX or RI at an appropriate dilution</td></tr>
<tr><td colspan="3">Example: If the CCVL has 50 S/N X 0.25 (25% IDA) = 12.5. 12.5 > 10 and RL is supported.

If the CCVL has 50 S/N X 0.15 (15% IDA) = 7.5. 7.5 < 10, therefor the RL must be elevated.

Note: if the RL is to be elevated add a comment into the worksheet about such for the 2nd level reviewer.</td></tr>
</table>

9.8.2.4. Re-extraction of samples should be performed if the signal-to-noise for any IDA is less than 10:1 or if the IDA recoveries fall below 10% (<5% for Me/Et-FOSE).

9.8.2.4.1. Re-extraction may be necessary under other circumstances when data quality has been determined to be adversely affected.

9.8.2.5. For samples analyzed in accordance with version 5.1 or higher of the DoD/DOE QSM, the IDA recovery criteria is 50-150%. If QC or field samples do not meet these criteria then see sections 9.8.2.1, 9.8.2.2, and 9.8.2.3for actions.

9.9. Internal Standard

9.9.1. The Internal Standard (IS) is added to each field and QC samples prior to analysis. The CCV IS response (peak area) must not deviate by more than 50% from the average response (peak area) of the initial calibration.

9.9.2. Sample IS response (peak area) must be within ±50% of the response (peak area) in the most recent CCV.

9.9.3. If the IS does not meet criteria, re-analyze the extract, at dilution if needed. If the IS meets criteria in the second analysis, report that analysis. If the IS does not meet criteria in the second analysis, report the first analysis with narration.

9.10. TOP Oxidation Efficiency

9.10.1. If the field sample data indicates incomplete oxidation (i.e. the Post-TOP M2-4:2 FTS recovery is greater than 10% or the Post-TOP precursor concentration is greater than 10% of the Pre-TOP concentration), but the laboratory QA are in control, report the data with narration and/or contact the client to prepare a second billable aliquot (10 mL or a 0.1g equivalent) to be processed.

9.10.2. A reduced sample size may be used initially if sample history or other information indicates the sample is grossly contaminated.

9.11. Ion Ratio

9.11.1. Compare the quantifier/qualifier SRM transition ratio in the sample to the SRM transition ratio in the standard.

Equation 1 $$Ion\ Ratio = \frac{Area\ Quantitation\ Ion\ (1°\ Transition)}{Area\ Qualitative\ Ion\ (2°\ Transition)}$$

9.11.2. The quantifier/qualifier SRM ion ratio should be within ±50% of the average of the quantifier/qualifier SRM ion ratios calculated from the midlevel ICAL point or from the CCV, if an ICAL is not run.

9.11.3. At this time the ion ratio evaluation is a quantitative identification tool. Analyst judgement should be used if the ratio does not meet criteria. Data should be qualified "I" if the ratio is not met.

9.11.4. If the ion ration >2X the target, then do not report the analyte. It should be either ND at the RL or elevate the RL as needed (G flag).

9.11.5. For samples analyzed in accordance with the DoD/DOE QSM version 5.3; if the quantitation ion peak does not meet the maximization criteria the peak shall be included in the summed integration. The result should be flagged "estimated, high bias". As there is not a default qualifier for this in the TALS formatter, use the "see case narrative" flag and NCM the issue.

10. CALIBRATION

10.1. For details of the calculations used to generate the regression equations, and how to use the factors generated by these equations, refer to SOP CA-Q-P-003 "Calibration Curves and Selection of Calibration Points".

10.2. Routine instrument operating conditions are listed in the table in Section 11.17.

10.3. Instrument Tuning & Mass Calibration

10.3.1. Mass Calibration is performed by instrument manufacturer service representatives in accordance with the manufacturer's procedures during installation, and annually thereafter. Mass calibration is performed such that all precursor and product ions for primary and confirmation transitions are bracketed.

10.3.2. Instrument tuning is done initially when the method is first developed and thereafter as needed during troubleshooting. Tuning is done by infusing each individual compound (native and/or IDA) into the mobile phase using a tee fitting at a point just before the entrance to the electrospray probe. The responses for the parent and daughter ions for each compound are observed and optimized for sensitivity and resolution. Mass assignments are reviewed and updated as needed. The mass assignments must be within ± 0.5 amu of the values shown in the table in Section 11.17.

10.3.3. Once the optimal mass assignments (within ±0.5 amu of true) are made immediately following the initial tune, the lowest level standard from the initial calibration curve is assessed to ensure that a signal to noise ratio greater than 10 to 1 (S/N > 10:1) is achieved for each PFAS analyte. The first level standard from the initial calibration curve is used to evaluate the tune stability on an ongoing basis. The instrument mass windows are set initially at ± 0.5 amu of the true value; therefore, continued detection of the analyte transition with S/N > 10:1 serves as verification that the assigned mass remains within ± 0.5 amu of the true value, which meets the DoD/DOE QSM 5.1 tune criterion. For QSM 5.1 or higher work, the instrument sensitivity check (section 10.12.4) is also evaluated to ensure that the signal to noise criteria is met.

10.3.3.1. For samples run in accordance with the DoD/DOE QSM version 5.3, the instrument must have a valid mass calibration prior to sample analysis. This is verified through the acquisition of a full scan continuum mass spectrum of a PFAS stock standard. All masses must be verified to be within ± 0.5 amu of true value.

10.4. A new calibration curve must be generated after major changes to the system or when the continuing calibration criteria cannot be met. Major changes include, but are not limited to, new columns or pump seals. A new calibration is not required after minor maintenance.

10.5. With the exception of the circumstances delineated in policy CA-Q-P-003, it is not acceptable to remove points from a calibration curve. In any event, at least five points must be included in the calibration curve. Average Response Factor and linear fit calibrations require five points, whereas Quadratic (second order) calibrations require six points.

10.6. A fixed injection volume is used for quantitation purposes and is to be the same for both the sample and standards.

10.7. All units used in the calculations must be consistently uniform, such as concentration in ng/mL.

10.8. Some jurisdictions may have additional requirements for PFAS in initial and continuing calibrations. These are detailed in the document WS-WI-0066, “Agency Specific Criteria for PFAS in Matrices Other Than Drinking Water”.

10.9. Retention Times

10.9.1. Retention time windows of at least ± 0.25 minutes are set within the data system to facilitate peak identification.

10.9.2. The retention times of the target and reference compounds are initially set in the data system using the mid-range standard from the initial calibration. They are updated using the first CCV on days when an initial calibration is not performed.

10.10. Initial Calibration

10.10.1. A number of analytical standards of different analyte concentrations are used to generate the curve. Each standard is injected once to obtain the peak response for each analyte at each concentration. These standards define the working range of the analysis.

10.10.1.1. A minimum of five analytical standards is used when using average response factor and/or linear calibration fits.

10.10.1.2. A minimum of six analytical standards is used when a quadratic fit is used to generate the curve.

10.10.2. Calibration is by average response factor, linear fit, or by quadratic fit. Quadratic fit is used for the analyte if the response is non-linear.

10.10.2.1. For average response factor (RFa), the relative standard deviation (RSD) for all compounds except those identified as poor performers must be < 30% for the curve to be valid.

10.10.2.2. Poor performing analytes are: 6:2 FTS, PFHxDA and PFODA.

10.10.2.3. For average response factor (RFa), the relative standard deviation (RSD) for poor performing compounds must be < 50% for the curve to be valid.

10.10.2.4. For linear fit, the intercept of the line must be less than ½ the reporting limit, and the coefficient of determination (r2) must be greater than or equal to 0.990 for the curve to be considered valid (or the correlation coefficient (r) > 0.995).

10.10.2.5. For quadratic fits, the intercept of the line must be less than ½ the reporting limit, and the coefficient of determination (r2) must be greater than or equal to 0.990 for the curve to be considered valid.

10.10.2.6. The Internal Standard (IS) response (peak area) must not deviate by more than 50% from the average response (peak area) of the initial calibration.

10.10.2.7. Criteria for samples analyzed in accordance with QSM 5.3 or higher:

- The %RSD of the RFs for all analytes must be <20%.
- Linear or non-linear calibrations must have r^2>0.99 for each analyte.
- Analytes must be within 70-130% of their true value for each calibration standard.

10.11. Calibration Curve Fits

10.11.1. Linear regression or quadratic curves may be used to fit the data to a calibration function. Detailed descriptions and formulas for each fitting type can be found in SOP CA-Q-P-003, "Calibration Curves and Selection of Calibration Points".

10.11.2. The Chrom data system is programmed to complement the calibration evaluation guidelines in policy CA-Q-P-003 by evaluating calibration curve fits in the order listed below. An optimal fit is recommended to the analyst, who may override based on evaluation of the residuals for each calibration level, as per policy CA-Q-P-003.

- Average Response Factor
- Linear, 1/concentration^2 weighting
- Linear, 1/concentration weighting, forced through zero
- Quadratic, 1/concentration^2 weighting

10.11.3. The linear curve uses the following function:

Equation 2

$$y = bx + c$$

Where:

y = $\frac{Area\ (Analyte)}{Area\ (IDA)} \times Concentration(IDA)$

x = concentration

b = slope

c = intercept

10.11.4. The quadratic curve uses the following function:

Equation 3

$$y = ax^2 + bx + c$$

Where y, x, b, and c are the same as above, and a = curvature.

10.11.5. Evaluation of Calibration Curves

The following requirements must be met for any calibration to be used:

- Response must increase with increasing concentration.

- The absolute value of the intercept of a regression line (linear or non-linear) at zero response must be less than the reporting limit.
- There should be no carryover at or above 1/2 MRL after a high CAL standard.

If these criteria are not met, instrument conditions and standards will be checked, and the ICAL successfully repeated before continuing.

10.11.6. Weighting of Calibration Points

In linear and quadratic calibration fits, the points at the lower end of the calibration curve have less absolute variance than points at the high concentration end of the curve. This can cause severe errors in quantitation at the low end of the calibration. Because accuracy at the low end of the curve is very important for this analysis, it is preferable to increase the weighting of the lower concentration points. 1/concentration or 1/x weighting is encouraged. Visual inspection of the line fitted to the data is important in selecting the best fit.

10.12. Initial Calibration Blank (ICB)

10.12.1. Immediately following the ICAL, a calibration blank is analyzed that consists of an injection of 80:20 methanol:water blank containing both IDA and IS.

10.12.2. The result for the calibration blank must be less than the reporting limit.

10.12.3. If the ICB is greater than the reporting limit then the source of contamination must be identified and any necessary cleaning completed, and then the instrument should be recalibrated.

10.12.4. Criteria for samples analyzed in accordance with QSM 5.3 or higher:

- Instrument blanks are required immediately following the highest standard analyzed and *daily prior to sample analysis*.
- The instrument blank must be < ½ the LOQ.

10.13. Initial Calibration Verification (ICV)

10.13.1. Following the ICAL and the ICB, an ICV standard obtained from a different source or vendor than the ICAL standards is analyzed. This ICV standard is a mid-range standard.

10.13.2. The recovery for the ICV must meet the appropriate following criteria:

10.13.2.1. The native analyte must be within or equal to 70-130% for all native analytes. The native analyte must be within or equal to 50-150% for all poor performing analytes. The IDA must be within or equal to 50-150%.

10.13.3. Criteria for samples analyzed in accordance with QSM 5.3 or higher: Analyte concentrations must be within ±30% of their true values for all analytes, IDA and target.

10.13.4. See Section 9.7 for corrective actions in the event that the ICV does not meet the criteria above.

10.14. Continuing Calibration Verification (CCV)

Analyze a CCV at the beginning of a run, the end of a run, and after every 10 samples to determine if the calibration is still valid. The exception is after an acceptable curve and ICV are run 10 samples can be analyzed before a CCV is required. The CCVs are usually at the mid-level range of the curve and should vary throughout the run from low level (LOQ/RL) to mid-level. The curve and ICV do not need to be run every day. To start an analytical on days when an ICAL is not performed, a CCV and CCVL (low standard at or below the RL and at or above the lowest levels used in the ICAL) are analyzed and if they meet acceptance criteria a run can be started.

10.14.1. The recovery for the CCV standards must be equal to or within 70-130% for all natives and equal to or within 50% to 150% for all poor performers. The recovery for the IDA must be within or equal to 50-150%.

10.14.2. The recovery for the CCVL (low-level CCV at or below the RL) standards must be equal to or within 50-150% for all natives and IDA.

10.14.3. The Internal Standard (IS) response (peak area) must be within ± 50% from the response (peak area) from the midpoint of the initial calibration.

10.14.3.1. Sample IS response (peak area) must be within ± 50% of the response (peak area) in the most recent CCV.

10.14.4. If this is not achieved, the instrument has drifted outside the calibration limits. The instrument must be recalibrated.

10.14.5. Criteria for samples analyzed in accordance with QSM 5.3 or higher:

- All analyte concentrations must be within ± 30% of their true value.
- Additionally, prior to analysis and at least once every 12 hours an instrument sensitivity check (ISC/CCVL) must be analyzed. The analyte

concentrations must be at LOQ and the concentrations must be within ± 30% of their true value. This can be used as a CCV.

11. PROCEDURE

11.1. One-time procedural variations are allowed only if deemed necessary in the professional judgment of a supervisor to accommodate variation in sample matrix, chemistry, sample size, or other parameters. Any variation in procedure shall be completely documented using a non-conformance memo (NCM). The NCM process is described in more detail in SOP WS-QA-0023. The NCM shall be filed in the project file and addressed in the case narrative.

Any deviations from this procedure identified after the work has been completed must be documented in an NCM, with a cause and corrective action described.

11.2. Water Sample Preparation

11.2.1. Visually inspect samples for the presence of settled and/or suspended sediment/particulates. If present or if the sample is biphasic add IDA prior to any sample decanting or centrifugation. If the sample requires decanting or centrifugation contact the client for guidance prior to such action. Decanting or filtering of the sample can lead to a low bias. Filtration is discouraged.

11.2.2. If authorized by the client to filter the sample, filter the water sample through a glass fiber filter (Whatman GF/F Cat No 1825 090 or equivalent). Gravity or vacuum can be used to pass the sample through the filter. Prepare a filtration blank with any samples requiring filtration. File an NCM noting the need for filtration.

11.2.2.1. Filters are rinsed with 4 mL of 0.3% NH_4OH/methanol when the bottle is rinsed (Section 11.5.1).

Warning: The use of a vacuum system creates the risk of glassware implosion. Inspect all glassware prior to use. Glassware with chips, scratches, rub marks or cracks must not be used.

11.2.3. Weigh the sample container prior to extraction and then weigh the sample container after extraction to determine the initial volume. Unless otherwise directed by client, use the entire sample volume, and spike directly into the sample container.

11.2.4. Prepare additional aliquots of a field sample for the MS/MSD, if requested.

11.2.5. Prepare two 250 mL aliquots of HPLC-grade water for the method blank

and LCS.

11.2.6. Vortex the LCS/Matrix PFC Spike and IDA PFC solutions prior to use.

11.2.7. Spike the LCS and MS/MSD (if requested) with 0.5 mL of the LCS/Matrix PFC Spike solution (Section 7.3). This will result in a sample concentration of 40 ng/L.

11.2.8. Add 0.5 mL of the IDA PFC solution (Section 7.4) into each sample and QC sample, for a fixed concentration of 1.25-2.5 ng/mL in the final sample vial. Allow the spikes to equilibrate with the samples for at least 10 minutes before loading onto the SPE cartridge (Section 11.3.5).

11.3. Solid Phase Extraction (SPE) of Aqueous Samples

The automated Zymark Auto-Trace Workstation can be used as long as the program follows these conditions and passes the background check.

11.3.1. Condition the SPE cartridges (Section 6.9.2, [redacted] or equivalent) by passing the following without drying the column.

Note: *The cartridges should not be allowed to go dry until the final elution step with methanol. At all of the other transition steps, the solvent/sample level should be stopped at the top of the column before the next liquid is added.*

WARNING: The use of a vacuum system creates the risk of glassware implosion. Inspect all glassware prior to use. Glassware with chips, scratches, rub marks or cracks must not be used.

11.3.2. Wash with 5.0 mL of [redacted]

11.3.3. Wash with 5.0 mL of 0.1N NaOH/water. Close valve when ~ 200 uL remains on top to keep column wet. After this step, the columns cannot go dry until the completion of loading and rinsing samples.

11.3.4. Appropriately label the columns and add the reservoir to the column. Be certain to rotate method blank samples through each sample port on the SPE manifold, such that each new batch uses a different port for the MB.

11.3.5. Add samples to the columns and with vacuum, pull the entire 250 mL aliquot of the sample through the cartridge at a rate of approximately 2 to 5 drops per second.

11.3.5.1. If the SPE column should plug (flow rate <1 drop per minute) prior to the entire content of the sample container passing through the column do the following:

1. Stop adding sample to the reservoir.
2. Return any remaining sample volume back to the original container.
3. Weigh the original container and record this weight into the worksheet notes field within the TALS extraction batch.
4. Determine the full volume of sample fortified by using the "Gross Weight" – (remaining sample volume – default tare weight of a sample container (26.1 g)).
5. Enter this value into the "Initial Amount" field in the TALS extraction batch.
6. Proceed to Section 11.4, noting that additional vacuum or pressure might be needed to elute the SPE column.

11.3.6. After the entire sample has been loaded onto the column, rinse the sample bottle with two 5 mL aliquots of reagent water and pour onto the column reservoir.

11.3.7. After the final loading of the sample but before completely passed through the column, rinse the SPE column with 1 mL of water.

11.3.8. After the sample and water rinse have completely passed through the cartridge, allow the column to dry well with vacuum for 15 minutes.

11.4. SPE Column Wash of Aqueous Samples with 30:70 methanol:water.

11.4.1. Load the first 5 mL of 30:70 methanol:water wash and let soak for five minutes and then elute to waste.

11.4.2. Load the second 5 mL of 30:70 methanol:water wash and elute to waste (without a soaking period).

11.4.3. Allow the column to dry with vacuum for 10 minutes. Columns must be dried before continuing.

11.5. SPE Elution of Aqueous Samples – using 15 mL polypropylene test tubes as receiving tubes in the SPE manifold.

11.5.1. Rinse sample bottles with 4 mL [redacted] and transfer to the column reservoir onto the cartridge. Allow the solution to soak for 5 minutes and then elute into the 15 mL collection tube.

11.5.2. Repeat sample bottle to column reservoir rinse and cartridge elution with a second 4 mL aliquot [redacted] The total collection should be approximately 8 mL.

11.5.3. Proceed to Section 11.6 for final volume.

11.6. Final volume for extract

11.6.1. Vortex the IS solution prior to use.

11.6.2. Add 0.5 mL of IS (Section 7.6) at 25 ng/mL concentration and 2 mL of water to the extract, for a final volume of 10 mL. Verify that the volume 10 mL using the graduations on the tube. This will create an extract with a final solvent composition of 80:20 methanol:water.

11.6.2.1. Seal the test tube tightly. Invert container several times and then vortex. Allow extract to settle for 10 minutes prior to moving to the next step. This permits particulates (SPE resin and/or residual carbon) to settle to the bottom of the tube so that they are not transferred to the autosampler vial.

11.6.3. Transfer a portion of the extract to a 300 uL polypropylene autosampler vial (7 drop-wise or approximately ½ filled is sufficient). Archive the rest of the extracts for re-injection and dilution.

11.6.4. Seal the vial with a polypropylene screw cap. Note: Teflon lined caps cannot be used due to detection of low level concentration of PFAS.

11.7. Soil, Sediment and Tissue Sample Preparation and Extraction

11.7.1. Visually inspect soil samples. Homogenize the entire sample in accordance with SOP WS-QA-0018. If the sample cannot be mixed in the container, pour into a larger QC'd PFAS-free container and mix thoroughly. Transfer the sample label to the new container.

11.7.2. Weigh a representative 5 g aliquot of sample (1g for tissues) into a 50 mL centrifuge tube. Weigh additional sample amounts for the matrix spike and matrix spike duplicate analyses if they are requested.

11.7.3. For the method blank and LCS matrix, use 5 g each of Ottawa sand (solids) or 0.02 g of vegetable oil (tissues).

11.7.4. Vortex the LCS/Matrix PFC Spike and IDA PFC solutions prior to use.

11.7.5. Spike the LCS and MS/MSD (if requested) with 0.5 mL of the LCS/Matrix PFC Spike solution (Section 7.3).

11.7.6. Add 0.5 mL of the IDA PFC solution (Section 7.4) into each sample and QC sample, for a fixed concentration of 1.25 ng/mL in the final sample vial.

11.7.7. Cap the tubes and allow the spike to settle into the sample matrix. Gently shake the tubes to mix the spike into the matrix.

11.7.8. Add 10 mL of [redacted] to each sample. Cap and vortex.

11.7.9. Extract the samples in an ultrasonic water bath for 1 hour.

11.7.10. After the completion of extraction, centrifuge each sample at 3500 rpm for 5 minutes.

11.7.11. Collect and decant the [redacted] into a new container.

11.7.12. Add another 4 mL of [redacted] solution to the residue, briefly shake to mix and centrifuge at 3500 rpm for 5 minutes.

11.7.13. Combine the rinsate to the first corresponding containers.

11.7.14. To the final [redacted] extract, bring the volume up to 125 mL with HPLC-grade water.

11.7.15. Neutralize with glacial acetic acid and/or sodium hydroxide, and mix the contents well with vortex mixer. Check the pH to ensure pH is between 6 and 8.

11.8. Solid Extract Cleanup by SPE

Set up SPE cartridges (Section 6.9.2, [redacted] or equivalent) for sample cleanup using vacuum manifold.

11.8.1. Condition the SPE cartridges by passing the following without drying the column.

Note: *The cartridges should not be allowed to go dry until the final elution step with methanol. At all of the other transition steps, the solvent/sample level should be stopped at the top of the column before the next liquid is added.*

WARNING: The use of a vacuum system creates the risk of glassware implosion. Inspect all glassware prior to use. Glassware with chips, scratches, rub marks or cracks must not be used.

11.8.2. Wash with 5.0 mL of [redacted]

11.8.3. Wash with 5 mL of 0.1 N NaOH/water. Close valve when ~ 500 µL remains on top of column to keep column wet. *After this step, the columns cannot go dry until the completion of loading and rinsing samples.*

11.8.4. Be certain to rotate method blank samples through each sample port on the

SPE manifold, such that each new batch uses a different port for the MB.

11.8.5. Add extracts to the columns and with vacuum, pull the entire extracts through the cartridge at rate of approximately 3 to 5 drops per second.

11.8.6. Rinse the sample tube with 5 mL of water and add to the SPE column.

11.8.7. Dry the columns with vacuum for 15 minutes.

11.9. SPE Column Wash of Solid Extracts with 30:70 methanol:water.

11.9.1. Load the first 5 mL of the 30:70 methanol:water wash to soak for five minutes, and elute to waste.

11.9.2. Load the second 5 mL of the 30:70 methanol:water wash and elute to waste (without a soaking period).

11.9.3. Allow the column to dry with vacuum for 10 minutes. Columns must be dried before continuing.

11.10. SPE Elution of Solid Extracts – using 15 mL polypropylene test tube as receiving tube in the SPE manifold.

11.10.1. Rinse extraction bottles with 4 mL of [redacted] and transfer to the column reservoir onto the cartridge. Allow the solution to soak for 5 minutes and then elute into the 15 mL collection tube.

11.10.2. Repeat extract bottle to column reservoir rinse and cartridge elution with a second 4 mL aliquot of [redacted] The total collection should be approximately 8 mL.

11.10.3. Proceed to Section 11.6 for final volume.

11.11. Product/Waste Samples

Note: Please see WS-WI-0065 for the preparation of methanol extracts and dispersion samples.

Vortex all spike solutions immediately prior to use.

11.11.1. Check the solubility of the material in both methanol and water

11.11.1.1. If the material is soluble in water, dilute 0.5 mL of sample into 250 mL of DI water and proceed to Section 11.3 (follow water extraction procedures). Fortify sample appropriately with IDA or PFC spike solution, see Section 11.2.

11.11.1.2. If the material is soluble in methanol, dilute 1 g (if solid) or 1 mL (if liquid) of material into 10 mL of methanol (MeOH).

- If the material does not completely dissolve, contact your immediate supervisor.

11.11.2. Take 100 µL of the 10 mL solution and dilute it to 10 mL in MeOH.

11.11.3. Take a 1 mL aliquot of this solution (effective dilution of 1000x (1 mg for solid or 0.001 mL for liquid)) and fortify with 0.5 mL of labeled IDA solution (Section 7.4).

11.11.4. Prepare two 1.0 mL/g aliquots of HPLC-grade water for the method blank and LCS.

11.11.5. Spike the LCS and MS/MSD (if requested) with 0.5 mL of the LCS/Matrix PFC Spike solution (Section 7.3).

11.11.6. Add 0.5 mL of the IDA PFC solution (Section 7.4) into each QC sample.

11.11.7. DO NOT PASS EXTRACT THROUGH SPE CARTIRIDGE (omit steps 11.9 – 11.11).

11.11.8. Proceed to Section 11.6 for final volume.

11.12. TOP (Total Oxidizable Precursor) Assay for Aqueous Samples

11.12.1. Prepare 3-250 mL HDPE containers with HPLC grade water to create the needed QC Samples (MB, LCS/LCSD).

11.12.2. Prepare enough 125 mL HDPE containers as needed for all “Pre” and “Post” samples, including QC. Label each appropriately.

11.12.3. Vortex reverse surrogate and LCS spike solutions immediately prior to use.

11.12.4. Spike the “Pre” and “Post” MB 125 mL containers with 25 µL of the reverse surrogate solution of M2-4:2 FTS (Section 7.8).

11.12.5. Spike the “Pre” and “Post” LCS/LCSD 125 mL containers with 0.5 mL of the LCS Spike solution (Section 7.6), and 25 uL of the reverse surrogate solution (Section 7.8).

11.12.6. Add 2g of [redacted] and 1.9 mL of [redacted] to each “Post” sample container.

11.12.7. Subsample 100 mL aliquots of water from each field sample and QC from the 250 mL containers into each of the corresponding 125 mL containers for both the "Pre" and "Post" samples. Spike all "Pre" and "Post" samples with 25uL of the reverse surrogate solution (Section 7.9).

11.12.8. Set aside all "Pre" sample containers.

11.12.9. Cap each "Post" sample container, invert 2-3 times prior to placing container into water bath.

11.12.10. Add 2 g of [redacted] and 1.9 mL of [redacted] to each "Post" sample container.

11.12.11. Heat each "Post" sample container in a water bath (KD) at 85°C for 6 hours.

11.12.12. After digestion for 6 hours, place the "Post" sample containers in an ice bath for 30 minutes.

11.12.13. Adjust the pH of "Post" samples and associated QC aliquots to 7 with concentrated HCl. Use pH paper to determine the pH.

11.12.14. Spike both "Pre" and "Post" samples and their associated QC samples with 0.5 mL of TOP IDA solution (Section 7.10). Vortex the IDA solution prior to use.

11.12.15. Proceed to Section 11.13.26 SPE for TOP Assay for both "Pre" and "Post" aliquots.

11.13. TOP (Total Oxidizable Precursor) Assay for Soil Samples

11.13.1. Weigh representative 1 g aliquots of soil for each "Pre" and "Post" sample into a 50 mL centrifuge tube.

11.13.2. For the method blank and LCS matrix, use 1 g each of Ottawa sand for each "Pre" and "Post" QC sample.

11.13.3. Add 20 mL of [redacted] to each sample.

11.13.4. Extract the samples in an ultrasonic water bath for 1 hour.

11.13.5. After the completion of extraction, centrifuge each sample at 3500 rpm for 5 minutes.

11.13.6. Collect and decant the [redacted] extract to a new 50 mL centrifuge tube.

11.13.7. Add another 2 mL of [redacted] solution to the residue, briefly shake to mix and centrifuge at 3500 rpm for 15 minutes.

11.13.8. Combine the rinsate to the first corresponding tubes.

11.13.9. Proceed to Section 11.15.2 (Envi-carb clean up), following steps 11.15.2.1. through 11.15.2.4.

11.13.10. To the final [redacted] extract, add 0.5 mL of water to each.

11.13.11. Concentrate the [redacted] extract under nitrogen to less than 0.25 mL.

11.13.12. Dilute extract up to 50 mL with water in the centrifuge tube and vortex.

11.13.13. Prepare enough 125 mL HDPE containers as needed for all “Pre” and “Post” samples, including QC. Label each appropriately.

11.13.14. Vortex reverse surrogate and LCS spike solutions immediately prior to use.

11.13.15. Spike the “Pre” and “Post” MB 125 mL containers with 25 µL of the reverse surrogate solution of M2-4:2 FTS (Section 7.9).

11.13.16. Spike the “Pre” and “Post” LCS/LCSD 125 mL containers with 0.5 mL of the LCS Spike solution and 25 µL of the reverse surrogate solution (Sections 7.3 and 7.9).

11.13.17. Add 2 g of [redacted] and 1.9 mL of [redacted] to each “Post” sample container.

11.13.18. Transfer extract from the centrifuge tube to the appropriate 125 mL container.

11.13.19. Rinse the centrifuge container with an additional 50 mL of water and transfer to the appropriate 125 mL container.

11.13.20. Set aside all “Pre” sample containers.

11.13.21. Cap each “Post” sample container, invert 2-3 times prior to placing container into water bath.

11.13.22. Heat each “Post” sample container in a water bath (KD) at 85°C for 6 hours.

11.13.23. After digestion for 6 hours, place the “Post” sample containers in an ice bath for 30 minutes.

11.13.24. Adjust the pH of both "Pre" and "Post" samples and associated QC aliquots to 7 with concentrated HCl. Use pH paper to determine the pH.

11.13.25. Spike both "Pre" and "Post" samples and their associated QC samples with 0.5 mL of TOP IDA solution (Section 7.10). Vortex the IDA solution prior to use.

11.13.26. Proceed to Section 11.13.26 SPE for TOP Assay for both "Pre" and "Post" aliquots.

11.14. SPE Extraction for TOP Assay

Use the following SPE procedure for both "Pre" and "Post" samples for both solid and aqueous samples:

11.14.1. Set up [redacted] SPE columns for sample extraction using a vacuum manifold.

11.14.2. Establish a sample loading flow rate of 3-5 drops per second for each port of the vacuum manifold, for as many ports as will be used simultaneously during sample loading.

11.14.3. Wash/condition the SPE column with 5 mL of [redacted] then 5 mL water.

11.14.4. Load 100 mL of sample onto the SPE cartridge at a flow rate of 3-5 drops per second.

11.14.5. Add 5 mL rinse water

11.14.6. After the sample and water rinse have completely passed through the column, allow it to dry well using vacuum with a flow rate of 1 mL/minute for 15 minutes.

11.14.7. Wash the SPE column with 10 mL hexane rinse eluting all to waste.

11.14.8. Allow the column to dry well using vacuum for 5 minutes. Columns must be dry before continuing.

11.14.9. Elute the samples into 15 mL polypropylene test tubes in the SPE manifold by rinsing each 125 mL sample container with 4 mL of [redacted], and add to the SPE cartridge as eluent.

11.14.10. Repeat with another 4 mL of [redacted].

11.14.11. Collect the eluent and proceed to Section 11.6 for final volume.

11.15. Other Types of Sample Cleanup

11.15.1. Freezing technique to remove lipids.

If samples contain lipids then freeze the methanolic extract and QC extracts at -20^{o}C for at least 1 hour. Collect the solvent layer.

11.15.2. Additional cleanup with graphitized carbon can be applied to those samples with severe matrix impacts that can benefit from an additional treatment of carbon.

11.15.2.1. Add 100 mg of graphitized carbon to each sample extract and QC extracts.

11.15.2.2. Shake vigorously and then let sit for 10 minutes.

11.15.2.3. Centrifuge each sample for 2 minutes at 1000 rpm.

11.15.2.4. Decant the solvent layer.

11.15.2.5. Proceed to Section 11.6.

11.16. AFFF Sample Preparation

11.16.1. QC for AFFF samples consists of a method blank, a laboratory control sample and a sample or matrix duplicate only. No matrix spike or matrix spike duplicate is needed.

11.16.2. Perform a 1,000,000 X serial dilution of the AFFF sample. Dilute 1 mL of AFFF sample to 1 L with laboratory supplied water. Then dilute 1mL of this dilution to 1 L with laboratory supplied water.

11.16.2.1. Be sure to retain all dilutions should the initial analysis warrant re-analysis at higher concentration.

11.16.3. Subsample 2.0 mL of this dilution and fortify with 0.5 mL IDA solution and 0.5 mL of IS (1.25-2.5 ng/mL) solution: then add 7.0 mL of methanol. Vortex the IDA solution prior to use.

11.16.4. Vortex the subsample, then transfer a portion of the sample to a 300 µL polypropylene autosampler vial (7 drop-wise or approximately ½ filled is sufficient). Archive the rest of the sample for re-injection or dilution.

11.17. Instrument Analysis

Suggested operating conditions are listed in Tables 1-4 for the SCIEX LCMS systems:

Table 11.17 - 1 **Recommended Instrument Operating Conditions**				
HPLC Conditions ([redacted])				
Column (Column temp = [redacted]°C)	[redacted]			
Mobile Phase Composition	A = [redacted] B = [redacted]			
Gradient Program	**Time**	**%A**	**%B**	**Flow Rate - mL/min**
	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]
	[redacted]	[redacted]	[redacted]	[redacted]
	Maximum pressure limit = 5,000 psi			
Injection Size	[redacted] (fixed amount throughout the sequence).			
Run Time	~[redacted]			
Mass Spectrometer Interface Settings ([redacted])				
MS Interface Mode	ESI Negative Ion. Minimum of 10 scans/peak.			
Ion Spray Voltage (kV)	[redacted]			
Entrance Potential (V)	[redacted]			
Declustering Potential (V)	[redacted]			
Desolvation Temp	[redacted]°C			
Curtain Gas	[redacted]			
Collision Gas	[redacted]			

Table 11.17 - 2 **Recommended Instrument Operating Conditions**								
Mass Spectrometer Scan Settings ([redacted])								
Compound	Comments	Reaction (MRM)	Dwell (sec)	Ent. Pot. (V)	Col. Energy (V)	Declu. Pot. (V)	Cell Exit Pot. (V)	Typ RT (Min)
PFPrA	Native Analyte	162.95>119	0.011	[redacted]				
MTP	Native Analyte	175>97	0.011	[redacted]				
PFMOAA	Native Analyte	179>84.9	0.011	[redacted]				
PFBA	Native Analyte	212.9>169	0.011	[redacted]				
13C4_PFBA	IDA	217>172	0.011	[redacted]				
PFECA F	Native Analyte	229>85	0.011	[redacted]				
PMPA	Native Analyte	229>185	0.011	[redacted]				
3:3 FTCA	Native Analyte	241>177.1	0.011	[redacted]				
3:3 FTCA_2	Native Analyte	241>116.9	0.011	[redacted]				
PFO2HxA	Native Analyte	245>85	0.011	[redacted]				
PFPrS	Native Analyte	249.1>80	0.011	[redacted]				

Table 11.17 - 2
Recommended Instrument Operating Conditions
Mass Spectrometer Scan Settings ([redacted])

Compound	Comments	Reaction (MRM)	Dwell (sec)	Ent. Pot. (V)	Col. Energy (V)	Declu. Pot. (V)	Cell Exit Pot. (V)	Typ RT (Min)
PFPeA	Native Analyte	262.9>219	0.011	[redacted]				
13C5_PFPeA	IDA	267.1>223	0.011					
PEPA	Native Analyte	278.9>234.9	0.011					
PFECA A	Native Analyte	278.95>84.9	0.011					
HFPO-DA	Native Analyte	285>169	0.011					
HFPO-DA_2	Native Analyte	285>185	0.011					
13C3_HFPO-DA	IDA	287>169	0.011					
PFECA B	Native Analyte	295.1>201	0.011					
NVHOS	Native Analyte	297>135	0.011					
PFBS	Native Analyte	298.9>80	0.011					
PFBS_2	Native Analyte	298.9>99	0.011					
M3-PFBS	IDA	301.9>80	0.011					
PFO3OA	Native Analyte	311.1>85.2	0.011					
PFHxA	Native Analyte	313>269	0.011					
PFHxA_2	Native Analyte	313>119	0.011					
PES	Native Analyte	314.8>135	0.011					
13C2_PFHxA	IDA	315>270	0.011					
4:2 FTS	Native Analyte	327>307	0.011					
4:2FTS_2	Native Analyte	327>80	0.011					
M2-4:2FTS	IDA	329>81	0.011					
5:3 FTCA	Native Analyte	340.88>236.9	0.011					
5:3 FTCA_2	Native Analyte	340.88>216.9	0.011					
PFPeS	Native Analyte	349>80	0.011					
PFPeS_2	Native Analyte	349>99	0.011					
6:2 FTUCA	Native Analyte	356.86>292.9	0.011					
6:2 FTUCA_2	Native Analyte	356.86>243	0.011					
6:2 FTUCA_3	Native Analyte	356.95>93	0.011					
13C-6:2 FTUCA	IDA	358.86>293.9	0.011					
PFHpA	Native Analyte	363>319	0.011					
PFHpA_2	Native Analyte	363>169	0.011					
13C4_PFHpA	IDA	367>322	0.011					
PFO4DA	Native Analyte	376.9>85	0.011					
DONA	Native Analyte	377>251	0.011					
DONA_2	Native Analyte	377>85	0.011					
6:2 FTCA	Native Analyte	377.1>313.1	0.011					
6:2 FTCA_2	Native Analyte	377.1>63	0.011					
13C-6:2 FTCA	IDA	378.88>293.9	0.011					
PFECA G	Native Analyte	378.9>184.9	0.011					
R-PSDCA	Native Analyte	397>217	0.011					
PFHxS	Native Analyte	399>80	0.011					
PFHxS_2	Native Analyte	399>99	0.011					
18O2_PFHxS	IDA	403>84	0.011					
R-EVE	Native Analyte	405>217	0.011					
PFOA	Native Analyte	413>369	0.011					
PFOA_2	Native Analyte	413>169	0.011					
13C2_PFOA	IDA	415>370	0.011					
13C4_PFOA	IDA	417>372	0.011					

Table 11.17 - 2
Recommended Instrument Operating Conditions

Mass Spectrometer Scan Settings ([redacted])

Compound	Comments	Reaction (MRM)	Dwell (sec)	Ent. Pot. (V)	Col. Energy (V)	Declu. Pot. (V)	Cell Exit Pot. (V)	Typ RT (Min)
13C8_PFOA	IDA	421>376	0.011	[redacted]				
6:2 FTS	Native Analyte	427>407	0.011					
Hydro-EVE Acid	Native Analyte	427>282.9	0.011					
6:2 FTS_2	Native Analyte	427>79.96	0.011					
M2-6:2FTS	IDA	429>81	0.011					
7:3 FTCA	Native Analyte	441>337	0.011					
7:3 FTCA_2	Native Analyte	441>317	0.011					
PS Acid	Native Analyte	442.8>146.8	0.011					
PFO5DoA (TAF)	Native Analyte	442.9>85	0.011					
PFHpS	Native Analyte	449>80	0.011					
PFHpS_2	Native Analyte	449>99	0.011					
8:2 FTUCA	Native Analyte	456.86>392.9	0.011					
8:2 FTUCA_2	Native Analyte	456.86>343	0.011					
13C-8:2 FTUCA	IDA	458.86>393.6	0.011					
EVE Acid	Native Analyte	407 > 262.9	0.011					
PFECHS_2	Native Analyte	460.8>98.9	0.011					
PFECHS	Native Analyte	460.8>380.9	0.011					
PFNA	Native Analyte	463>419	0.011					
PFNA_2	Native Analyte	463>169	0.011					
Hydro-PS Acid	Native Analyte	463>263	0.011					
13C5_PFNA	IDA	468>423	0.011					
8:2 FTCA	Native Analyte	477>393.1	0.011					
8:2 FTCA_2	Native Analyte	477>63.2	0.011					
13C-8:2 FTCA	IDA	478.85>393.9	0.011					
PFOSA	Native Analyte	498>78	0.011					
PFOS	Native Analyte	499>80	0.011					
PFOS_2	Native Analyte	499>99	0.011					
13C4_PFOS	IDA	503>80	0.011					
13C8_PFOSA	IDA	506>78	0.011					
13C8_PFOS	IDA	507>99	0.011					
MeFOSA	Native Analyte	512>169	0.011					
MeFOSA_2	Native Analyte	512>218.99	0.011					
PFDA	Native Analyte	513>469	0.011					
PFDA_2	Native Analyte	513>169	0.011					
13C2_PFDA	IDA	515>470	0.011					
d3MeFOSA	IDA	515>169	0.011					
EtFOSA	Native Analyte	526>169	0.011					
EtFOSA_2	Native Analyte	526>218.99	0.011					
8:2 FTS	Native Analyte	527>507	0.011					
8:2 FTS_2	Native Analyte	528.97>79.96	0.011					
M2-8:2FTS	IDA	529>81	0.011					
d5EtFOSA	IDA	531>169	0.011					
9Cl-PF3ONS	Native Analyte	531>351	0.011					
9Cl-PF3ONS_2	Native Analyte	531>79.96	0.011					
PFNS	Native Analyte	549>80	0.011					
PFNS_2	Native Analyte	549>99	0.011					
10:2 FTUCA	Native Analyte	556.86>492.9	0.011					

Table 11.17 - 2
Recommended Instrument Operating Conditions
Mass Spectrometer Scan Settings ([redacted])

Compound	Comments	Reaction (MRM)	Dwell (sec)	Ent. Pot. (V)	Col. Energy (V)	Declu. Pot. (V)	Cell Exit Pot. (V)	Typ RT (Min)
10:2 FTUCA_2	Native Analyte	556.97>472.99	0.011	[redacted]				
13C-10:2 FTUCA	IDA	558.86>493.9	0.011					
PFUdA	Native Analyte	563>519	0.011					
PFUdA_2	Native Analyte	563>169	0.011					
13C2_PFUdA	IDA	565>520	0.011					
N-MeFOSAA	Native Analyte	570>419	0.011					
N-MeFOSAA_2	Native Analyte	570>483	0.011					
d3-MeFOSAA	IDA	573>419	0.011					
10:2 FTCA	Native Analyte	576.8>493	0.011					
10:2 FTCA_2	Native Analyte	576.8>63.1	0.011					
13C-10:2 FTCA_3	IDA	578.8>493.9	0.011					
N-EtFOSAA	Native Analyte	584>419	0.011					
N-EtFOSAA_2	Native Analyte	584>526.1	0.011					
d5-EtFOSAA	IDA	589>419	0.011					
PFDS	Native Analyte	599>80	0.011					
PFDS_2	Native Analyte	599>99	0.011					
PFDoA	Native Analyte	613>569	0.011					
PFDoA_2	Native Analyte	613>169	0.011					
13C2_PFDoA	IDA	615>570	0.011					
N-MeFOSE	Native Analyte	616>59	0.011					
d7N-MeFOSE	IDA	623>59	0.011					
10:2 FTS	Native Analyte	627>607	0.011					
10:2 FTS_2	Native Analyte	627>79.96	0.011					
N-EtFOSE	Native Analyte	630>59	0.011					
11Cl-PF3OUdS	Native Analyte	631>451	0.011					
11Cl-PF3OUdS_2	Native Analyte	631>79.96	0.011					
M2-10:2FTS	IDA	634.21>612	0.011					
d9N-EtFOSE	IDA	639>59	0.011					
PFTrDA	Native Analyte	663>619	0.011					
PFTrDA_2	Native Analyte	663>169	0.011					
PFDoS	Native Analyte	699>80	0.011					
PFDoS_2	Native Analyte	699>99	0.011					
PFTeDA	Native Analyte	713>169	0.011					
PFTeDA_2	Native Analyte	713>219	0.011					
13C2_PFTeDA	IDA	715>670	0.011					
PFHxDA	Native Analyte	813>769	0.011					
PFHxDA_2	Native Analyte	813>169	0.011					
13C2_PFHxDA	IDA	815>770	0.011					
PFODA	Native Analyte	913>869	0.011					
PFODA_2	Native Analyte	913>169	0.011					

Table 11.17 – 3
Retention Times & Quantitation

Native Compounds	Typical Native RT (minutes)	IDA analog	Typical IDA RT (minutes)	Quantitation Method
PFPrA	[redacted]	13C4_PFBA	[redacted]	Isotope Dilution
PFMOAA		13C4_PFBA		Isotope Dilution
R-EVE		13C4_PFBA		Isotope Dilution
PFBA		13C4_PFBA		Isotope Dilution
PMPA		13C4_PFBA		Isotope Dilution
PFPrS		13C3-PFBS		Isotope Dilution
NVHOS		13C4_PFBA		Isotope Dilution
PFECA F		13C5_PFPeA		Isotope Dilution
PFO2HxA		13C5_PFPeA		Isotope Dilution
PEPA		13C5_PFPeA		Isotope Dilution
3:3 FTCA		13C3-PFBS		Isotope Dilution
PFPeA		13C5_PFPeA		Isotope Dilution
PFBS		M3-PFBS		Isotope Dilution
PFECA A		13C5_PFPeA		Isotope Dilution
PES		13C3-PFBS		Isotope Dilution
PFECA B		13C2_PFHxA		Isotope Dilution
4:2 FTS		M2-4:2FTS		Isotope Dilution
PFO3OA		13C2_PFHxA		Isotope Dilution
PFHxA		13C2_PFHxA		Isotope Dilution
PFPeS		13C3-PFBS		Isotope Dilution
HFPO-DA		13C3_HFPO-DA		Isotope Dilution
R-PSDCA		13C4_PFHpA		Isotope Dilution
Hydro-EVE Acid		13C4_PFHpA		Isotope Dilution
5:3 FTCA		13C-6:2 FTCA		Isotope Dilution
PFO4DA		13C4_PFHpA		Isotope Dilution
PFECA_G		13C-6:2 FTCA		Isotope Dilution
PFHpA		13C4_PFHpA		Isotope Dilution
PFHxS		18O2_PFHxS		Isotope Dilution
6:2 FTUCA		13C-6:2 FTUCA		Isotope Dilution
6:2 FTCA		13C-6:2 FTCA		Isotope Dilution
DONA		13C4_PFOS		Isotope Dilution
PS Acid		13C4_PFOA		Isotope Dilution
EVE Acid		13C4_PFOA		Isotope Dilution
PFECHS		13C4_PFOA		Isotope Dilution
6:2 FTS		M2-6:2FTS		Isotope Dilution
PFOA		13C4_PFOA		Isotope Dilution
PFHpS		13C4_PFOS		Isotope Dilution
Hydro-PS Acid		13C4_PFHpA		Isotope Dilution
PFO5DoA (TAF		13C4_PFOA		Isotope Dilution
7:3 FTCA		13C-8:2 FTCA		Isotope Dilution

Table 11.17 – 3
Retention Times & Quantitation

Native Compounds	Typical Native RT (minutes)	IDA analog	Typical IDA RT (minutes)	Quantitation Method
8:2 FTUCA	[redacted]	13C-8:2 FTUCA	[redacted]	Isotope Dilution
PFOS		13C4_PFOS		Isotope Dilution
PFNA		13C5_PFNA		Isotope Dilution
8:2 FTCA		13C-8:2 FTCA		Isotope Dilution
9Cl-PF3ONS		13C4_PFOS		Isotope Dilution
PFOSA		13C8_PFOSA		Isotope Dilution
PFNS		13C4_PFOS		Isotope Dilution
PFDA		13C2_PFDA		Isotope Dilution
8:2 FTS		M2-8:2FTS		Isotope Dilution
N-MeFOSAA		d3-MeFOSAA		Isotope Dilution
PFDS		13C4_PFOS		Isotope Dilution
10:2 FTUCA		13C-10:2 FTUCA		Isotope Dilution
10:2 FTCA		13C-10:2 FTCA		Isotope Dilution
PFUdA		13C2_PFUdA		Isotope Dilution
N-EtFOSAA		d5-EtFOSAA		Isotope Dilution
N-MeFOSE		d7N-MeFOSE		Isotope Dilution
MeFOSA		d3MeFOSA		Isotope Dilution
11Cl-PF3OUdS		13C4_PFOS		Isotope Dilution
N-EtFOSE		d9N-EtFOSE		Isotope Dilution
EtFOSA		d5EtFOSA		Isotope Dilution
PFDoA		13C2_PFDoA		Isotope Dilution
10:2 FTS		M2-10:2FTS		Isotope Dilution
PFDoS		13C4_PFOS		Isotope Dilution
PFTrDA		13C2_PFDoA		Isotope Dilution
PFTeDA		13C2_PFTeDA		Isotope Dilution
PFHxDA		13C2_PFHxDA		Isotope Dilution
PFODA		13C2_PFHxDA		Isotope Dilution

11.17.1. Post Spike Sample Analysis for AFFF samples

11.17.1.1. This section only applies to aqueous samples prepared by serial dilution instead of SPE that have reported value of <LOQ (RL) for any analyte.

11.17.1.2. Spike aliquots of the sample at the final dilution reported for the sample with all analytes that have reported of <LOQ in the final dilution. The spike must be at the LOQ concentration to be reported with the sample (the < LOQ value).

11.17.1.3. When analyte concentrations are calculated as <LOQ, the spike must recover within 70-130% of its true value.

11.17.1.4. It the recovery does not meet this criteria, the sample, sample duplicate and post spike sample must be reanalyzed at consecutively higher dilutions until the criteria is met.

11.17.2. Tune and calibrate the instrument as described in Section 10.

11.17.3. A typical run sequence is as follows:

- Rinse Blank (RB, not linked to anything)
- Start ICAL with CCVL but called IC in TALS (starts the 12 hour clock or time 0:00)
- Rest of ICAL
- ICB: link to midpoint of ICAL and samples
- ICV: link to midpoint of ICAL and samples (If ICAL good)
- CCB: link to midpoint of ICAL and samples
- PFOA RT marker
- Rinse Blank (RB, not linked to anything)
- 10 samples: link to midpoint of ICAL
- CCV: link to midpoint of ICAL
- 10 more samples: link to midpoint of ICAL
- CCV: link to midpoint of ICAL
- Etc.
- CCVL (within 12 hours from CCVL in ICAL, can be the ending CCV and starts 12 hours all over again): if this occurs link to the midpoint of the ICAL/toggle it as opening/closing CCV.
- CCV: link to midpoint of ICAL
- 10 samples: link to midpoint of ICAL
- CCV: link to midpoint of ICAL
- If no ICAL run that day
- CCB: link to CCVIS
- CCVL (starts 12 hour clock): link to CCVIS
- CCVIS: link to midpoint of ICAL
- 10 samples: link to CCVIS
- CCV: link to CCVIS
- 10 samples: link to CCVIS
- CCV: link to CCVIS
- Etc.

- If going over 12 hours in the sequence: CCVL (within 12 hours from CCVL at item 2 above, can be the ending CCV and starts 12 hours all over again): if this occurs link to the CCVIS /toggle as opening and closing CCV.
- CCV: link to CCVIS
- 10 samples: link to CCVIS
- CCV: link to CCVIS

11.18. Vortex all sample aliquots and standards prior to placing on the autosampler.

11.19. Samples analyzed subsequent to any sample with results at or above the upper calibration limit must be evaluated for potential carryover, and corrective actions taken, as detailed below.

11.19.1. If carryover is suspected, those samples are to be re-analyzed from a fresh extract aliquot (i.e. go the archive of the extract).

11.19.2. Should there be instrument contamination, as evident by sample carryover, any sample >5X the UCL or instrument blanks with detections > RL:

- Analyze 20 blanks alternating between 1% formic acid/methanol and 1% formic acid/water.
- Then analyze 3 methanol only blanks.
- If the system is clean resume analyses. Proceed to 11.19.4. If not clean, proceed as directed below.

11.19.3. If the system is still contaminated the following items might need to be cleaned or replaced:

- Reverse flush the analytical column
- Reverse flush the isolation column
- Replace the column (isolation, analytical or both)
- Clean the cones/entry port
- Replace the PEEK tubing in the sample pathway
- Then, repeat 11.19.2.

11.19.4. Should a high level sample be analyzed that triggers these steps then detections for those analytes over the next 2-3 days require additional evaluation (are all instrument blanks from the sequence < ½ RL) and possible re-analysis. If sample results replicate and the associated instrument blanks from the sequences are <1/2 RL then one can assume the system is under control and confirmation of positive detections can stop.

12. CALCULATIONS / DATA REDUCTION

12.1. If the concentration of the analyte ions exceeds the working range as defined by the calibration standards, then the sample might require to be diluted and reanalyzed, based upon client need. It may be necessary to dilute samples due to matrix.

12.2. Extracts can be diluted up to 100X without diluting out the IDA and thus preserving quantitation via isotope dilution. Dilutions greater than 100X can be performed but additional IDA must be added. The quantitation will now be via internal standard as a result and will have a low bias as extraction losses will no longer be taken into account. Consult the client for authorization of such a dilution.

12.3. Results less than the reporting limit are flagged in the client report as estimated. Generally, the "J" flag is used to denote ≥ MDL and ≤ RL, but the specific flag may change based on client requirements.

12.4. Qualitative Identification

12.4.1. The retention times of PFAS with labeled standards should be the same as that of the labeled IDA's to within 0.1 min. For PFAS with no labeled standards, the RT must be within ± 0.3 minutes of the ICV if analyzed immediately following the ICAL or the most recent CCV standard.

Note: The IDA RT and native RT may be offset by 0.02 to 0.04 minutes.

12.4.1.1. Criteria for samples analyzed in accordance with QSM 5.3: The peak RT must be within 0.4 mins of the CCV or midpoint of the ICAL.

12.4.2. PFBS, PFHxS, PFOS, NMeFOSAA, and NEtFOSAA have multiple chromatographic peaks using the LC conditions specified in the method due to the linear and branch isomers of these compounds. Most PFAS compounds are produced by one of two processes. One gives rise to linear PFAS only while the other process produces both linear and branched isomers. Both branched and linear PFAS compounds can potentially be found in the environment. For the aforementioned compounds that give rise to more than one peak, all chromatographic peaks observed in the standard must be integrated and the areas totaled. Chromatographic peaks in the sample must be integrated in the same way as the calibration standard and concentrations reported as a total for each of these analytes.

12.4.3. The expected retention times (RT) are established in the Chrom data processing module during the processing of the ICAL by selecting Edit>Method>Update RT. Once the retention times are established Chrom

will look for a peak within ± 0.25 minutes of the RT. The analyst confirms that the branched isomers present in the quantitative calibration standards for PFOS, PFHxS, NEtFOSAA and NMeFOSAA are within the ± 0.25 minute window. If they are not, an adjustment to the RT window is made. The analyst confirms the presence of the branched isomers in the technical (qualitative) PFOA standard as well, and adjusts the RT window for PFOA if they are not present within the ± 0.25 minute window.

12.4.3.1. If a peak is detected within this window of ±0.25 minutes, Chrom will assign the absolute retention time at the apex of the peak. Chrom assigns the RT to the most predominant peak within this window. As the linear peak is the predominant peak in calibration solutions for those PFAS that are calibrated with the combination of both branched and linear isomers, those PFAS require additional evaluation in the event that the branched isomer is the predominant peak in a field sample and Chrom has not positively identified the peak due to the RT shift, as the apex may now be the branched isomer.

12.4.3.2. Additional evaluation is required if the field samples contain branched isomers not present in the quantitative or qualitative standards. The analyst confirms that only the peaks present in the calibration standards are included in the peak integration, or adjusts the peak integration to assure that only the peaks present in the standards are identified and quantitated.

12.4.3.3. RT are updated as needed based upon evaluation of the daily CCV.

12.4.4. The signal to noise ratio for both quantitative and qualitative ions/transitions must be ≥ 3:1 for a baseline deflection to be considered a peak. If this criterion is not met, the analyte is not considered and reported as "non-detect".

12.5. The ICAL established in Section 10 is used to calculate concentrations for the extracts.

12.6. Extract concentrations are calculated as below. The first equation applies Average Response Factor model, the second to a linear fit, and the third to the quadratic line fit.

Equation 4 $$Concentration\ (ng/mL) = \frac{y}{RRF}$$

Equation 5 $$Concentration\ (ng/mL) = \frac{y-c}{b}$$

Equation 6 $$Concentration\ (ng/mL) = \frac{-b \pm \sqrt{b^2 - 4a(c-y)}}{2a}$$

Where:

y = $\frac{Area_{Target}}{Area_{IDA}} \times Concentration(IDA)$

RRF = Relative Response Factor

x = concentration

a = curvature

b = slope

c = intercept

12.7. Water Sample Result Calculation:

Equation 7 $$Concentration\ (ng/L) = \frac{C_{ex}V_t}{V_0}$$

Where:

C_{ex} = Concentration measured in sample extract (ng/mL)

V_t = Volume of total extract (mL)

V_o = Volume of water extracted (L), i.e. total volume fortified with IDA

12.8. Soil Sample Result Calculation:

Equation 8 $$Concentration\ (ng/g) = \frac{C_{ex}V_t}{W_s D}$$

Where ng/g = µg/kg and:

C_{ex} = Concentration measured in sample extract (ng/mL)

V_t = Volume of total extract (mL)

W_s = Weight of sample extracted (g)

D = Fraction of dry solids, which is calculated as follows:

$\frac{100 - \%\ moisture\ in\ sample}{100}$ (for dry weight result)

12.9. IDA Recovery Calculation:

Equation 9 $$\%\ Recovery = \frac{A_{IDA}Q_{IS}}{A_{IS}Q_{IDA}RRF_{IDA}} \times 100$$

Where:

RRF_{IDA} = Response Factor for IDA compound

A_{IDA} = Area response for IDA compound

A_{IS} = Area Response for IS compound

Q_{IS} = Amount of IS added

Q_{IDA} = Amount of IDA added

12.10. Raw data, calibration summaries, QC data, and sample results are reviewed by the analyst. These must also be reviewed thoroughly by a second qualified person. See the Data Review Policy (WS-PQA-0012). These reviews are documented in TALS.

13. METHOD PERFORMANCE

13.1. The group/team leader has the responsibility to ensure that this procedure is performed by an associate who has been properly trained in its use and has the required expertise.

13.2. Method Detection Limit

The laboratory must generate a valid method detection limit for each analyte of interest. The MDL must be below the reporting limit for each analyte. The procedure for determination of the method detection limit is given in 40 CFR Part 136, Appendix B, and further defined in SOP WS-QA-0006 and policy WS-PQA-003. MDLs are available in the Quality Assurance Department.

13.3. Initial Demonstration of Capability (IDOC)

Each analyst performing this procedure must successfully analyze four LCS QC samples using current laboratory LCS control limits. IDOCs are approved by the Quality Assurance Manager and the Technical Director. IDOC records are maintained by the QA staff in the central training files.

14. POLLUTION PREVENTION

14.1. All waste will be disposed of in accordance with Federal, State and Local regulations.

14.2. Solid phase extraction used for water samples greatly reduces the amount of solvent used compared to liquid-liquid extraction.

14.3. Standards and reagents are purchased and prepared in volumes consistent with laboratory use to minimize the volume of expired standards and reagents requiring disposal.

14.4. Where reasonably feasible, technological changes have been implemented to minimize the potential for pollution of the environment. Employees will abide by this method and the policies in Section 13 of the Corporate Safety Manual for “Waste Management and Pollution Prevention.”

14.5. Do not allow waste solvent to vent into the hoods. All solvent waste is stored in capped containers unless waste is being transferred.

14.6. Transfer waste solvent from collection cups (tri-pour and similar containers) to jugs and/or carboys as quickly as possible to minimize evaporation.

15. WASTE MANAGEMENT

The following waste streams are produced when this method is carried out:

15.1. Assorted test tubes, autovials, syringes, filter discs and cartridges. Dump the solid waste into a yellow contaminated lab trash bucket. When the bucket is full or after no more than one year, tie the plastic bag liner shut and put the lab trash into the hazardous waste – landfill steel collection drum in the H3 closet. When the drum is full or after no more than 75 days, move it to the waste collection area for shipment.

15.2. Extracted soil samples, used sodium sulfate, paper funnel filters, glass wool, thimbles, and extracted solids saturated with solvents. Dump these materials into an orange contaminated lab trash bucket. When the bucket is full or after no more than one year, tie the plastic bag liner shut and put the lab trash into the incineration steel collection drum in the H3 closet. When the drum is full or after no more than 75 days, move it to the waste collection area for shipment.

15.3. Waste Methanol. Collect the waste solvents in tripours during use. Empty the tripours into a 1-liter to 4-liter carboy at the fume hood. When the carboy is full, or at the end of your shift, whichever comes first, empty the carboy into the steel flammable solvent drum in the H3 closet. When the drum is full to between four and six inches of the top, or after no more than 75 days, move the steel flammable solvent drum to the waste collection area for shipment.

15.4. Mixed water/methanol waste from soil extraction. Collect the waste in the HPLC waste carboy. When full, or after no more than one year, dump into the blue plastic HPLC collection drum in the H3 closet. When the drum is full to between four and six inches of the top or after no more than 75 days, move it to the waste collection area for shipment.

15.5. Aqueous acidic waste from the LCMS instrument contaminated with methanol. This is collected in a 1-gallon carboy at the instrument. When the carboy is full, or after no more than one year, it is emptied into the blue plastic HPLC collection drum in the H3 closet. When the drum is full to between four and six inches of the top or after no more than 75 days, move it to the waste collection area for shipment.

15.6. Autovials contaminated with methanol. As the autovials are removed from the instrument after analysis, they are collected in open containers at the instrument. After all autovials are removed, the open container must be dumped into a closed satellite collection container in a fume hood, as the punctured septa in the autovial can allow methanol and other contaminants to evaporate into the atmosphere. The satellite collection containers are transferred to the waste disposal area when full or after no more than one year, where they are disposed through the vial eater.

16. REFERENCES

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16.11. U.S. Department of Denfense (DoD)/Department of Energy (DOE) Consolidated Quality Systems Manual (QSM) for Environmental Laboratories, Version 5.1.1, dated 2017.

16.12. U.S. Department of Denfense (DoD)/Department of Energy (DOE) Consolidated Quality Systems Manual (QSM) for Environmental Laboratories, Version 5.3 dated 2019.

17. METHOD MODIFICATIONS

17.1. Modifications from Method 537 are detailed below:

17.1.1. Target analyte results are quantitated via isotope dilution.

17.1.2. Two ion transitions (precursor to quant ion and precursor to confirmation ion) are monitored for those analytes that have two transitions. Ion ratios are monitored as well for these analytes.

17.1.3. Water sample containers are not preserved with Trizma.

17.1.4. The method has been modified to address soil/solid matrices. The extraction holding time is set at 14 days.

17.1.5. The analyte list has been expanded. The number of labeled analytes has been expanded as well to improve quantitation.

17.1.6. The reporting limits differ as they are all set at one consistent value whenever possible.

17.1.7. Calibration levels differ from the referenced method.

17.1.8. More labeled analytes are fortified into the samples prior to the extraction process. Most target analytes are quantitated against a labeled analyte.

17.1.9. There is no symmetry requirement.

17.1.10. Calibration, both initial and continuing, has different acceptance criteria due to the longer list of analytes, and the use of isotope dilution quantitation.

17.1.11. The eluents and HPLC configuration differs. As a result the final extract is in 80:20 methanol:water.

17.1.12. The LCS and MS/MSD are spiked at one concentration and do not rotate between a low to high levels.

17.1.13. Samples are not checked for residual chlorine or pH.

17.1.14. A different SPE cartridge [redacted] is used for the extraction process. As a result solvents and elution procedures are different.

18. ATTACHMENTS

There are no attachments to this SOP.

19. REVISION HISTORY

Revisions prior to 9/1/2020 have been removed and are available in previous versions of this SOP.

19.1. Comparison of WS-LC-0025 Revision 3.6 to WS-LC-0025 Revision 4.0, Effective 01/27/2021

19.1.1. This SOP has undergone substantial revision to incorporate many new analytes. The addition of parameters affects Sections 1, 7.4 through 7.9, and 11.16.

19.1.2. Removed all references to Waters LCMS systems – this affects sections 6 and 11.

19.1.3. Removed all references and procedures for concentrating extracts.

19.1.4. All references to the DOD/DOE QSM have been updated to refer to Version 5.3.

19.1.5. The soil preparation has been revised to use a shorter extraction process. Table 1.3, Section 2.2 and Section 11.7 have been updated to reflect these changes.

19.1.6. Agency-specific criteria for calibration, holding times, QC acceptance, etc., have been codified in SOP WS-WI-0066. With the exception of DOD/DOE requirements, they are not included in this document.

19.1.7. Specific instruction for preparation of standard solutions (intermediate and working) has been included in Sections 7.4 through 7.9. This includes information formerly present in WS-LC-0025 Revision 3.6 Attachment 2. The tables have been updated to reflect changed concentration values for specific IDA. The IDA that were at 5x the others are now at the same concentration.

19.1.8. The information present in Attachment 1 of Revision 3.6 (describing an in-

line SPE process for analysis of water samples) has been moved to SOP WS-LC-0025 Att1.

19.1.9. The SPE cartridges (Section 6.9) used for water extraction and solid extract cleanup have been changed. The new cartridges incorporate graphitized carbon, which is now applied to all sample extracts. The solvent systems used with this cartridges have been updated as needed in Section 11.

19.1.10. Columns and SPE cartridges no longer used have been removed from Section 6.

19.1.11. Section 4.5 (discussion of branched and linear isomers) has been revised to include Et-FOSAA and Me-FOSAA.

19.1.12. Section 7.15 (Ammonium Acetate preparation) has been revised to remove the filtration prior to use, and to correct the weight of the salt to 1.54g.

19.1.13. Section 8 (Sample Collection, Preservation, and Holding Times) has been reformatted with a table to clarify containers, preservation, and holding times. Guidance for bi-phasic samples has been incorporated into this section.

19.1.14. Corrective actions for IDA have been added to Section 9.8

19.1.15. Updated the guidance and criteria for the TOP Assay in the event of incomplete oxidation of field samples in Section 9.10.

19.1.16. Guidance and criteria for ion ratios has been added as Section 9.11.

19.1.17. Section 10.3 updated to clarify mass calibration process and frequency for the LCMS.

19.1.18. Section 11.7, Tables 2 and 3 have been combined. The transitions monitored for HFPO-DA and 13C-HFPO-DA have been updated to match those detailed in Method 537.1.

19.1.19. Added guidance in Section 12 regarding the maximum dilution before IDA are diluted out.

19.1.20. Added a note in Section 12 regarding flagging of results less than the reporting limit.

19.1.21. Section 16 has been updated to include reference to QSM version 5.1.1 and 5.3, as well as Method 537.1

19.1.22. Updated Section 17 to include mention of isotope dilution, monitoring two ion transitions, and ion ratios.

19.1.23. Editorial changes

19.2. Comparison of WS-LC-0025 Revision 3.8 to WS-LC-0025 Revision 4.0, Effective 09/30/2020

19.2.1. This SOP has undergone substantial revision to incorporate many new analytes. The addition of parameters affects Sections 1, 7.4 through 7.9, and 11.16.

19.2.2. All references to the DOD/DOE QSM have been updated to refer to Version 5.3.

19.2.3. The soil preparation has been revised to use a different aliquot size, and a shorter extraction process. Table 1.3, Section 2.2 and Section 11.7 have been updated to reflect these changes.

19.2.4. References to agency-specific criteria for calibration, holding times, QC acceptance, as codified in SOP WS-WI-0066 have been added.

19.2.5. The SPE cartridges (Section 6.9) used for water extraction and solid extract cleanup have been changed. The new cartridges incorporate graphitized carbon, which is now applied to all sample extracts. The solvent systems used with this cartridges have been updated as needed in Section 11.

19.2.6. Section 4.5 (discussion of branched and linear isomers) has been revised to include Et-FOSAA and Me-FOSAA.

19.2.7. Section 7.1.2 (Ammonium Acetate preparation) has been revised to remove the filtration prior to use, and to correct the weight of the salt to 1.54g.

19.2.8. Section 7.10 now directs the user to make the TOP reverse surrogate solution in water, rather than Methanol.

19.2.9. Section 8 (Sample Collection, Preservation, and Holding Times) has been reformatted with a table to clarify containers, preservation, and holding times. Guidance for bi-phasic samples has been incorporated into this section.

19.2.10. Expanded the guidance and corrective actions for IDAs in Section 9.8. Also updated the criteria for the FOSE IDA.

19.2.11. Updated the guidance and criteria for the TOP Assay in the event of incomplete oxidation of field samples in Section 9.10.

19.2.12. Updated the guidance and criteria for ion ratios has been added in Section 9.11.

19.2.13. Section 11.7, Tables 2 and 3 have been combined. The transitions monitored for HFPO-DA and 13C-HFPO-DA have been updated to match those detailed in Method 537.1.

19.2.14. Section 12.4 has been expanded to include more detail regarding retention times and qualitative identification.

19.2.15. Editorial changes.