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EPA Method 537.1 – PFAS in Drinking Water by LC-MS/MS

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1. Scope and Application

1.1. This is a solid phase extraction (SPE) liquid chromatography/tandem mass spectrometry (LC-MS/MS) method for the determination of selected per- and polyfluorinated alkyl substances (PFAS) in drinking water.

1.2. The target list includes:

Analyte	Acronym	CAS Number
Perfluorobutanesulfonic acid	PFBS	375-73-5
Perfluorohexanoic acid	PFHxA	307-24-4
Hexafluoropropylene oxide dimer acid	HFPO-DA	13252-13-6
Perfluoroheptanoic acid	PFHpA	375-85-9
Perfluorohexanesulfonic acid	PFHxS	355-46-4
4,8-dioxa-3H-perfluorononanoic acid	ADONA	919005-14-4
Perfluorooctanoic acid	PFOA	335-67-1
Perfluorooctanesulfonic acid	PFOS	1763-23-1
Perfluorononanoic acid	PFNA	375-95-1
9-chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	9Cl-PF3ONS	756426-58-1
Perfluorododecanoic acid	PFDoA	307-55-1
N-methyl perfluorooctanesulfonamidoacetic acid	NMeFOSAA	2355-31-9
Perfluoroundecanoic acid	PFUnA	2058-94-8
N-ethyl perfluorooctanesulfonamidoacetic acid	NEtFOSAA	2991-50-6
11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF3OUdS	763051-92-9
Perfluorodecanoic acid	PFDA	335-76-2
Perfluorotridecanoic acid	PFTrDA	72629-94-8
Perfluorotetradecanoic acid	PFTA	376-06-7

1.3. Restricted Procedure

1.3.1. This procedure is restricted to use by an analyst experienced in solid phase extractions, the operation of LC-MS/MS instruments, and the interpretation of the associated data. Additionally, the analyst must complete the requirements of the GA EPD Initial Demonstration of Analyst Proficiency prior to the analysis of actual samples. Analysts are further warned that performance of this analysis involves the use of potentially hazardous chemicals; refer to the GA EPD Chemical Hygiene Plan for additional information regarding chemicals required by this method.

2. Definitions

2.1. Analysis Batch – A set of samples that is analyzed on the same instrument during a 24-hour period, including no more than 20 Field Samples, that begins and ends with the analysis of the appropriate CCC standards.

2.2. Calibration Standard (CAL) – A solution prepared from the PDS and spiked with ISs and SURs that is used to calibrate the instrument response with respect to analyte concentration.

2.3. Collisionally Activated Dissociation (CAD) – The process of converting the precursor ion's translational energy into internal energy by collisions with neutral gas molecules to bring about dissociation into product ions.

2.4. Continuing Calibration Check/ Continuing Calibration Verification (CCC/CCV) – A CAL standard that is analyzed periodically to verify the accuracy of the existing calibration for those analytes.

2.5. Detection Limit/ Method Detection Limit (DL/MDL) – The minimum concentration of an analyte that can be identified, measured, and reported with 99% confidence that the reported value is greater than zero. This is a statistical determination of precision, and accurate quantitation is not expected at this level. The DL is compound dependent and is dependent on extraction efficiency, sample matrix, fortification concentration, and instrument performance.

2.6. Extraction Batch – A set of up to 20 Field Samples and the required QC samples extracted together by the same person during a workday using the same lot of SPE devices, solvents, ISs, and SURs. Required QC samples include an LRB, an LFB, an LFSM, and an LFSMD.

2.7. Field Duplicates (FD1 and FD2) – Two separate samples collected at the same time and place under identical circumstances and treated exactly the same throughout field and laboratory procedures. Analyses of FD1 and FD2 give a measure of the precision associated with sample collection, preservation, and storage, as well as laboratory procedures.

2.8. Field Reagent Blank/ Field Blank (FRB/FB) – An aliquot of reagent water that is placed in a sample container in the laboratory and treated as a sample in all respects, including shipment to the sampling site, exposure to sampling site conditions, storage, preservation, and all analytical procedures. The purpose of the FRB is to determine if method analytes or other interferences are present in the field environment.

2.9. Internal Standard (IS) – A pure chemical added to an extract or standard solution in a known amount and used to measure the relative response of other method analytes and surrogates that are components of the same solution. The internal standard must be a chemical that is structurally similar to the method analytes, has no potential to be present in water samples, and is not a method analyte.

2.10. Laboratory Fortified Blank/ Laboratory Control Standard (LFB/LCS) – A volume of reagent water to which known quantities of the method analytes and the preservation compound are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.

2.11. Laboratory Fortified Sample Matrix/ Matrix Spike (LFSM/MS) – A preserved Field Sample to which known quantities of the method analytes are added in the

laboratory. The LFSM is processed and analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate sample extraction and the measured values in the LFSM corrected for background concentrations.

2.12. Laboratory Fortified Sample Matrix Duplicate/ Matrix Spike Duplicate (LFSMD/MSD) – A duplicate of the Field Sample used to prepare the LFSM. The LFSMD is fortified, extracted, and analyzed identically to the LFSM. The LFSMD is used instead of the Field Duplicate to assess method precision when the occurrence of method analytes is low.

2.13. Laboratory Reagent Blank/ Method Blank (LRB/MBLK) – An aliquot of reagent water that is treated exactly as a sample including exposure to all equipment, solvents and reagents, sample preservatives, internal standards, and surrogates that are used in the analysis batch. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.

2.14. Lowest Concentration Minimum Reporting Level (LCMRL) – For a single laboratory, the lowest true concentration for which the future recovery is expected, with 99% confidence, to be between 50 and 150% recovery.

2.15. Minimum Reporting Level/ Reporting Limit (MRL/RL) – The minimum concentration that can be reported as a quantitated value for a method analyte in a sample following analysis. This defined concentration can be no lower than the concentration of the lowest calibration standard for that analyte and can only be used if acceptable QC criteria for this standard are met.

2.16. Precursor Ion – The deprotonated molecule ([M-H]-) of the method analyte.

2.17. Primary Dilution Standard (PDS) – A solution diluted from the concentrated SSS. The IS PDS is used to spike the 1 mL autosampler vials prior to analysis. The SUR PDS is used to spike the 250 mL bottles prior to extraction. The PFAS PDS is used to fortify the LFBs, LFSMs, and LFSMDs prior to extraction. The PFAS PDS is further diluted to a PFAS Secondary Dilution Standard (SDS) and both are used to prepare the CAL standards.

2.18. Product Ion – The fragment ion of the method analyte produced by collisionally activated dissociation of the precursor ion. The fragment ion has a smaller mass to charge ratio (m/z) than the precursor ion.

2.19. Qualitative Standard – A standard for which either the concentration is estimated, or method analyte impurities exist at a concentration >1/3 of the MRL in the highest concentration calibration standard. For the purposes of this method, qualitative standards are used to identify retention times of branched isomers of method analytes and are not used for quantitation purposes.

2.20. Quality Control Sample/Initial Calibration Verification (QCS/ICV) – A solution of method analytes of known concentrations that is obtained from a source external to the laboratory and different from the source of calibration standards. The second source SSS is used to fortify the QCS at a known concentration. The QCS is used to check calibration standard integrity.

2.21. Quantitative Standard – A standard of known concentration and purity. The quantitative standard must not contain any impurities of the method analytes at concentrations >1/3 of the MRL in the highest concentration calibration standard.

2.22. Safety Data Sheet (SDS) – Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.
2.23. Stock Standard Solution (SSS) – A concentrated solution containing method analytes purchased from a reputable commercial source.
2.24. Surrogate Analyte (SUR) – A pure chemical which chemically resembles method analytes and is extremely unlikely to be found in any sample. This chemical is added to a sample aliquot in known amounts prior to extraction and is measured with the same procedures used to measure other method analytes. The purpose of the SUR is to monitor method performance with each sample.

3. Interferences

3.1. Samples and extracts should not come in contact with any glass containers or pipettes as these analytes can potentially adsorb to glass surfaces.
3.2. Method interferences may be caused by contaminants in solvents, reagents, sample bottles and caps, and other sample processing hardware that lead to discrete artifacts and/or elevated baselines in the chromatograms. The analytes in this method can also be found in many common laboratory supplies and equipment such as PTFE products, LC solvent lines, methanol, aluminum foil, SPE cartridges, SPE sample transfer lines, etc. All items such as these must be routinely demonstrated to be free from interferences (less than 1/3 the MRL for each method analyte). Analyses of laboratory reagent blanks provide information about the presence of contaminants. Subtracting blank values from sample results is not permitted.
3.3. Matrix interferences may be caused by contaminants that are co-extracted from the sample. Humic and/or fulvic material can be co-extracted during SPE and high levels can cause enhancement and/or suppression in the electrospray ionization source or low recoveries on the SPE sorbent.
3.4. Relatively large quantities of the preservative Trizma are added to sample bottles. The potential exists for trace-level organic contaminants in these reagents. Interferences from these sources should be monitored by analysis of laboratory reagent blanks, particularly when new lots of reagents are acquired.

4. Safety

4.1. Refer to Laboratory Safety/Chemical Hygiene Plan and Fire Safety Plan.
4.2. Methanol is used during the extraction. Store the 4 L bottle in the flammable storage cabinet. Keep squeeze bottles inside the hood. If a Methanol spill occurs, activated carbon (large yellow bucket) should be used to soak up the spill and prevent fumes from building up in the room.
4.3. Keep solvent squeeze bottles inside the hood.
4.4. Wear safety glasses and polypropylene lab coats and gloves while handling field and QC samples during weighing, spiking, extraction, concentration, and LC-MS/MS analysis.
4.5. The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined. Each chemical should be treated as a potential health hazard, and exposure to these chemicals should be minimized.

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

5. Equipment and Apparatus

5.1. Polypropylene protective gown (Uline Prod. No. S-17099 or equivalent).
5.2. Nitrile gloves (Kimtech Prod. No. 62993 (L) or equivalent).
5.3. 250 mL polypropylene bottles with polypropylene screw caps (Wheaton Prod. No.: 209668 or equivalent).
5.4. 15 mL conical polypropylene centrifuge tubes with polypropylene plug seal caps (Corning Prod. No.: 430766 or equivalent).
5.5. 2 mL and 300 µL polypropylene autosampler vials (ThermoFisher Cat. No.: C4000-14 and C4000-11 or equivalent) with polypropylene caps (ThermoFisher Cat. No.: C5000-50 or equivalent).
5.5.1. NOTE: Polypropylene vials and caps are necessary to prevent contamination of the sample from PTFE coated septa. However, polypropylene caps do not reseal, so evaporation occurs after injection. Thus, multiple injections from the same vial are not possible.
5.6. 10 and 250 mL Polypropylene graduated cylinders (Nalgene Prod. No.: 3662-0010 and 3662-0250 or equivalent).
5.7. 25, 50, 100, 1000 mL Polypropylene volumetric flasks (Corning Prod. No.: 5640P-25, 5640P-50, 5640P-100, and 5640P-1L or equivalent).
5.8. 5-100 µL and 50-1000 µL electronic pipettes (Eppendorf Cat. No.: 2231000779 and 2231000782 or equivalent).
5.9. 1-200 µL and 100-1000 µL polypropylene pipette tips (Fisher Cat. No.: 02-707-501 and 02-707-508 or equivalent).
5.10. 7.7 mL polypropylene disposable transfer pipettes (Fisher Cat. No.: 13-711-7M or equivalent).
5.11. 500 mL autoclavable wash bottle (Dynalon Prod. No.: 506985 or equivalent).
5.12. Analytical balance capable of weighing to the nearest 0.0001 g (Mettler Toledo AT200 or equivalent).
5.13. Solid Phase Extraction system:
5.13.1. Vacuum aspiration system (Vactrap Prod. No.: 305-4001-FLS or equivalent).
5.13.2. SPE vacuum manifold with large volume sampler (Supelco Cat. No.: 57030 and 57275 or equivalent).
5.13.3. 1/16” O.D. x 0.030” I.D. Green Stripe PEEK transfer tubing system (Restek Cat. No. 27756 or equivalent).
5.13.4. Peek Hex-Head Fittings (https://www.restek.com/pdfs/GNSS2788A-UNV.pdf)
5.13.5. Tubing to SPE cartridge Vacuum Stopper.
5.13.6. 500 mg, 6 mL SPE cartridges containing styrenedivinyl-benzene (SDVB) polymeric sorbent phase (Clean Prep Prod. No.: O-P0001-06P or equivalent).
5.14. Extract concentration system using evaporation with nitrogen and a water bath set at 65 °C (Organomation N-Evap 112 Cat. No.: 11250 or equivalent).
5.15. LC-MS/MS with data system:
5.15.1. LC-MS/MS capable of negative ion electrospray ionization (ESI) with an LC flow rate of 0.3 mL/min, production of unique product ions for the method analytes

within specified retention times, and a minimum of 10 scans across the chromatographic peak (Sciex Triple Quad 6500+).

5.15.2. 2.1 x 150 mm Atlantis dC18 analytical column packed with 5 µm silica solid phase particles (Waters Prod. No.: 186001301).

5.15.3. Data system to acquire, store, reduce, and output mass spectral data. The software processes stored LC-MS/MS data to allow recognition of LC peaks, integration of ion abundance of a specific ion within a specified retention time, calculation of relative response factors and analyte concentrations, and construction of linear regressions and quadratic calibration curves (Sciex OS).

5.15.4. Operating parameters for LC are as follows:

Table 5.15.4.1 – Sciex LC Operating Parameters

Parameter	Setting
Injection Volume	2µL
Injector sampling Speed	5 µL/sec
Inlet Temp	Oven Track
Sample Tray Temperature	15°C
Rinse mode before/after aspiration/dip time	10 sec
Sample tray 1.5ml vials needle stroke	50mm
Injection stroke	50mm
Rinse speed	35µL/sec
Rinse volume	500µL
Column Oven	35°C

Table 5.15.4.2 – Sciex LC Operating Parameters

Time (min)	Flow (ml/min)	Ammonium Acetate (%)	Methanol (%)
0	0.3	60	40
0.2	0.3	60	40
0.5	0.3	45	55
2.5	0.3	25	75
7	0.3	10	90
7.9	0.3	6	94
8	0.3	0	100
9	0.3	0	100
9.5	0.3	60	40

5.15.5. Operating parameters for Sciex 6500+ MS/MS are as follows:

Table 5.15.5.1 – Sciex Triple Quad 6500+ MS/MS Operating Parameters	
Parameter	**Setting**
Run Time	12 min
Mass Scanning	Scheduled MRM
Cone Gas Flow	50 L/hr
Nitrogen Desolvation Gas	800 L/hr
Desolvation Gas Temperature	350 °C
Target Scan Time	0.5 sec
Pause between masses	5.007 ms
SRM minimum dwell time	3ms
SRM maximum dwell time	250ms
Cycles	1440
Cycle time	0.5 sec
MRM window	60 sec
Waste Valve	0.0 min=waste 3.4 min=inject 10.0 min=waste
Purge Time	25 min

Table 5.15.5.2 – Sciex Triple Quad 6500+ MS/MS Operating Parameters			
Compound	**Q1**	**Q3**	**Retention Time (min)**
11Cl-PF3OUdS	631	451	8.08
9Cl-PF3ONS	531	351	6.84
ADONA	377	251	5.56
HFPO-DA (GenX)	285	169	5.18
N-EtFOSAA	584	419	7.89
N-MeFOSAA	570	419	7.56
PFBS	298.9	80	4.46
PFDA	513	469	7.19
PFDoA	613	569	8.47
PFHpA	363	319	5.52
PFHxA	313	269	5.02
PFHxS	399	80	5.51
PFNA	463	419	6.58
PFOA	413	369	6.03
PFOS	499	80	6.53
PFTeDA	713	669	9.59
PFTrDA	663	619	9.06
PFUnA	563	519	7.83
IS-13C2_PFOA	415	370	6.03
IS-13C4_PFOS	503	80	6.53
IS-d3-NMeFOSAA	573	419	7.54
SUR-13C2_PFDA	515	470	7.19
SUR-13C2-PFHxA	315	270	5.01
SUR-13C3-HFPO-DA	287	169	5.17
SUR-d5-NEtFOSAA	589.1	418.8	7.87

5.16 The Sciex 6500+ has maintenance and tuning of masses performed every 6 months by service engineer.

6. Reagents and Standards

6.1. Gases, Reagents, and Solvents: Reagent grade or better chemicals should be used.

6.1.1. Reagent Water (H_2O, CAS #: 7732-18-5) – Purified water which does not contain any measurable quantities of any method analytes or interfering compounds greater than 1/3 the MRL for each method analyte of interest. Prior to daily use, at least 3 L of reagent water should be flushed from the purification system to rinse out any build-up of analytes in the system's tubing (Fisher LC/MS Grade).

6.1.2. Methanol (CH_3OH, CAS#: 67-56-1) – High purity, demonstrated to be free of analytes and interferences (Fisher LC/MS grade).

6.1.3. Ammonium Acetate ($NH_4C_2H_3O_2$, CAS#: 631-61-8) – High purity, demonstrated to be free of analytes and interferences (Fisher HPLC Grade).

6.1.4. 20 mM Ammonium Acetate/Reagent Water – To prepare 1 L, add 1.54 g ammonium acetate to 1 L of reagent water. This solution is volatile and must be replaced at least once a week. More frequent replacement may be necessary if unexplained loss in sensitivity or retention time shifts are encountered and attributed to loss of the ammonium acetate.

6.1.5. Trizma Preset Crystals pH 7.0 ($C_4H_{11}NO_3$ and $C_4H_{12}ClNO_3$, Sigma Cat No.: T-7193) – A premixed blend of Tris [Tris(hydroxymethyl)aminomethane] and Tris HCL [Tris(hydroxymethyl)aminomethane hydrochloride]. Alternatively, a mix of the two components with a weight ratio of 15.5/1 Tris HCL/Tris may be used. Trizma functions as a buffer and removes free chlorine in chlorinated finished waters (Reagent Grade).

6.1.6. Nitrogen gas – Ultra high purity, used for the following purposes:

6.1.6.1. Nitrogen aids in aerosol generation of the ESI liquid spray and is used as collision gas in the MS/MS instrument.

6.1.6.2. Nitrogen is used to concentrate sample extracts.

6.2. Standard Solutions: When a compound purity is assayed to be 96% or greater, the weight can be used without correction to calculate the concentration of the stock standard. IS, SUR, and PFAS analyte standards commercially purchased in glass ampoules are acceptable; however, all subsequent transfers or dilutions performed by the analyst must be prepared and stored in polypropylene containers. Standards for sample fortification generally should be prepared in the smallest volume that can be accurately measured to minimize the addition of excess organic solvent to aqueous samples. PDSs are stored at ≤4 °C. Allow PDSs adequate room temperature stabilization to minimize daily imprecision. Vortex well prior to use to prevent adsorption of the method analytes onto the container surfaces that may occur when refrigerated. PDS and calibration standards need to be replaced every six months.

6.2.1. Internal Standard Solutions – These isotopically labeled ISs encompass all the functional groups of the method analytes. Different isotopic labels of the same ISs are acceptable (e.g., $^{13}C_2$-PFOA and $^{13}C_4$-PFOA) but will require modification of the MS/MS precursor and product ions.

Table 6.2.1.1 – Internal Standard Analytes

IS	Acronym
Perfluoro-[1,2-$^{13}C_2$]octanoic acid	$^{13}C_2$-PFOA
Sodium perfluoro-1-[1,2,3,4-$^{13}C_4$]octanesulfonate (acid form)	$^{13}C_4$-PFOS
N-deuteriomethylperfluoro-1-octanesulfonamidoacetic acid	d_3-NMeFOSAA

6.2.1.1. Internal Standard Stock Standard Solution – The 1-4 ng/µL group IS SSS was purchased from Wellington Labs. Analysis of the ISs is less complicated if the ISs purchased contain only the linear isomer. (Wellington Cat. No.: EPA-537IS).

6.2.1.2. Internal Standard Primary Dilution Standard – The 1-4 ng/µL IS SSS is diluted using a 96% methanol/ 4% reagent water solution to create the IS PDS with a final concentration of 10-40 ng/mL. Use 100 µL of this 10-40 ng/mL solution to fortify the 1 mL extracts. This will yield a final concentration of 4-16 ng/L of each IS in the 1 mL extracts.

Table 6.2.1.2.1 – 10-40 ng/mL IS Dilution			
IS Aliquot	**96% MeOH/ 4%Water**		**Final Volume**
250 µL	24.75 mL		25 mL
Internal Standard Analyte	**Wellington Name**	**Purchased Conc. (ng/µL)**	**Final Conc. (ng/mL)**
$^{13}C_2$-PFOA	M2PFOA	1.0	10
$_{13}C_4$-PFOS	MPFOS	2.87	28.7
d_3-NMeFOSAA	d_3-N-MeFOSAA	4.0	40

6.2.2. Surrogate Standard Solutions – These isotopically labeled SURs encompass most of the functional groups as well as the water solubility range of the method analytes.

Table 6.2.2.1 – Surrogate Standard Analytes	
SUR	**Acronym**
Perfluoro-n-[1,2-$^{13}C_2$]hexanoic acid	$^{13}C_2$-PFHxA
Perfluoro-n-[1,2-$^{13}C_2$]decanoic acid	$^{13}C_2$-PFDA
N-deuterioethylperfluoro-1-octanesulfonamidoacetic acid	d_5-NEtFOSAA
Tetrafluoro-2-heptafluoropropoxy-$^{13}C_3$-propanoic acid	$^{13}C_3$-HFPO-DA

6.2.2.1. Surrogate Stock Standard Solution – The 1-4 ng/µL group SUR SSS was purchased from Wellington Labs. Analysis of the SURs is less complicated if the SURs purchased contain only the linear isomer. (Wellington Cat. No.: EPA-537SS-R1).

6.2.2.2. Surrogate Primary Dilution Standard – The 1-4 ng/µL SUR SSS is diluted using a 96% methanol/ 4% reagent water solution to create the SUR PDS with a final concentration of 10-40 ng/mL. Use 100 µL of this 10-40 ng/mL solution to fortify all field and QC samples. This will yield a final concentration of 4-16 ng/L of each SUR in the 1 mL extracts.

Table 6.2.2.2.1 – 10-40 ng/mL SUR Dilution			
SUR Aliquot	**96% MeOH/ 4%Water**		**Final Volume**
250 µL	24.75 mL		25 mL
Surrogate Analyte	**Wellington Name**	**Purchased Conc. (ng/µL)**	**Final Conc. (ng/mL)**
$^{13}C_2$-PFHxA	MPFHxA	1.0	10
$^{13}C_2$-PFDA	MPFDA	1.0	10
d_5-NEtFOSAA	d_5-N-EtFOSAA	1.0	10
$^{13}C_3$-HFPO-DA	M3HFPO-DA	4.0	40

6.2.3. PFAS Standard Solutions – The linear only PFOA standard must be used for quantitation until a quantitative PFOA standard containing the branched and linear isomers becomes commercially available.

Table 6.2.3.1 – PFAS Standard Analytes	
PFAS	**Acronym**
Perfluorobutanesulfonic acid (acid form)	PFBS
Perfluorohexanoic acid	PFHxA
Hexafluoropropylene oxide dimer acid	HFPO-DA
Perfluoroheptanoic acid	PFHpA
Perfluorohexanesulfonic acid (acid form)	PFHxS
4,8-dioxa-3H-perfluorononanoic acid (acid form)	ADONA
Perfluorooctanoic acid	PFOA
Perfluorooctanesulfonic acid (acid form)	PFOS
Perfluorononanoic acid	PFNA
9-chlorohexadecafluoro-3-oxanonane-1-sulfonic acid (acid form)	9Cl-PF3ONS
Perfluorododecanoic acid	PFDoA
N-methyl perfluorooctanesulfonamidoacetic acid	NMeFOSAA
Perfluoroundecanoic acid	PFUnA
N-ethyl perfluorooctanesulfonamidoacetic acid	NEtFOSAA
11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid (acid form)	11Cl-PF3OUdS
Perfluorodecanoic acid	PFDA
Perfluorotridecanoic acid	PFTrDA
Perfluorotetradecanoic acid	PFTA

6.2.3.1. PFAS Stock Standard Solution – The 0.5-2.5 ng/µL PFAS SSS was purchased from Wellington Labs. (Wellington Cat. No.: EPA-537PDSL-R1).

6.2.3.2. PFAS Primary Dilution Standard – The 0.5-2.5 ng/µL PFAS SSS is diluted using a 96% methanol/ 4% reagent water solution to create the PFAS PDS with a final concentration of 175-200 ng/mL. The PFAS PDS is used to prepare the 5 ng/mL, 10 ng/mL, 20 ng/mL, and 30 ng/mL CAL standards and fortify the LFBs, LFSMs, and LFSMDs with the method analytes. If the PFAS PDS is stored cold, care must be taken to ensure that these standards are at room temperature and adequately vortexed before usage.

Table 6.2.3.2.1 – 175-200 ng/mL PFAS Primary Dilution			
PFAS Aliquot	**96% MeOH/ 4%Water**		**Final Volume**
100 µL	900 µL		1 mL
PFAS Analyte	**Wellington Name**	**Purchased Conc. (ng/µL)**	**Final Conc. (ng/mL)**
PFBS	L-PFBS	1.77	177
PFHxA	PFHxA	2.0	200
HFPO-DA	HFPO-DA	2.0	200
PFHpA	PFHpA	2.0	200
PFHxS	L-PFHxS	1.9	190
ADONA	NaDONA	1.89	189
PFOA	PFOA	2.0	200
PFOS	L-PFOS	1.92	192
PFNA	PFNA	2.0	200
9Cl-PF3ONS	9Cl-PF3ONS	1.87	187
PFDoA	PFDoA	2.0	200
NMeFOSAA	N-MeFOSAA	2.0	200
PFUnA	PFUdA	2.0	200

Table 6.2.3.2.1 – 175-200 ng/mL PFAS Primary Dilution			
NEtFOSAA	N-EtFOSAA	2.0	200
11Cl-PF3OUdS	11Cl-PF3OUdS	1.89	189
PFDA	PFDA	2.0	200
PFTrDA	PFTrDA	2.0	200
PFTA	PFTeDA	2.0	200

6.2.3.3. PFAS Secondary Dilution Standard – Some of the 175-200 ng/mL PFAS PDS is further diluted using a 96% methanol/ 4% reagent water solution to a final concentration of 17.5-20 ng/mL. The PFAS SDS is used to prepare the 1.25 ng/mL and 2.0 ng/mL CAL standards. If the PFAS SDS is stored cold, care must be taken to ensure that these standards are at room temperature and adequately vortexed before usage.

Table 6.2.3.3.1 – 17.5-20 ng/mL PFAS Secondary Dilution			
200 ng/mL PFAS Aliquot	**96% MeOH/ 4%Water**		**Final Volume**
100 µL	900 µL		1 mL
PFAS Analyte	**Wellington Name**	**Initial Conc. (ng/mL)**	**Final Conc. (ng/mL)**
PFBS	L-PFBS	177	17.7
PFHxA	PFHxA	200	20
HFPO-DA	HFPO-DA	200	20
PFHpA	PFHpA	200	20
PFHxS	L-PFHxS	190	19
ADONA	NaDONA	189	18.9
PFOA	PFOA	200	20
PFOS	L-PFOS	192	19.2
PFNA	PFNA	200	20
9Cl-PF3ONS	9Cl-PF3ONS	187	18.7
PFDoA	PFDoA	200	20
NMeFOSAA	N-MeFOSAA	200	20
PFUnA	PFUdA	200	20
NEtFOSAA	N-EtFOSAA	200	20
11Cl-PF3OUdS	11Cl-PF3OUdS	189	18.9
PFDA	PFDA	200	20
PFTrDA	PFTrDA	200	20
PFTA	PFTeDA	200	20

6.3. Calibration Standards: Prepare the six CAL standards over the concentration range from 1.25-30 ng/mL from dilutions of the PFAS PDS and SDS in a 96% methanol/ 4% reagent water solution. The IS and SUR are added to the CAL standards at a constant concentration. The lowest concentration CAL standard must be at or below the MRL. The CAL standards are to be stored at -10° C. The CAL standards may also be used as CCCs.

6.3.1. The 1.25 and 2.0 ng/mL CAL standards are prepared by diluting an aliquot of the PFAS SDS with a 96% methanol/ 4% reagent water solution and adding an IS spike and a SUR spike into a 1 mL autosampler vial.

Table 6.3.1.1 – 1.25 and 2.0 ng/mL CAL Standards

CAL Conc.	20 ng/mL PFAS Aliquot	96% MeOH/ 4%Water	IS Spike	SUR Spike	Final Volume
1.25 ng/mL	62.5 µL	737.5 µL	100 µL	100 µL	1 mL
2.0 ng/mL	100 µL	700 µL	100 µL	100 µL	1 mL

PFAS Analyte	Initial Conc. (ng/mL)	1.25 CAL Final Conc. (ng/mL)	2.0 CAL Final Conc. (ng/mL)
PFBS	17.7	1.11	1.77
PFHxA	20	1.25	2.0
HFPO-DA	20	1.25	2.0
PFHpA	20	1.25	2.0
PFHxS	19	1.19	1.9
ADONA	18.9	1.18	1.89
PFOA	20	1.25	2.0
PFOS	19.2	1.20	1.92
PFNA	20	1.25	2.0
9Cl-PF3ONS	18.7	1.17	1.87
PFDoA	20	1.25	2.0
NMeFOSAA	20	1.25	2.0
PFUnA	20	1.25	2.0
NEtFOSAA	20	1.25	2.0
11Cl-PF3OUdS	18.9	1.18	1.89
PFDA	20	1.25	2.0
PFTrDA	20	1.25	2.0
PFTA	20	1.25	2.0

6.3.2. The 5.0, 10, 20, and 30 ng/mL CAL standards are prepared by diluting an aliquot of the PFAS PDS with a 96% methanol/ 4% reagent water solution and adding an IS spike and a SUR spike into a 1 mL autosampler vial.

Table 6.3.2.1 – 5.0, 10, 20, and 30 ng/mL CAL Standards

CAL Conc.	200 ng/mL PFAS Aliquot	96% MeOH/ 4%Water	IS Spike	SUR Spike	Final Volume
5.0 ng/mL	25 µL	775 µL	100 µL	100 µL	1 mL
10 ng/mL	50 µL	750 µL	100 µL	100 µL	1 mL
20 ng/mL	100 µL	700 µL	100 µL	100 µL	1 mL

Table 6.3.2.1 – 5.0, 10, 20, and 30 ng/mL CAL Standards					
30 ng/mL	150 µL	650 µL	100 µL	100 µL	1 mL
PFAS Analyte	**Initial Conc. (ng/mL)**	**5.0 CAL Final Conc. (ng/mL)**	**10 CAL Final Conc. (ng/mL)**	**20 CAL Final Conc. (ng/mL)**	**30 CAL Final Conc. (ng/mL)**
PFBS	177	4.425	8.85	17.7	26.55
PFHxA	200	5.0	10	20	30
HFPO-DA	200	5.0	10	20	30
PFHpA	200	5.0	10	20	30
PFHxS	190	4.75	9.5	19	28.5
ADONA	189	4.725	9.45	18.9	28.35
PFOA	200	5.0	10	20	30
PFOS	192	4.8	9.6	19.2	28.8
PFNA	200	5.0	10	20	30
9Cl-PF3ONS	187	4.675	9.35	18.7	28.05
PFDoA	200	5.0	10	20	30
NMeFOSAA	200	5.0	10	20	30
PFUnA	200	5.0	10	20	30
NEtFOSAA	200	5.0	10	20	30
11Cl-PF3OUdS	189	4.725	9.45	18.9	28.35
PFDA	200	5.0	10	20	30
PFTrDA	200	5.0	10	20	30
PFTA	200	5.0	10	20	30

6.4. Quality Control Sample Standard: For the analytes PFHxS, PFOS, NMeFOSAA, and NEtFOSAA, the linear and branched isomers appear in the QCS SSS. For PFHxS and PFOS, Sciex is able to detect and calculate both the linear and branched isomers. For NMeFOSAA and NEtFOSAA, Sciex is only able to detect and calculate the linear isomer. This difference is reflected in Table 6.4.3.1.

Table 6.4.1 – QCS Isomer Concentrations						
PFAS Analyte	**Isomer**	**Isomer Amount (%)**	**QCS SSS (ng/mL)**	**QCS PDS (ng/mL)**	**10 QCS Std (ng/mL)**	**30 QCS Std (ng/mL)**
PFHxSK	Linear	81.1	1480	148	7.4	22.2
PFHxSK	Total Branched	18.9	345	34.5	1.72	5.16
PFHxSK	P1MPeSK	2.9	52.925	5.2925	0.26448	0.7946
PFHxSK	P2MPeSK	1.4	25.55	2.555	0.12768	*0.3836
PFHxSK	P3MPeSK	5.0	91.25	9.125	0.456	1.37
PFHxSK	P4MPeSK	8.9	162.425	16.2425	0.81168	2.4386
PFHxSK	Remaining Branched[1]	0.7	12.775	1.2775	0.06388	*0.19163
PFOSK	Linear	78.8	1460	146	7.3	21.9
PFOSK	Total Branched	21.2	392	39.2	1.96	5.88
PFOSK	P1MHpSK	1.2	22.224	2.2224	0.11112	*0.3336
PFOSK	P2MHpSK	0.6	11.112	1.1112	0.05556	*0.1668
PFOSK	P3MHpSK	1.9	35.188	3.5188	0.17594	0.5282

Table 6.4.1 – QCS Isomer Concentrations						
PFOSK	P4MHpSK	2.2	40.744	4.0744	0.20372	0.6116
PFOSK	P5MHpSK	4.5	83.34	8.334	0.4167	1.251
PFOSK	P6MHpSK	10	185.2	18.52	0.926	2.78
PFOSK	Remaining Branched[2]	0.8	14.816	1.4816	0.07408	*0.22224
N-MeFOSAA	Linear	76	1520	152	7.6	22.8
N-MeFOSAA	Total Branched	24	480	48	2.4	7.2
N-MeFOSAA	N-Me3MHpSAA	0.7	14	1.4	0.07	*0.21
N-MeFOSAA	N-Me4MHpSAA	2.0	40	4.0	0.2	0.6
N-MeFOSAA	N-Me5MHpSAA	6.0	120	12	0.6	1.8
N-MeFOSAA	N-Me6MHpSAA	14	280	28	1.4	4.2
N-MeFOSAA	Remaining Branched[3]	1.3	26	2.6	0.13	*0.39
N-EtFOSAA	Linear	77.5	1550	155	7.75	23.25
N-EtFOSAA	Total Branched	22.5	450	45	2.25	6.75
N-EtFOSAA	N-Et3MHpSAA	2.3	46	4.6	0.23	0.69
N-EtFOSAA	N-Et4MHpSAA	2.2	44	4.4	0.22	0.66
N-EtFOSAA	N-Et5MHpSAA	5.4	108	10.8	0.54	1.62
N-EtFOSAA	N-Et6MHpSAA	10.4	208	20.8	1.04	3.12
N-EtFOSAA	Remaining Branched[4]	2.2	44	4.4	0.22	0.66

[1]Remaining PFHxSK Branched Isomers: P33DMBuSK-0.2% and unidentified isomers-0.5%.
[2]Remaining PFOSK Branched Isomers: P35DMHxSK-0.07%, P44DMHxSK-0.03%, P45DMHxSK-0.4%, and P55DMHxSK-0.2%.
[3]Remaining N-MeFOSAA Branched Isomers: N-Me55DMHxSAA-0.2% and unidentified isomers-1.1%..
[4]Remaining N-EtFOSAA Branched Isomers: N-Et35DMHxSAA-0.3%, N-Et45DMHxSAA-0.3%, N-Et55DMHxSAA-0.3%, and unidentified isomers-1.3%.
**Not >1/3 MRL (0.4167 ng/mL).*

6.4.1. QCS Stock Standard Solution – The 1.75-2.0 ng/µL QCS SSS was purchased from Wellington Labs. Second source target analytes from a different source than the calibration standards are used to check for calibration standard integrity. (Wellington Cat. No.: EPA-537PDS-R1).

6.4.2. QCS Primary Dilution Standard – The 1.75-2.0 ng/µL QCS SSS is diluted using a 96% methanol/ 4% reagent water solution to create the QCS PDS with a final concentration of 175-200 ng/mL.

Table 6.4.2.1 – 150-200 ng/mL QCS Dilution			
PFAS Aliquot	**96% MeOH/ 4%Water**	**Final Volume**	
100 µL	900 µL	1 mL	
PFAS Analyte	**Wellington Name**	**Purchased Conc. (ng/mL)**	**Final Conc. (ng/mL)**
PFBS	L-PFBS	1770	177
PFHxA	PFHxA	2000	200
HFPO-DA	HFPO-DA	2000	200
PFHpA	PFHpA	2000	200
PFHxSK (Linear)	L-PFHxSK	1480	148
PFHxSK (Branched)	br-PFHxSK	345	34.5

Table 6.4.2.1 – 150-200 ng/mL QCS Dilution			
ADONA	NaDONA	1890	189
PFOA	PFOA	2000	200
PFOSK (Linear)	L-PFOSK	1460	146
PFOSK (Branched)	br-PFOSK	392	39.2
PFNA	PFNA	2000	200
9Cl-PF3ONS	9Cl-PF3ONS	1870	187
PFDoA	PFDoA	2000	200
NMeFOSAA (Linear)	L-N-MeFOSAA	1520	152
NMeFOSAA (Branched)	br-N-MeFOSAA	480	48
PFUnA	PFUdA	2000	200
NEtFOSAA (Linear)	L-N-EtFOSAA	1550	155
NEtFOSAA (Branched)	br-N-EtFOSAA	450	45
11Cl-PF3OUdS	11Cl-PF3OUdS	1890	189
PFDA	PFDA	2000	200
PFTrDA	PFTrDA	2000	200
PFTA	PFTeDA	2000	200

6.4.3. The 7.5-10 ng/mL QCS standard solution is prepared by diluting an aliquot of the QCS PDS with a 96% methanol/ 4% reagent water solution and adding an IS spike and a SUR spike into a 1 mL autosampler vial. The 7.5-10 ng/mL QCS standard solution concentration matches the 10 ng/mL calibration curve midpoint concentration.

Table 6.4.3.1 – 7.5-10 ng/mL QCS Standard				
200 ng/mL QCS Aliquot	**96% MeOH/ 4%Water**	**IS Spike**	**SUR Spike**	**Final Volume**
50 µL	750 µL	100 µL	100 µL	1 mL

PFAS Analyte	Initial Conc. (ng/mL)	Final Conc. (ng/mL)
PFBS	177	8.85
PFHxA	200	10
HFPO-DA	200	10
PFHpA	200	10
PFHxSK (L and B)	182.5	9.12
ADONA	189	9.45
PFOA	200	10
PFOSK (L and B)	185.2	9.26
PFNA	200	10
9Cl-PF3ONS	187	9.35
PFDoA	200	10
NMeFOSAA (L)	152	7.6
PFUnA	200	10
NEtFOSAA (L)	155	7.75
11Cl-PF3OUdS	189	9.45
PFDA	200	10

Table 6.4.3.1 – 7.5-10 ng/mL QCS Standard		
PFTrDA	200	10
PFTA	200	10

6.4.4. The 20-30 ng/mL QCS standard solution is prepared by diluting an aliquot of the QCS PDS with a 96% methanol/ 4% reagent water solution and adding an IS spike and a SUR spike into a 1 mL autosampler vial. The 20-30 ng/mL QCS standard solution is used to verify the detection of the branched isomers within the second source target analytes.

Table 6.4.4.1 – 20-30 ng/mL QCS Standard				
200 ng/mL QCS Aliquot	**96% MeOH/ 4%Water**	**IS Spike**	**SUR Spike**	**Final Volume**
150 µL	650 µL	100 µL	100 µL	1 mL
PFAS Analyte	**Initial Conc. (ng/mL)**		**Final Conc. (ng/mL)**	
PFBS	177		26.5	
PFHxA	200		30	
HFPO-DA	200		30	
PFHpA	200		30	
PFHxSK (L and B)	182.5		27.4	
ADONA	189		28.3	
PFOA	200		30	
PFOSK (L and B)	185.2		27.8	
PFNA	200		30	
9Cl-PF3ONS	187		28.1	
PFDoA	200		30	
NMeFOSAA (L)	152		22.8	
PFUnA	200		30	
NEtFOSAA (L)	155		23.25	
11Cl-PF3OUdS	189		28.3	
PFDA	200		30	
PFTrDA	200		30	
PFTA	200		30	

6.5. T-PFOA Qualitative Standard: The qualitative PFOA standard contains both the linear and branched isomers for PFOA and is used to identify the retention times of the branched PFOA isomers. Specifically, the P6MHpA branched isomer must be visible as a retention peak in the chromatogram.

6.5.1. T-PFOA Stock Standard Solution – The 50 ng/µL T-PFOA SSS was purchased from Wellington Labs (Wellington Labs Cat. No.: T-PFOA).

6.5.2. T-PFOA Primary Dilution Standard – The 50 ng/µL T-PFOA SSS is diluted using a 96% methanol/ 4% reagent water solution to create the T-PFOA PDS with a final concentration of 30 ng/mL.

Table 6.5.2.1 – 30 ng/mL T-PFOA Dilution Isomer Concentrations				
50 ng/µL T-PFOA Aliquot		**96% MeOH/ 4%Water**		**Final Volume**
15 µL		24.985 mL		25 mL
PFAS Analyte	**Isomer**	**Isomer Amount (%)**	**Purchased Conc. (ng/µL)**	**Final Conc. (ng/mL)**
T-PFOA	Linear	79	39.5	23.7
T-PFOA	Total Branched	21	10.5	6.3
T-PFOA	P3MHpA	3.0	1.5	0.9
T-PFOA	P4MHpA	4.0	2.0	1.2
T-PFOA	P5MHpA	4.5	2.25	1.35
T-PFOA	P6MHpA	9.0	4.5	2.7
T-PFOA	Remaining Branched[1]	0.5	0.25	*0.15

[1]*Remaining Branched Isomers: P2MHpA, P35DMHxA, P44DMHxA, P45DMHxA, and P55DMHxA at a combined percent composition of 0.5%.*
**Not >1/3 MRL (0.4167 ng/mL).*

7. Sample Collection

7.1. Pre-Shipment Cooler Preparation: For each site being sampled, a cooler is prepared with a proper label identifying the water system being tested and the entry point number on top and with the necessary bottles, the instructions and chain of custody paperwork, a bag for ice, and two sets of nitrile gloves inside. For every site being sampled, 10 total bottles need to be prepared: two bottles for sample collection, four bottles for FRB, and four bottles for LFSM and LFSMD. The duplicate bottle amounts allow for the mandatory testing of backup sampling if the sample comes back positive for method analytes.

7.1.1. Sample: The two sample bottles are filled with 1.25 g of Trizma, labeled properly, and put into individually sealed bags.

7.1.2. FRB: The four FRB bottles are separated into pairs. The first FRB bottle is filled with 250 mL of reagent water and labeled properly. The second bottle is filled with 1.25 g of Trizma and labeled properly. The pairs are put into sealed bags. At the site, the sampler will pour the 250 mL of reagent water from the full bottle into the bottle with the 1.25 g of Trizma, seal, and treat as a sample from there on out.

7.1.3. LFSM and LFSMD: The four LFSM and LFSMD bottles are filled with 1.25 g of Trizma, labeled properly, and put into individually sealed bags.

7.1.4. Ice Bag: A large bag is included in the cooler to be filled with ice by the sampler. A proper label is included on the bag with a reminder to keep the ice separate from the bottles to avoid contamination.

7.1.5. Instructions and CoC Paperwork: The paperwork is put into its own bag and included within the cooler.

7.2. Sample Shipment and Storage: Water samples are collected in a 250 mL polypropylene bottle containing 1.25 g of Trizma to balance the pH and dechlorinate the sample. All sample bottles must be cooled to 4° C after sample collection.

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

7.2.2. Holding time of 250 mL samples for extraction is 14 days from the collection date. Samples must be stored at or below 6 °C but must not be frozen. Holding time of 1 mL extractions for instrument analysis is 28 days from the extraction date. Extracts must be stored at room temperature.

8. Calibration

8.1. A certified Sciex engineer arrives every six months to perform scheduled machine maintenance, cleaning, calibration, and tuning. Members of the EPD Laboratory are not allowed to perform tuning or maintenance on the Sciex Triple Quad 6500+.

8.2. Initial Calibration Curve:

8.2.1. Prepare the set of six CAL standards ranging from 1.25 ng/mL-30 ng/mL as described in Section 6.3. The lowest concentration CAL standard is prepared at the MRL.

8.2.2. The LC-MS/MS system is calibrated using the IS technique. Use the LC-MS/MS data system software to generate a linear regression for each of the analytes. This curve must always be forced through zero to allow for a better estimate of the background levels of method analytes.

8.2.3. Calibration Acceptance Criteria – Validate the initial calibration by calculating the concentration of each analyte as an unknown against its regression equation. The analyte concentrations for the lowest calibration point for each analyte must be within ± 50% of the true value and the analyte concentrations for the rest of the calibration points must be within ± 30% of the true value. If these criteria are not met, it is recommended that corrective action is taken to reanalyze the CAL standards or restrict the range of calibration.

8.2.4. Peak Asymmetry Factor – Calculate the peak asymmetry factors for the first two eluting peaks in the midpoint CAL standard using Calculation 11.2. The peak asymmetry factors must be within the range of 0.8 to 1.5.

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

8.2.6. The initial calibration curve must be repeated every six months. The calibration curve can be re-run any time the samples or QC lose their analyte or SUR recoveries.

9. Quality Control

9.1. The QC parameters, their required frequencies, and their performance criteria are necessary to meet EPA quality objectives. The QC criteria discussed in the following sections are summarized in Table 14.2.1.

9.2. Initial Demonstration of Capability: The IDC must be successfully performed prior to analyzing any Field Samples. Prior to conducting the IDC, an acceptable Initial Calibration must be generated following the procedure outlined in Section 8.2. The IDC must be performed every six months.

9.2.1. Initial Demonstration of Branched vs Linear Isomer Profile for PFOA in a Qualitative Standard – Prepare and analyze a qualitative technical standard in order to identify the retention times of branched isomers of PFOA. This qualitative PFOA standard is not used for quantitation.

9.2.1.1. The T-PFOA analysis is repeated every six months, any time changes occur that affect analyte retention times, or any time a new calibration curve is set.

9.2.2. Initial Demonstration of Low System Background – Prepare and analyze an LRB to confirm that potential background contaminants are not interfering with the identification of method analytes. Background from method analytes or other contaminants that interfere with the measurement of method analytes must be below 1/3 of the MRL.

9.2.2.1. If the LRB produces a peak within the retention time window of any analyte that would prevent the determination of that analyte, determine the source of contamination, and eliminate the interference before processing samples.

9.2.2.2. The low system background analysis is repeated every six months or any time a new lot of SPE cartridges, solvents, centrifuge tubes, disposable pipettes, and autosampler vials are used.

9.2.3. Initial Demonstration of Precision (IDP) – Prepare and analyze four replicate LFBs fortified at the midpoint of the initial calibration curve along with an LFB Blind. The LFB Blind is fortified by a manager at an unknown concentration. The concentration is revealed post-analysis. Calculate the relative standard deviation (RSD) for each analyte. The RSD must be less than 20% using Calculation 11.1.

9.2.3.1. The IDP is repeated every six months.

9.2.4. Initial Demonstration of Accuracy (IDA) – Using the same set of replicate and blind LFB data generated for Section 9.2.3, calculate the average recovery for each analyte. The average recovery must be within ± 30% of the expected value.

9.2.4.1. The IDA is repeated every six months.

9.2.5. Initial Demonstration of Peak Asymmetry Factor – Using the same set of replicate and blind data generated for Section 9.2.3, calculate the peak asymmetry factors for the first two eluting peaks using Calculation 11.2. The peak asymmetry factors must be within the range of 0.8 to 1.5.

9.2.5.1. If broad, split, or fronting peaks are observed, change the initial mobile phase conditions to higher aqueous content until the peak asymmetry factor is acceptable.

9.2.5.2. The Peak Asymmetry Factor analysis is repeated every six months.

9.2.6. Detection Limit (DL) Determination – Prepare and analyze seven replicate LRBs and seven replicate LFBs at the proposed MRL concentration over the course of three days. Calculate the DL for each analyte for both the LRBs and the LFBs using Calculation 11.3.

9.2.6.1. Do not subtract blank values when performing DL calculations. The DL is a statistical determination of precision only. If the DL replicates are fortified at a low enough concentration, it is likely that they will not meet the precision and accuracy criteria for CCCs. Therefore, no precision and accuracy criteria are specified.

9.2.6.2. Between the two DL values of the LRB and the LFB, use the higher value to set the DL for each analyte.

9.2.6.3. The DL analysis is repeated every six months.

9.2.7. Minimum Reporting Level Confirmation – Establish a target concentration for the MRL based on the intended use of the method. The MRL for this procedure is established at 5 ng/L, or 1.25 ng/250 mL. Establishing the MRL concentration too low may cause repeated failure of ongoing QC requirements.

9.2.7.1. Using the same set of replicate LFB data for section 9.2.6, calculate the Prediction Interval for Results for each analyte and confirm that the upper PIR limit of ≤ 150% recovery and the lower PIR limit of ≥ 50% recovery are met using Calculation 11.4.

9.2.7.2. If the upper and lower PIR limits are not met, set a higher MRL and repeat the LFB analysis.

9.2.7.3. The MRL analysis is repeated every six months.

9.2.8. Calibration Confirmation – Analyze a QCS from a second source prepared at the midpoint concentration to confirm the accuracy of the calibration curve. The calculated concentrations of each analyte must be within ± 30% of the expected value.

9.2.8.1. If measured analyte concentrations are not of acceptable accuracy, check the entire analytical procedure to locate and correct the problem.

9.2.8.2. The QCS analysis is repeated every three months or any time new standards are prepared.

9.3. Ongoing Quality Control Requirements: When processing and analyzing Field Samples, these conditions must be met at regular intervals corresponding to each QC requirement.

9.3.1. Laboratory Reagent Blank Verification – An LRB is required with each extraction batch to confirm that potential background contaminants are not interfering with the identification of method analytes. Analyze one LRB for every 20 Field Samples.

9.3.1.1. Background from method analytes or other contaminants that interfere with the measurement of method analytes must be below 1/3 of the MRL. If the method analytes are detected in the LRB at concentrations equal to or greater than this level, then all data for the problem analytes must be considered invalid for all samples in the extraction batch. Because background contamination is a significant problem for several method analytes, maintaining a historical record of LRB data is highly recommended.

9.3.2. Detection Limit Verification – An LFB fortified at the low point concentration of the calibration curve is required with each extraction batch to compile a continuous format DL/MDL Study. The LRB and the low point fortified LFB data are compiled on a yearly basis as the MDL_{Blank} and the MDL_{Spike}. Calculate the DL for each analyte for both the LRBs and LFBs using Calculation 11.3.

9.3.2.1. Do not subtract blank values when performing DL calculations. The DL is a statistical determination of precision only. If the DL replicates are fortified at a low enough concentration, it is likely that they will not meet the precision and accuracy criteria for CCCs. Therefore, no precision and accuracy criteria are specified.

9.3.2.2. Between the two DL values of the LRB and the LFB, use the higher value to set the DL for each analyte.

9.3.2.3. The DL analysis is repeated every six months.

9.3.3. Laboratory Fortified Blank Verification – An LFB is required with each extraction batch to confirm that the laboratory is capable of making accurate and precise measurements. Analyze one LFB for every 20 Field Samples. The fortified concentration of the LFB must be rotated between the low point, the midpoint, and the high point concentrations of the initial calibration curve from batch to batch.

9.3.3.1. Results of the low point LFB analyses must be 50-150% of the true value. Results of the midpoint and high point LFB analyses must be 70-130% of the true value. If the LFB results do not meet these criteria for method analytes, then all data for the problem analytes must be considered invalid for all samples in the extraction batch.

9.3.4. Laboratory Fortified Sample Matrix Verification – An LFSM is required with each extraction batch to confirm that the sample matrix does not adversely affect method accuracy. Analyze one LFSM for every 20 Field Samples. The fortified concentration of the LFSM must be rotated between the low point, the midpoint, and the high point concentrations of the initial calibration curve from batch to batch.

9.3.4.1. The percent recovery for the LFSM must take into account the sample matrix sample recovery when trying to calculate the recovery of just the fortified concentrations. This is reflected when using Calculation 11.5. Results of the low point LFSM analyses must be 50-150% of the true value. Results of the midpoint and high point LFSM analyses must be 70-130% of the true value. If the LFSM results do not meet these criteria for method analytes, and the laboratory performance for that analyte is shown to be in control in the CCCs, then the data for the problem analytes is judged to be suspect due to matrix bias in both the fortified and unfortified samples.

9.3.5. Laboratory Fortified Sample Matrix Duplicate Verification – An LFSMD is required with each extraction batch to confirm the precision associated with sample collection, preservation, storage, and laboratory procedures. The LFSMD is fortified at the same concentration as the LFSM in each batch. An LFSMD, rather than a FD, is used since the occurrence of method analytes in the samples is low.

9.3.5.1. Calculate the relative percent difference for the duplicate analyte concentration values using Calculation 11.6. Results of the low point RPD analyses must be $\leq$ 50%. Results of the midpoint and high point RPD analyses must be $\leq$ 30%. If the

RPD results do not meet these criteria for method analytes, and the laboratory performance for that analyte is shown to be in control in the CCCs, then the data for the problem analytes is judged to be suspect due to matrix bias in both the fortified and unfortified samples.

9.3.6. Continuing Calibration Check – CCC Standards are analyzed at the beginning of the analysis batch, after every 10 Field Samples, and at the end of the analysis batch. The beginning CCC of each analysis batch must be at or below the MRL to verify instrument sensitivity prior to any analyses. Subsequent CCCs should alternate between a medium and high concentration CAL standard.

9.3.6.1. Determine that the retention peak areas of ISs are within 70-140% of the areas measured in the most recent continuing calibration check, and within 50-150% from the average areas measured during initial calibration. If any of the IS areas has changed by more than these amounts, adjustments must be made to restore system sensitivity and may include cleaning of the MS ion source. Major instrument maintenance, such as cleaning the electrospray probe, atmospheric pressure ionization source, or mass analyzer; replacing the LC column; etc., requires recalibration and verification of sensitivity by analyzing a CCC at or below the MRL.

9.3.6.2. Calculate the concentration of each analyte and SUR in the CCC. The calculated amount for each analyte and SUR for medium and high level CCCs must be within ± 30% of the true value. The calculated amount for the lowest calibration point for each analyte must be within ± 50% of the true value and the SUR must be within ± 30% of the true value. If these conditions do not exist, then all data for the problem analyte must be considered invalid. Any Field or QC Samples that have been analyzed since the last acceptable calibration verification that are still within holding time must be reanalyzed after adequate calibration has been restored, with the following exception. If the CCC fails because the calculated concentration is greater than the upper limit for a particular method analyte, and Field Sample extracts show no detection for that method analyte, non-detects may be reported without re-analysis.

9.3.7. Internal Standard Recovery – The retention peak areas of the ISs in all injections during all analyses must be within 70-140% of the response in the most recent CCC and must not deviate by more than 50% from the average area measured during initial analyte calibration.

9.3.7.1. If the IS areas in a chromatographic run do not meet these criteria, inject a second aliquot of that extract aliquot into a new capped autosampler vial. Random evaporation losses have been observed with the polypropylene caps causing high IS areas. If the extract reanalysis produces an acceptable IS response, report results only for the reanalyzed extract. If the reinjected extract fails again, the calibration should be checked by analyzing the most recently acceptable CAL standard. If the CAL standard fails the criteria of Section 9.3.6, recalibration is necessary. If the CAL standard is acceptable, extraction of the sample should be repeated provided the sample is still within the holding time. Otherwise, report all data for that sample as suspect due to IS recovery. Alternatively, collect a new sample and re-analyze.

9.3.8. Surrogate Standard Recovery – The SUR recovery for all samples, blanks, and CCCs must be within 70-130%.

9.3.8.1. If the SUR recovery does not meet this criterion, check the standard solutions for degradation, check for contamination, and check for instrument performance. Correct the problem and reanalyze the extract. If the extract reanalysis meets the SUR recovery criterion, report results only for the reanalyzed extract. If the reinjected extract fails again, the calibration should be checked by analyzing the most recently acceptable CAL standard. If the CAL standard fails the criteria of Section 9.3.6, recalibration is necessary. If the CAL standard is acceptable, extraction of the sample should be repeated provided the sample is still within the holding time. Otherwise, report all data for that sample as suspect due to SUR recovery. Alternatively, collect a new sample and re-analyze.

9.3.9. Field Reagent Blank Verification – Analysis of an FRB is required only if a Field Sample contains method analytes at or above the MRL to confirm that PFAS measured in the Field Samples were not inadvertently introduced into the sample during sample collection and handling. The FRB is processed, extracted, and analyzed in exactly the same manner as a Field Sample. If the method analytes found in the Field Sample are present in the FRB at a concentration greater than 1/3 the MRL, then all samples collected with that FRB are invalid and must be recollected and reanalyzed.

9.3.10. See Appendix A for control limits of QC samples.

10. Procedure

10.1. Sample Preparation:

10.1.1. Before interacting with the samples, don a pair of nitrile gloves and a polyethylene gown to prevent contamination from clothing and touch transference.

10.1.2. The SPE cartridges and sample bottles described in this Section are designed as single use items and must be discarded after use. They may not be refurbished for reuse in subsequent analyses.

10.1.3. Each sample batch cooler contains six filled sample bottles, two filled FRB bottles, and two empty FRB transfer bottles from the sampling site. Samples were collected and stored according to Section 7.2. Each extraction batch requires an LFSM and an LFSMD to assess sample matrix bias. For each extraction batch, collect three sample bottles from one sample location and a sample bottle from every subsequent sample location from their storage refrigerator and set them aside to begin acclimating to room temperature. The three sample bottles are for the Field Sample, the LFSM, and the LFSMD. The other three sample bottles are backups in case of the need for retesting. The FRB only needs to be tested if a sample contains analytes above the MRL.

10.1.4. Collect the SUR PDS, PFAS PDS, and PFAS SDS from their storage refrigerator to begin acclimating to room temperature.

10.1.5. Perform the pipette verification for each volume that will be drawn prior to performing any pipetting. The pipette must measure within ± 3% of the true volume for the average of two pulls. The volume is measured by pulling and weighing water while assuming a density of 1 g/mL. An analytical scale capable of measuring out to four decimal places is used. These volumes typically include 100 µL for the SUR and IS, 900 µL for the 96% methanol/ 4% reagent water solution used to dilute the IS, and volumes for the PFAS PDS/SDS dependent on the low point, midpoint, or high point fortification concentration of the LFB, LFSM, and LFSMD.

Table 10.1.5.1 – LFB and LFSM PFAS Dilutions

Desired Final Conc	200 ng/mL PFAS Aliquot	20 ng/mL PFAS Aliquot	Sample Volume	Final Volume
1.25 ng/mL	N/A	62.5 µL	~250 mL	1 mL
2.0 ng/mL	N/A	100 µL	~250 mL	1 mL
5.0 ng/mL	25 µL	N/A	~250 mL	1 mL
10 ng/mL	50 µL	N/A	~250 mL	1 mL
20 ng/mL	100 µL	N/A	~250 mL	1 mL
30 ng/mL	150 µL	N/A	~250 mL	1 mL

10.1.6. Begin preparation of one LRB and one LFB for every 20 Field Samples being analyzed while waiting for the samples to acclimate to room temperature. The LFSM and LFSMD do not count as Field Samples. Weigh out the Trizma by zeroing the scale with the empty bottle on it and adding the 1.25 g needed for each LRB and LFB. Fill the bottles containing the Trizma with 250 mL of reagent water measured using a 250 mL polypropylene graduated cylinder.

10.1.7. Once the samples and standards are at room temperature, verify that the sample pH is 7 ± 0.5. For every bottle that the pH falls outside of the range, test one of

the backups. If a total of three bottles do not pass the pH test, run the three bottles with the closest pH and flag the sample as pH imbalanced.

10.1.8. Fortify the bottles with the proper standards. A spike witness is necessary to verify the proper amounts of fortifications were used in the proper bottles.

10.1.8.1. Fortify the Field Samples, LRBs, LFBs, LFSMs, and LFSMDs with 100 µL of SUR standard prepared at 10-40 ng/mL. This will yield a concentration of 1-4 ng/mL of each SUR in the 1 mL final volume.

10.1.8.2. Fortify the LFBs, LFSMs, and LFSMDs with the proper amount of PFAS PDS or SDS according to Table 10.1.5.1. The fortified concentration of the LFB, LFSM, and LFSMD must be rotated between the 1.25 ng/mL low point, the 10 ng/mL midpoint, and the 30 ng/mL high point concentrations from batch to batch. The LFSM and LFSMD need to be fortified at the same concentration within a single batch.

10.1.8.3. Once all the bottles are properly fortified, cap and invert the bottles to mix.

10.1.9. Mark the water level of the samples on the outside of the bottles. This indirect measurement will be used to determine the sample volume after the extraction. Some of the PFAS adsorb to surfaces, thus the sample may not be transferred to a graduated cylinder for volume measurement.

10.2. Extraction:

10.3. Holding time of 250 mL samples for extraction is 14 days from the collection date. Samples must be stored at or below 6 °C but must not be frozen.

10.3.1. Before the samples and QCs can be extracted, the SPE cartridges need to be conditioned. During the conditioning phase, do not allow the cartridge packing material to go dry. If the cartridge goes dry at any point during the conditioning phase, the conditioning must be started over.

10.3.1.1. Rinse each cartridge with 15 mL of methanol. Allow the packing material to soak for two minutes.

10.3.1.2. Without letting the packing material go dry, rinse each cartridge with 18 mL of reagent water. Allow the packing material to soak for two minutes.

10.3.1.3. Refill the cartridges with reagent water and attach the sample transfer tubes.

10.3.2. Turn on the vacuum to a low setting (3-5” Hg) to allow for controlled dropwise sample extraction.

10.3.3. Begin extracting the samples and QCs at a flow rate of 10-15 mL/min which translates to a steady drip and a total extraction time of 15-25 min. Do not allow the cartridge to go dry before all the sample has passed through.

10.3.4. After the entire sample has passed through the cartridge, rinse the sample bottles with two 7.5 mL aliquots of reagent water, and draw each aliquot through the sample transfer tubes and the cartridges.

10.3.5. Open the manifolds completely and draw air through the cartridges to dry them for 5 min at high vacuum (10-15” Hg).

10.3.6. Close the manifolds and insert the 15 mL centrifuge tubes into the extraction tank to collect the extracts as they are eluted from the cartridges. Reset the vacuum to a low setting.

10.3.6.1. *Do not forget to place the collection tubes into the correct racks before elution.*

10.3.7. Rinse the sample bottles with 4 mL of methanol and elute the analytes from the cartridges by pulling the 4 mL of methanol through the sample transfer tubes and the cartridges in a dropwise fashion. Allow the packing material to soak for two minutes and then open the manifold to collect all the methanol.

10.3.8. Close the manifold again. Rinse the sample bottles with a second 4 mL of methanol and elute the remaining analytes from the cartridges by pulling the 4 mL of methanol through the sample transfer tubes and the cartridges in a dropwise fashion. Allow the packing material to soak for two minutes and then open the manifold to collect all the methanol.

10.3.9. Set aside the centrifuge tubes for concentration and clean the extraction station.

10.3.9.1. Rinse the transfer tubing with methanol by partially filling a 250 mL bottle and pulling it through all the transfer tubing and used cartridges.

10.3.9.2. Determine the sample volumes by filling the sample bottles with water to the lines marked in Section 10.1.9. Measure the volume to the nearest 2 mL using a graduated cylinder. The sample volume will be used in the final calculations of the analyte concentration.

10.4. Concentration:

10.4.1. If the concentration cannot be performed on the same day as the extraction, store the centrifuge tubes in the sample refrigerator overnight.

10.4.2. If the centrifuge tubes were refrigerated overnight, allow them to acclimate to room temperature before concentration.

10.4.3. Concentrate the extracts collected in the centrifuge tubes to dryness under a gentle stream of Nitrogen in a heated water bath set to 60-65 °C to remove all the methanol and water mixture.

10.4.4. Collect the IS PDS and the 96% methanol/ 4% reagent water from their storage refrigerator to begin acclimating to room temperature. Once at room temperature, vortex the solutions prior to use.

10.4.5. Add 900 µL of 96% methanol/ 4% reagent water and 100 µL of IS prepared at 10-40 ng/mL to the dry centrifuge tubes. This will yield a concentration of 1-4 ng/mL of each IS in the 1 mL final volumes.

10.4.6. Store the centrifuge tubes filled with 1 mL of solution for analysis runs and dilutions at room temperature.

10.5. Sample Analysis:

10.5.1. Holding time of 1 mL extractions for instrument analysis is 28 days from the extraction date. Extracts must be stored at room temperature.

10.5.2. Collect the double blank and the CCCs from their storage refrigerator to begin acclimating to room temperature. The double blank is a 2 mL autosampler vial filled with the 96% methanol 4% reagent water solution. The double blank is run three times before the analysis batch to further clear the instrument of built up PFAS contamination. The CCCs are the 1.25 ng/mL low point, the 10 ng/mL midpoint, and the 30 ng/mL high point CAL standards. The low point CCC is run at the beginning of the analysis batch, the midpoint CCC is run after 10 Field Samples of the analysis batch, and the high point CCC is run at the end of the analysis batch. If the analysis batch does not contain over 10 Field Samples, then the midpoint and high point CCC should be ran at the end of the analysis batch and be alternated between batches.

10.5.3. Transfer a small aliquot of the samples, LFSM, LFSMD, LRB, DL, and LFB from the 15 mL centrifuge tubes into the 300 µL polypropylene autosampler vials for injection. Polypropylene caps do not reseal after injection, so evaporation losses occur in the autosampler vials. Thus, multiple injections from the same vial are not possible.

10.5.4. Prior to running the samples, check to make sure the Detailed Status icon (left icon with two parallel bars) in the bottom right of the Analyst application is not red. If the icon is red, the system must be rebooted. This icon turns red when the Sciex Triple Quad 6500+ instrument is disconnected from power.

10.5.4.1. The first reboot operation is to reconfigure the hardware. In Analyst, double click on Hardware Configuration in the left panel. Highlight and Deactivate the LC LCMS profile. Wait a minute and reactivate the profile. The green check mark should reappear next to the LC LCMS profile.

10.5.4.2. If the Detailed Status icon is still red, the second reboot operation is to restart all the auxiliary systems. Turn off the AC Pump, AC Autosampler, Controller, and AC Column Oven. Wait a minute and turn the AC Pump, AC Autosampler, and AC Column Oven back on. Once all the front facing lights on the systems are green, turn the Controller back on last.

10.5.4.3. If the Detailed Status icon is still red, the third reboot operation is to restart the Sciex Triple Quad 6500+. On the back-left side of the instrument is a red button. Hold down this button for six seconds. This should trigger the instrument to turn off and begin resetting.

10.5.4.4. If the Detailed Status icon is still red, a Sciex certified engineer is required to fix the issue.

10.5.5. Prior to running the samples, the instrument must be flushed and equilibrated.

10.5.5.1. Prior to daily use, flush the column with 100% methanol for at least 20 minutes before initiating a sequence. If background contamination begins to rise over time, it may become necessary to flush the column for a longer period. This is achieved by double clicking on the Integrated System icon (middle icon with three left arrows) in the bottom right of the Analyst application. Expand the Binary Gradient tab and change the concentration corresponding to methanol to 100%. Be sure to change the correct concentration; the two concentrations A and B correspond to Ammonium Acetate and methanol. Press the pump icon aligned with “AC” to initiate the pump. The icon will turn blue, and the pressure will increase when running.

10.5.5.2. Use the 20 minutes that the column is being flushed to create the sample batch.

10.5.5.2.1. To create a sample batch, double click on Build Acquisition Batch in the left panel to pull up the Batch Editor.

10.5.5.2.2. Set the Acquire Mode to EPA Method 537/Reports (or the most recently set acquire mode) by selecting the mode in the drop-down tab in the top bar (next to yellow folder icon). Input the desired Set Name to include the date of analysis and the samples being ran, change the Quantitation drop-down tab to EPA 537 (or the most recently set quantitation method), and change the Acquisition drop-down tab to EPA 537-SMRM with delay column (or the most recently set acquisition method). Select Add Set.

10.5.5.2.3. Select Add Samples. Clear the Sample Name and Data File Prefixes set to *Sample* and *Data* and leave blank. Set the Number of New Samples to the correct number. For a single drinking water site sample this will include three for the initial double blank, the initial CCC, LRB, DL, LFB, Field Sample, LFSM, LFSMD, and closing CCC for a total of 11 New Samples.

10.5.5.2.4. Right click in the chart and select Add/ Hide Columns. Add the sample ID column and the Comments column. Add the Sample IDs, including the sample ID with the LFSM added to the QC vials. Put the LabWorks test codes into the

Comments column. Change the sample names to include the sample ID and the extraction date. Change the initial CCC name to start with Ini CCC. The CCC IS Area calculation column uses the beginning characters to detect the IS Areas to compare to. Change the vial positions in the table to match the samples being ran. The Rack Code, Rack Position, Plate Code, and Plate Position should not need to be adjusted. For the double blank, the sample will be listed three times and the vial position will be the same for all three.

10.5.5.2.5. Verify the vial positions are correct by selecting the Locations tab and double clicking on 1.5mL 105 vials.

10.5.5.2.6. Set the sample types for the vials being run by selecting the Quantitation tab and changing the column labeled Sample Type. The double blanks are set to double blank, the samples, LFSM, and LFSMD are left as unknowns, and the LRB, DL, LFB, and CCCs are set as QC.

10.5.5.2.7. Save the Acquisition Batch as a new .dab file and click submit to submit the batch for the Sample Queue.

10.5.5.2.8. Open the Sample Queue by selecting Acquire in the left tab, selecting the View tab in the top bar, and selecting Sample Queue (equal sign page icon). The newly submitted Acquisition Batch should be listed in the Sample Queue as Waiting.

10.5.5.3. After the 20-minute methanol column flush, disengage the pump by pressing the pump icon again. The icon will go grey and the pressure will return to near zero. Return the concentrations A and B to their starting values used for analysis, which is 60% Ammonium Acetate and 40% methanol.

10.5.5.4. Equilibrate the machine by selecting Acquire in the left panel, selecting the Acquire tab in the top bar, and selecting Equilibrate (heartbeat EKG icon). Set the equilibration time to 15 minutes. In order to equilibrate, be sure that the instrument is not reserved for tuning. Check for tuning by selecting Acquire in the left panel, selecting the Tools tab in the top bar, and making sure that Tune Instrument is not selected (purple T icon). Set a timer to be there exactly when the 15 minutes is up. LC system components, as well as the mobile phase constituents, contain many of the analytes in this method. Thus, these PFAS will build up on the head of the LC column during mobile phase equilibration. To minimize the background PFAS peaks and to keep background levels constant, the time the LC column sits at initial conditions must be as short as possible and kept constant.

10.5.5.5. Use the 15 minutes that the instrument is being equilibrated to insert the autosampler vials in the loading tray.

10.5.5.5.1. Before loading the autosampler vials into the loading tray, they should be acclimated to room temperature and vortexed. Put on nitrile gloves and a polypropylene gown before opening the instrument and working with the loading tray. The loading tray can be found inside the AC autosampler door of the instrument. Match the vial locations shown in the acquisition batch vial position tab with the correct sample names and vials. When replacing the tray, make sure that the tray is level, on the track, and pushed in completely.

10.5.6. After the 15-minute equilibration, start the sampling by selecting acquire in the left panel, selecting the acquire tab in the top bar, and selecting start samples (Erlenmeyer flask icon). Be sure to start the sampling as soon as possible after the equilibration. When the equilibration is done, the icons in the bottom right will

change from yellow to green, and the start samples icon will turn from faded to dark grey and will become selectable.

10.5.7. Track the completion of the sampling using the Sample Queue. Once the sampling is done, return the instrument to standby mode by selecting Acquire in the left tab, selecting Acquire in the top bar, and selecting Standby (hourglass icon). The data is automatically saved as a .wiff file under the same name as the Acquisition Batch.

10.5.8. Remove the autosampler vials from the loading tray. Replace all the punctured caps on the 2 mL autosampler vials with new caps. Store the 2 mL autosampler vials for further analysis or dilutions. Dispose of the remaining 300 µL sample solutions properly and dispose of the 300 µL autosampler vials.

10.6. Data Analysis:

10.6.1. A preview of the chromatogram is available in the Analyst software. All data analysis is done using Sciex OS.

10.6.2. In Sciex OS, select the Projects tab and select EPA Method 537/Reports (or the most recently set project).

10.6.3. Select the Results tab and create a new result.

10.6.3.1. Highlight the Analyst data file (.wiff) and click the right arrow to select the data file for analysis. Add the latest calibration curve data file to be analyzed to serve as the standard of comparison to calculate the concentrations of found analytes. Set the Sample Type for the calibration curve data to standard.

10.6.3.2. Under Processing Method, select browse and select 20201123MethodwithAcidandIsomers.qmethod (or the most recently set processing method). Select process to compile results.

10.6.4. The tandem MS identifies analytes by identifying both the precursor ion and the product ion. This two-step identification removes a level of uncertainty in identifying analytes. The Sciex OS software automatically returns retention times for detected analyte peaks.

10.6.5. Sciex OS displays the chromatogram as time (min) vs intensity (counts per second). The relationship of intensity to concentration is not direct and is relative to each analyte based on how readily the analyte ionizes. The initial calibration curve, which has known concentrations, is required to act as the standard of comparison by which each analyte and SUR concentration is calculated. Do not use daily calibration verification data to quantitate analyte concentrations in samples.

10.6.6. Final analyte concentrations are adjusted in LabWorks using Calculation 11.7 to reflect the sample volume determined in Section 10.3.9.2.

10.6.7. Chromatographic peaks in all Field Samples and QC samples must be integrated in the same way as the CAL standards for analytes with quantitative standards containing the branched and linear isomers. The PFAS Standard used to create the calibration curve contains only the linear isomers. Therefore, only the linear isomers can be identified and quantitated in Field Samples and QC samples because the retention time of the branched isomers cannot be confirmed. The exception for this is PFOA because of the analysis of the T-PFOA for branched isomers. For PFOA, integrate over both the branched and linear isomers. Check the peak integrations to make sure that only the linear peaks are integrated for all analytes other than PFOA.

10.6.8. For any samples with an analyte detect above the MRL, perform an extraction on the FRB to confirm there are no interferences present in the field environment. Follow all extraction batch QC when analyzing the FRB.

10.6.9. Extrapolation beyond the established calibration range is not allowed. If an analyte peak area exceeds the range of the initial calibration curve, the extract may be diluted with 96% methanol 4% reagent water solution and the appropriate amount of IS added to match the original concentration. It is recommended not to perform any less than a 4x dilution. When attempting a 2x dilution, 500 mL of the sample is needed out of the 1 mL extracted. If a mistake is made, then not enough of the sample will remain to perform another dilution. Re-inject the diluted extract. Incorporate the dilution factor into the final concentration calculations. Acceptable SUR performance should be determined from the undiluted sample extract. The resulting data must be documented as a dilution and MRLs adjusted accordingly.

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

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

10.6.12. Create a raw data report for the analyzed sample. If samples contained method analyte detects or needed a dilution, create a raw data report for the duplicate samples, FRBs, and/or dilutions. In the sample selection window on the left, highlight all samples except the double blank and the calibration standards. Select Reporting in the top tab, select Create Report and save Results Table…, select EPA Analyte Report in Template Name (or the most recently set template), select Create an individual report for each sample, and select Create.

10.6.13. Create a LabWorks report for data conversion into the LIMS system. In the sample selection window on the left, highlight the LRB, DL, LFB, samples, LFSM, and LFSMD to create the $RWBK537, $RWML537, $RWL537, $RW537, $RWS537, and $RWD537 test codes. Make sure that the Sample ID column is filled in and the Comments column contains the test codes. Select Reporting in the top tab, select Create Report and save Results Table…, select LabWorks in Template Name (or the most recently set template), select CSV in the Report Format, select Create an individual report for each sample, and select Create. For all samples with analyte detects, make a LabWorks report for the FRB as well to create the $RWFB537 test code.

10.6.14. Create the spike amount raw test codes. The necessary spike test codes are for the DL, LFB, LFSM, and LFSMD and are $RWMA537, $RWLA537, $RWAS537, and $RWAD537. These test codes are stored as templates without the Sample IDs written in. Select the right template based on the spike concentration between 1.25 ng/mL, 10 ng/mL, and 30 ng/mL and add the Sample ID in the first column.

10.6.15. Save all the test code data files in the same folder. The LabWorks reports need to be conditioned to remove the empty line at the beginning of the report to output in a format that LabWorks accepts.

10.7. LabWorks:

10.7.1. The samples are batched in the LIMS system LabWorks.

10.7.1.1. Under the QA/QC folder, select QA/QC Batching. Select Specify new batches by analysis. Select Unbatched samples with selected analyses pending and enter $537 as the Analyses available for batching. Checkmark the desired sample IDs that are being analyzed. Match the batch size to the number of samples being analyzed. Select either #Q$537L or #Q$537H for the QA Group Added depending on if the LFB/LFSM(D) are spiked at the low or medium/ high point concentration. Highlight the Sample being used for all the QC. The sample ID should show up under QA Sample ID. Select OK and create the QA/QC Batch. Select print QA/QC Batch sheet and select latest Batch or enter the batch number. Select INSTR for the print format and print the QA/QC Batch Report.

10.7.2. The analysis results are uploaded as raw results directly into LabWorks through Multi Component Results Transfer. The raw results are then sample adjusted to ng/L. Select the Results folder, select Multi Component Transfer, and select Result File Mode. Under Selected Result File, select Add Files to List. Set the Available result file types to + LABWORKS Flat .CSV Results File, navigate in the directory to where the CSV LabWorks reports are saved, set the Result file types to *.csv, highlight the files to input under File name, and select OK. Under Multicomponent Analyses for Selected Result Files, select Find Samples. The chart should populate with the selected results. Select Load Results to load the data in.

10.7.2.1. Click under the Review column for each result to review for mistakes.

10.7.2.2. For all analyte detects beyond the calibration curve which required a dilution, enter the diluted result multiplied by the dilution factor. For example, the original sample could show PFOA at 40 ng/mL. This is above the upper limit of the calibration curve set at 30 ng/mL. With a 4x dilution and being rerun, the value could come out as 9.8 ng/mL. Under PFOA, 39.2 ng/mL would be entered in the $RW537 test code. The MDL would be changed to match the dilution amount. For a 4x dilution, the original MDL of 5 ng/L would be multiplied by 4 to create a value of 20 ng/L.

10.7.2.3. Select Save Results when done reviewing and close the window.

10.7.2.4. Review the LabWorks calculated test codes by selecting the Results folder, selecting Results Entry, selecting the Sample IDs folder, entering the desired Sample IDs in the empty box, selecting View Selections after each Sample ID, and selecting Enter Selection when all Sample IDs are selected. The Results Entry spreadsheet should pop up. If it does not, select the Enter results for selected samples icon in the LABWORKS Results Entry Tab. Review the data by right clicking on each filled test code and selecting Modify results. If everything is correct, select Cancel Changes. If anything needs to be corrected, select Store Changes, and give the reason for modification.

10.7.2.5. The Sciex results are volume corrected for the LRB, samples, LFSM, LFSMD, and FRB using Excel calculation test codes from raw to reportable test codes to convert to units of ng/L.

Table 10.7.2.5.1 – Excel Setup Results Code		
Bottle Type	**Excel Calculation (S:\ Drive)**	**Excel Setup Results Code**
LRB	537BLK	537BLKRAW
Sample	537SAMPL	537RAW
LFSM	537Spike	537SpikeRaw
LFSMD	537Spike	537SpikeDupRaw
FRB	537FBLK	537FBLK

10.7.2.6. For all samples diluted, add a "D" in the Qualifier column under the $537 test code next to the diluted analyte. Add an Analysis Comment that says "$537-EPA 537.1-<D>-Sample required dilution for this compound. Analyzed on xx/xx/xxxx with all QC in compliance. Reporting Limits elevated due to high levels of target and/or non-target compounds." Input the date analyzed.

10.7.3. Any QC that flags red in LabWorks must have a corrective action generated to address the QC failure.

10.7.4. After all analytical data is entered into LabWorks, a QA/QC batch report is generated. Any flagged QC data must have a corrective action printed at the bottom of the page.

10.7.4.1. The QC batch is annotated depending on the spike amounts of the LFB and the LFSM(D). For a low point spike of both the LFB and the LFSM(D), the QC batch is annotated #Q$537L. For a mid or high point spike of both the LFB and the LFSM(D), the QC batch is named #Q$537H. These distinctions are necessary due to the acceptance criteria difference between the low point and mid and high point spikes.

10.7.5. The LabWorks test codes are as follows:

Table 10.7.5.1 - LIMS Test Codes	
Data Element	**LabWorks Test Code**
PFAS 537 Concentration (ng/L)	$537
PFAS 537 Raw Concentration (ng/mL)	$RW537
PFAS 537 Concentration for Calculations (ng/L)	$UNADJ537
LRB/MBLK Concentration (ng/L)	$B_537
LRB/MBLK Raw Concentration (ng/mL)	$RWBK537
DL/MDL Concentration (ng/L)	$ML537
DL/MDL Raw Concentration (ng/mL)	$RWML537
DL/MDL Spike Amount (ng/L)	$MA537
DL/MDL Raw Spike Amount (ng/mL)	$RWMA537
LFB/LCS Concentration (ng/L)	$LS537
LFB/LCS Raw Concentration (ng/mL)	$RWLS537
LFB/LCS Spike Amount (ng/L)	$LA537
LFB/LCS Raw Spike Amount (ng/mL)	$RWLA537
LFB/LCS Low Spike Recovery (%)	$LRL537
LFB/LCS Med High Spike Recovery (%)	$LRH537
LFSM/MS Concentration (ng/L)	$S_537
LFSM/MS Raw Concentration (ng/mL)	$RWS537
LFSM/MS Spike Amount (ng/L)	$AS537
LFSM/MS Raw Spike Amount (ng/mL)	$RWAS537
LFSM/MS Low Recovery (%)	$R_L537
LFSM/MS Med High Recovery (%)	$R_H537
LFSMD/MSD Concentration (ng/L)	$D_537

Table 10.7.5.1 - LIMS Test Codes	
Data Element	**LabWorks Test Code**
LFSMD/MSD Raw Concentration (ng/mL)	$RWD537
LFSMD/MSD Spike Amount (ng/L)	$AD537
LFSMD/MSD Raw Spike Amount (ng/mL)	$RWAD537
LFSMD/MSD Low Recovery (%)	$RDL537
LFSMD/MSD Med High Recovery (%)	$RDH537
LFSM(D)/MS(D) Low Precision	$P_L537
LFSM(D)/MS(D) Med High Precision	$P_H537
FRB/FB Concentration (ng/L)	$FB537
FRB/FB Raw Concentration (ng/mL)	$RWFB537
Sample Volume Extracted (mL)	537E
LFSM Volume Extracted (mL)	537SE
LFSMD Volume Extracted (mL)	537DE
Instrument	INSTR-537

11. Calculations

11.1. Relative Standard Deviation:

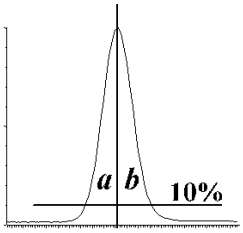
$$RSD = \frac{s}{|\bar{x}|} \times 100\%$$

11.1.1. Sample Standard Deviation:

$$s = \sqrt{\frac{1}{N-1}\sum_{i=1}^{N}(x_i - \bar{x})^2}$$

11.1.2. Where:
s = Sample standard deviation.
$\bar{x}$ = Sample mean.
N = Number of samples.
x_i = The i^{th} value of N samples.

11.2. Peak Asymmetry Factor:



$$A_s = \frac{b}{a}$$

11.2.1. Where:
b = The width of the back half of the peak measured at 10% peak height from the trailing edge of the peak to a line dropped perpendicularly from the peak.
a = The width of the front half of the peak measured at 10% peak height from the leading edge of the peak to a line dropped perpendicularly from the peak.

11.3. Detection Limit:

$$DL = s \times t_{(n-1,1-\alpha=0.99)}$$

11.3.1. Where:
s = Sample standard deviation.
$t_{(N-1,1-\alpha=0.99)}$ = Student's t value for the 99% confidence level with N-1 degrees of freedom.
N = Number of Samples.
α = Level of significance.

11.4. Prediction Interval of Results:

$$PIR = \frac{\bar{x} \pm HR_{PIR}}{x} \times 100\%$$

11.4.1. Half Range for the Prediction Interval of Results:

$$HR_{PIR} = 3.963s$$

11.4.2. Where:
$\bar{x}$ = Sample mean.
x = Sample value.
3.963 = A constant value dependent on degrees of freedom and level of confidence.
s = Standard deviation.

11.5. Percent Recovery (LFSM):

$$\%R = \frac{(f - x)}{c} \times 100\%$$

11.5.1. Where:
f = Measured concentration in the fortified sample.
x = Measured concentration in the unfortified sample.
c = Fortified concentration.

11.6. Relative Percent Difference:

$$RPD = \frac{|f - d|}{\frac{1}{2}(f + d)} \times 100\%$$

11.6.1. Where:
f = Measured concentration of fortified sample.
d = Measured concentration of fortified sample duplicate.

11.7. Final Concentration:

$$x' = c_f x$$

11.7.1. Final Concentration Factor:

$$c_f = \frac{1000\ mL}{V}$$

11.7.2. Where:
x' = Adjusted final concentration.
c_f = Final concentration factor to adjust final concentrations to ng/L.
x = Extraction concentration.
V = Sample volume extracted.

12. Waste Management

12.1. See GA EPD Laboratory SOP-EPD Laboratory Waste Management Standard Operating procedures, online revision (SOP reference 13.4).

13. References

13.1. Shoemaker, J. and Tettenhorst, D. *Method 537.1: Determination of Selected Per and Polyfluorinated Alkyl Substances in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS).* U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment, Washington, DC, 2018.
13.2. GA EPD Laboratory SOP's- Initial Demonstration of Capability SOP 6-001, online revision or Continuing Demonstration of Capability SOP 6-002, online revision.
13.3. GA EPD Laboratory SOP- EPD Laboratory Waste Management SOP, SOP 6-015, online revision.
13.4. GA EPD Laboratory SOP- Determination of Method Detection Limit, Method Detection Limit SOP 6-007, online revision.
13.5. GA EPD Laboratory Quality Assurance Plan, online revision.

14. Minimum Reporting Level, Precision and Accuracy Criteria, and Quality Control Approach

14.1. The MRL of all analytes analyzed by EPA Method 537.1 is 5 ng/L. This translates to 1.25 ng/ 250 mL for the volume of the polypropylene sample bottles.

14.2. The Summary of the Quality Control guidelines put forth in Section 9 are as follows:

Table 14.2.1 Summary of Quality Control

QC Check	Specification	Minimum Frequency	Acceptance Criteria	Corrective Action
8.2 Initial Calibration Curve	Prepare a set of at least five CAL standards with the lowest concentration CAL standard fortified at or below the MRL	Every six months or any time the CCC loses its analyte or SUR recoveries	Analyte recoveries must be within 50-150% for the low point CAL standard and within 70-130% for all other CAL standards when calculated as an unknown against the calibration curve Surrogate recoveries must be within 70-130% for all CAL standards Peak asymmetry factor of 0.8 - 1.5 for the first two eluting peaks	Correct problem and repeat initial calibration
Initial Demonstration of Capability				
9.2.1 Initial Demonstration of Branched vs Linear Isomer Profile for PFOA in a Qualitative Standard	Prepare and analyze a qualitative standard of T-PFOA	Every six months or any time changes occur that affect analyte retention times	Retention times of branched isomers in T-PFOA must be identified	Correct problem and repeat
9.2.2 Initial Demonstration of Low System Background	Prepare and analyze an LRB	Every six months or any time a new lot of SPE cartridges, solvents, centrifuge tubes, disposable pipettes, or autosampler vials are used	All method analyte recoveries are below 1/3 the MRL	Correct problem and repeat
9.2.3 Initial Demonstration of Precision (IDP)	Prepare and analyze four replicate LFBs fortified at the midpoint of the initial calibration curve and an LFB Blind	Every six months	%RSD must be <20%	Correct problem and repeat
9.2.4 Initial Demonstration of Accuracy (IDA)	Analyze the same four replicate LFBs and the LFB Blind from the IDP	Every six months	Mean recovery ± 30% of true value	Correct problem and repeat
9.2.5 Initial Demonstration of Peak Asymmetry Factor	Analyze the same four replicate LFBs and the LFB Blind from the IDP	Every six months	Peak asymmetry factor of 0.8 - 1.5 for the first two eluting peaks	Correct problem and repeat

9.2.6 Detection Limit (DL) Determination	Prepare and analyze seven replicate LRBs and LFBs prepared at the proposed MRL over the course of three days	Every six months	Data from DL replicates are not required to meet method precision and accuracy criteria	Correct problem and repeat
9.2.7 Minimum Reporting Level (MRL) Confirmation	Analyze the same seven replicate LFBs from the DL	Every six months	Upper PIR ≤ 150% Lower PIR ≥ 50%	Correct problem and repeat
9.2.8 Calibration Confirmation	Prepare and analyze a QCS from a second source fortified at the midpoint of the calibration curve	Every three months or any time new standards are prepared	Recovery must be within 70-130% of true value	Correct problem and repeat
Ongoing Quality Control				
7.2.2 Sample Holding Time	Samples are valid for extraction within 14 days of collection when stored below 6 °C and not frozen	Every sample	Results are valid only if samples are extracted within the sample holding time	Samples outside of 14 days of collection must be disposed of
7.2.2 Extract Holding Time	Extracts are valid for analysis within 28 days of extraction when stored at room temperature in polypropylene centrifuge tubes	Every extract	Results are valid only if extracts are analyzed within the extract holding time	Extracts outside of 28 days of extraction must be disposed of
9.3.7 Laboratory Reagent Blank (LRB) Verification	Prepare and analyze an LRB	Every extraction batch (one LRB for every 20 Field Samples)	All method analyte recoveries are below 1/3 the MRL	Results for the method analytes that exceed 1/3 of the MRL are invalid
9.3.8 Laboratory Fortified Blank (LFB) Verification	Prepare and analyze an LFB fortified at a rotation of the low point, midpoint, or high point of the initial calibration curve	Every extraction batch (one LFB for every 20 Field Samples)	Recoveries must be within 50-150% for the low point concentrations and within 70-130% for the mid and high point concentrations	Results for the analytes that fail these criteria are invalid for all samples within the extraction batch
9.3.9 Laboratory Fortified Sample Matrix (LFSM) Verification	Prepare and analyze an LFSM fortified at a rotation of the low point, midpoint, or high point of the initial calibration curve	Every extraction batch (one LFSM for every 20 Field Samples)	Recoveries must be within 50-150% for the low point concentrations and within 70-130% for the mid and high point concentrations	Results are labeled as suspect due to matrix bias for each sample that fails the criteria
9.3.10 Laboratory Fortified Sample Matrix Duplicate (LFSMD) Verification	Prepare and analyze an LFSMD fortified at the same concentration as the LFSM	Every extraction batch (one LFSMD for every 20 Field Samples)	RPDs must be ≤ 50% for the low point concentration and ≤ 30% for the mid and high point concentrations	Results are labeled as suspect due to matrix bias for each sample that fails the criteria
9.3.11 Continuing Calibration Check (CCC)	Analyze a low point CCC prior to analyzing samples, and subsequent CCCs every 10 samples, and after the last sample rotating concentrations to cover the calibrated range of the instrument	Every extraction batch (one CCC at MRL at beginning, one CCC every 10 Field Samples, one CCC at end alternating between midpoint and high point)	Analyte recoveries must be within 50-150% for the low point concentration and within 70-130% for the mid and high point concentrations when calculated as an unknown against the curve Surrogate recoveries must be within 70-130% for all concentrations	Results for the analytes that fail these criteria are invalid If the CCC concentration fails high for a particular analyte, and Field Samples show no detection for that analyte, non-detects may be reported without re-analysis
9.3.12 Internal Standard Recovery	Compare the IS areas to the average IS area in the initial calibration and to the most recent CCC	Every sample and QC	Peak area counts for all ISs must be within ± 50% of the average peak area calculated during the initial calibration and within 70-140% of the most recent CCC	Results for the samples that these criteria are invalid
9.3.13 Surrogate Standard Recovery	Calculate SUR recoveries	Every sample and QC	SUR recoveries must be within 70-130% of the true value	Results are labeled as suspect due to SUR recovery for each sample that fails the SUR criteria

Field Reagent Blank (FRB) Verification	Prepare and analyze an FRB in exactly the same manner as a Field Sample	Analysis of an FRB is required only if a sample contains method analytes above the MRL	All method analyte recoveries corresponding to the method analytes present in the Field Sample are below 1/3 the MRL	If the method analytes found in the Field Sample are present in the FRB at a concentration greater than 1/3 the MRL, then all samples collected with that FRB are invalid and must be recollected and reanalyzed

Appendix A – Quality Assurance Criteria for Method EPA 537.1

Table A.1 - Quality Assurance Criteria for EPA 537.1

		Accuracy (%R) At MRL level		Accuracy (%R) All other levels		Precision MS/MSD At MRL level	Precision MS/MSD All other levels
QC Type	**Analyte**	**LCL**	**UCL**	**LCL**	**UCL**	**(%RPD)**	**(%RPD)**
LCS MS/MSD	11Cl-PF3OUdS	50	150	70	130	≤50	≤30
	9Cl-PF3ONS	50	150	70	130	≤50	≤30
	ADONA	50	150	70	130	≤50	≤30
	HFPO-DA (GenX)	50	150	70	130	≤50	≤30
	N-EtFOSAA	50	150	70	130	≤50	≤30
	N-MeFOSAA	50	150	70	130	≤50	≤30

Table A.1 - Quality Assurance Criteria for EPA 537.1							
		Accuracy (%R) At MRL level		Accuracy (%R) All other levels		Precision MS/MSD At MRL level	Precision MS/MSD All other levels
QC Type	Analyte	LCL	UCL	LCL	UCL	(%RPD)	(%RPD)
	PFBS	50	150	70	130	≤50	≤30
	PFDA	50	150	70	130	≤50	≤30
	PFDoA	50	150	70	130	≤50	≤30
	PFHpA	50	150	70	130	≤50	≤30
	PFHxA	50	150	70	130	≤50	≤30
	PFHxS	50	150	70	130	≤50	≤30
	PFNA	50	150	70	130	≤50	≤30
	PFOA	50	150	70	130	≤50	≤30
	PFOS	50	150	70	130	≤50	≤30
	PFTeDA	50	150	70	130	≤50	≤30
	PFTrDA	50	150	70	130	≤50	≤30
	PFUnA	50	150	70	130	≤50	≤30
Surrogates	SUR-13C2_PFDA	70	130	70	130	NA	NA
	SUR-13C2-PFHxA	70	130	70	130	NA	NA
	SUR-13C3-HFPO-DA	70	130	70	130	NA	NA
	SUR-d5-NEtFOSAA	70	130	70	130	NA	NA