METHOD 8327

PER- AND POLYFLUOROALKYL SUBSTANCES (PFAS) USING EXTERNAL STANDARD CALIBRATION AND MULTIPLE REACTION MONITORING (MRM) LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY (LC/MS/MS)

TABLE OF CONTENTS

Disclaimer

SW-846 is not intended to be an analytical training manual. Therefore, method procedures are written based on the assumption that they will be performed by analysts formally trained in the basic principles of chemical analysis and in the use of the subject technology.

In addition, SW-846 methods, with the exception of required use for the analysis of method-defined parameters, are intended to be guidance methods which contain general information on how to perform an analytical procedure or technique, which a laboratory can use as a basic starting point for generating its own detailed standard operating procedure (SOP), either for its own general use or for a specific project application. Performance data included in this method are for guidance purposes only and must not be used as absolute quality control (QC) acceptance criteria for the purposes of laboratory QC or accreditation.

1.0 SCOPE AND APPLICATION

This method covers the analysis of selected per- and polyfluoroalkyl substances (PFAS) in prepared extracts of various matrices (e.g., waters and solids) by liquid chromatography/tandem mass spectrometry (LC/MS/MS) analysis.

The 24 PFAS that have been evaluated with this method are provided below. The suggested Lower Limit of Quantitation (LLOQ) and calibration ranges for these compounds listed in Table 1 (Sec. 17.0) are based on the example calibration standards and spiking solutions preparations described in Sec. 7. This method has been tested in reagent water, surface water, groundwater, and wastewater matrices. Some precision and bias (P&B) data are provided in Table 2 (Sec. 17.0).

Analyte		CAS RN*
PFAS sulfonic acids		
Perfluoro-1-butanesulfonic acid (PFBS)		375-73-5
Perfluoro-1-pentanesulfonic acid (PFPeS)		2706-91-4
Perfluoro-1-hexanesulfonic acid (PFHxS)		355-46-4
Perfluoro-1-heptanesulfonic acid (PFHpS)		375-92-8
Perfluoro-1-octanesulfonic acid (PFOS)		1763-23-1
Perfluoro-1-nonanesulfonic acid (PFNS)		68259-12-1
Perfluoro-1-decanesulfonic acid (PFDS)		335-77-3
1H, 1H, 2H, 2H-perfluorohexane sulfonic acid (4:2 FTS)		757124-72-4
1H, 1H, 2H, 2H-perfluorooctane sulfonic acid (6:2 FTS)	#	27619-97-2
1H, 1H, 2H, 2H-perfluorodecane sulfonic acid (8:2 FTS)	#	39108-34-4
PFAS carboxylic acids		
Perfluorobutanoic acid (PFBA)	#	375-22-4
Perfluoropentanoic acid (PFPeA)	#	2706-90-3
Perfluorohexanoic acid (PFHxA)	#	307-24-4
Perfluoroheptanoic acid (PFHpA)		375-85-9
Perfluorooctanoic acid (PFOA)		335-67-1
Perfluorononanoic acid (PFNA)		375-95-1
Perfluorodecanoic acid (PFDA)		335-76-2
Perfluoroundecanoic acid (PFUdA)	#	2058-94-8
Perfluorododecanoic acid (PFDoA)	#	307-55-1
Perfluorotridecanoic acid (PFTrDA)	#	72629-94-8

Analyte		CAS RN*
Perfluorotetradecanoic acid (PFTeDA)	#	376-06-7
PFAS sulfonamides and sulfonamidoacetic acids		
N-ethylperfluoro-1-octanesulfonamidoacetic acid (N-EtFOSAA)	#	2991-50-6
N-methylperfluoro-1-octanesulfonamidoacetic acid (N-MeFOSAA)	#	2355-31-9
Perfluoro-1-octanesulfonamide (FOSA)		754-91-6

*Standards for some target analytes may consist of mixtures of structural isomers; however, the Chemical Abstracts Service (CAS) Registry Number (RN) listed in the table is for the normal-chain isomer. All CAS RNs in the above table are for the acid form. Sulfonic acids in stock standard mixes are typically received as the sodium or potassium salt form. CAS RNs for the salt form are not included.

This analyte exhibits known difficulties with reproducibility, response, recovery, stability, and/or chromatography that may reduce the overall quality or confidence in the result when using this method. This analyte may require special care to ensure analytical performance will meet the needs of the project and, where necessary, may also require the use of appropriate data qualification. See Sec. 1.3 for specific information regarding this analyte.

1.1 Prior to employing this method, analysts are advised to consult the base method for each type of procedure that may be employed in the overall analysis (e.g., Methods 3500, 3600 and 8000) for additional information on QC procedures, development of QC acceptance criteria, calculations, and general guidance. Analysts also should consult the disclaimer statement at the front of the manual and the information in SW-846 Chapter Two for guidance on the intended flexibility in the choice of methods, apparatus, materials, reagents, and supplies; and (ii) the responsibilities of the analyst for demonstrating that the techniques employed are appropriate for the analytes of interest, in the matrix of interest, and at the levels of concern.

In addition, analysts and data users are advised that, except where explicitly specified in a regulation, the use of SW-846 methods is not mandatory in response to Federal testing requirements. The information contained in this method is provided by the U.S. Environmental Protection Agency (EPA) as guidance to be used by the analyst and the regulated community in making judgments necessary to generate results that meet the data quality objectives (DQOs) for the intended application.

1.2 This method is restricted to use by, or under supervision of, appropriately experienced and trained personnel. Each analyst must demonstrate the ability to generate acceptable results with this method.

1.3 During method development the following compounds showed a potential for reduced solubility either during standard preparation (resulting in low bias to calibration and high recoveries for samples) or during sample preparation (resulting in low recoveries). Extra care should be taken to ensure that the composition of the stock and intermediate standards

maintain enough organic cosolvent, ≥ 95%, to keep longer chain PFAS in solution. Sub-sampling a container will also result in a loss of these compounds to the container walls, the extent of which will be container dependent:

N-ethylperfluoro-1-octanesulfonamidoacetic acid (N-EtFOSAA)
N-methylperfluoro-1-octanesulfonamidoacetic acid (N-MeFOSAA)
Perfluorotetradecanoic acid (PFTeDA)
Perfluorotridecanoic acid (PFTrDA)
Perfluorododecanoic acid (PFDoA)
Perfluoroundecanoic acid (PFUdA)

During the multi-laboratory validation study, the following compounds may be difficult at low concentrations due to due to lack of qualifier transitions and interferences:

Perfluorobutanoic acid (PFBA)
Perfluoropentanoic acid (PFPeA)
Perfluorohexanoic acid (PFHxA)

During the multi-laboratory validation study, the following compounds had quality control failures at concentrations of 40 ng/L and below due to low response and/or high background:

Perfluorotridecanoic acid (PFTrDA)
N-ethylperfluoro-1-octanesulfonamidoacetic acid (N-EtFOSAA)
N-methylperfluoro-1-octanesulfonamidoacetic acid (N-MeFOSAA)
1H, 1H, 2H, 2H-perfluorodecane sulfonic acid (8:2 FTS)
1H, 1H, 2H, 2H-perfluorooctcane sulfonic acid (6:2 FTS)

During the multi-laboratory validation study, the following had frequent QC failures for calibration, sample preparation QC, high background, and/or analytical enhancement:

1H, 1H, 2H, 2H-perfluorooctane sulfonic acid (6:2 FTS)
1H, 1H, 2H, 2H-perfluoro-1-[1,2-$^{13}C_2$] octanesulfonic acid (M2-6:2 FTS)

For all the # indicated compounds a higher concentration range than the 5 to 200 ng/L used in the multi-laboratory study may be required to achieve data quality objectives.

2.0 SUMMARY OF METHOD

2.1 Samples are prepared using an appropriate sample preparation method (e.g., dilution of water samples using Method 3512 in Appendix B, extraction of solid samples using Method TBD) and, if necessary, an appropriate cleanup procedure (TBD). For Method 3512 water samples are diluted 1:1 with methanol, filtered, and acetic acid (0.1% by volume) is added to adjust pH to ~3 – 4. Acetic acid is added primarily because it improved sensitivity for some target analytes. Solids – TBD. Samples are then analyzed by LC/MS/MS using external standard calibration.

2.2 Target compounds are identified by comparing multiple reaction monitoring (MRM) transitions in the sample to MRM transitions in the standards (Table 3). The retention time (RT) and qualifier ion ratio (if available) are compared to a mid-level standard to support qualitative identification. Target compounds are quantitated based on the response of their quantifier MRM transitions utilizing external standard calibration.

3.0 DEFINITIONS

Refer to SW-846 Chapter One and the manufacturer's instructions for definitions that may be relevant to this procedure. See Glossary (Appendix A) for relevant terms and acronyms.

4.0 INTERFERENCES

4.1 In order to avoid compromising data quality, contamination of the analytical system by PFAS from the laboratory must be reduced to the lowest practical level. Method blanks (MBs) and reagent blanks (RBs) are prepared and analyzed with all samples and are used to demonstrate that laboratory supplies and preparation and analysis steps do not introduce interferences or PFAS artifacts at levels that would bias quantitation, especially near the lower limit of quantitation (LLOQ), or prevent the proper identification and integration of target analytes. Careful selection of reagents and consumables is necessary because even low levels of PFAS contamination may alter the precision and bias of the method as background introduced by these materials (and variability thereof) is cumulative. See Sec. 9.5 for MB and RB criteria. Refer to each method to be used for specific guidance on QC procedures and to SW-846 Chapter Four for general guidance on glassware cleaning.

4.2 Refer to Methods 3500, 3600 and 8000 for discussions of interferences. Matrix interferences can be caused by contaminants from the sample, sampling devices, or storage containers. The extent of matrix interferences will vary considerably from sample source to sample source, depending upon variations of the sample matrix.

4.3 The following are procedures employed to prevent or minimize problems with measurement precision and bias.

4.3.1 All solvents should be of pesticide residue purity or higher (or preferably LC/MS grade) to minimize interference problems.

4.3.2 PFAS contamination has been found in reagents, glassware, tubing, polytetrafluoroethylene (PTFE) LC vial caps, aluminum foil, glass disposable pipettes, filters, degassers, and other apparatus that release fluorinated compounds. All

supplies and reagents should be verified prior to use. If found, measures should be taken to remove the contamination, if possible, or find other suppliers or materials to use that meet method or project criteria.

4.3.3 The LC system used should have components replaced, where possible, with materials known to not contain PFAS target analytes of interest.

4.3.4 During method development, loss of some PFAS target analytes was observed during storage of standard solutions in 50:50 methanol-water containing 0.1% acetic acid in glass containers. Plastic containers (polypropylene or high density polyethylene [HDPE]) are recommended to be used for preparation and storage of samples and standards. Glass autosampler vials have been successfully used for analysis of standards and samples in addition to plastic containers.

4.3.5 Polyethylene LC vial caps are recommended. Alternate materials may be used if the blank criteria in Sec. 9.5 are met. PTFE lined caps should not be used.

4.3.6 Polyethylene disposable pipettes are recommended. Alternate materials may be used if the blank criteria in Sec. 9.5 are met. When a new batch of disposable pipettes is received, at least one should be checked for release of target analytes or interferences.

4.3.7 Degassers are important to continuous LC operation and are most commonly made of fluorinated polymers. To enable use, an isolator column should be placed after the pump mixer and before the sample injection valve to prevent contamination. The isolator column delays the contaminants to the analytical column and must be located in the gradient flowpath.

4.3.8 If labware is re-used, the procedure described for labware cleaning (Sec. 6.2.4) should be followed to minimize risk of contamination. The blank criteria in Sec. 9.5 can be used as a guideline for evaluating cleanliness.

4.4 Where measured analyte concentrations are suspected of being high-bias and/or false positive results due to contamination, the laboratory should inform the data user of any suspected data quality issues.

5.0 SAFETY

5.1 This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of U.S. Occupational Safety and Health Administration (OSHA) regulations regarding the safe handling of the chemicals specified in this method. A reference file of safety data sheets (SDSs) must be available to all personnel involved in these analyses.

5.2 Users of this method should operate a formal safety program.

5.3 The toxicity and carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound is treated as a health hazard. Exposure to these chemicals should be reduced to the lowest possible level and the appropriate personal protective equipment (PPE) should be utilized. Review SDSs for specific physical and health hazards including appropriate PPE to be used. SDSs can be accessed at multiple

locations (e.g., www.sigmaaldrich.com, www.well-labs.com, and www.isotope.com).

6.0 EQUIPMENT AND SUPPLIES

The mention of trade names or commercial products in this method is for illustrative purposes only and does not constitute an EPA endorsement or exclusive recommendation for use. The products and instrument settings cited in SW-846 methods represent those used during method development or subsequently evaluated by the Agency. Glassware, reagents, supplies, equipment, and settings other than those listed in this method may be employed provided that method performance appropriate for the intended application has been demonstrated and documented. This section does not list all common laboratory glassware (e.g., beakers and flasks) that might be used.

6.1 Equipment

6.1.1 Liquid chromatograph (LC) system: An ultraperformance liquid chromatograph (UPLC) with stainless steel flow through needle design was used to generate data during method development (PEEK needles may not puncture polyethylene caps; pre-slitting of caps is not allowed).

6.1.2 Analytical columns: The following were used to generate data during method development; other columns may be used, provided that method performance is appropriate for the application:

6.1.2.1 Acquity UPLC® CSHTM Phenyl-Hexyl, 2.1 x 100 mm and 1.7 µm particle size (Waters part no. 186005407)

6.1.2.2 ZORBAX RRHD Stable Bond C18, 2.1 x 100 mm and 1.8 µm particle size (Agilent part no. 858700-902)

6.1.2.3 Accucore RP 2.1 x 100 mm and 2.6 µm particle size (Thermo part no. 17626-102130)

6.1.2.4 Shim-pack SP-C18, 2.1 x 150 mm and 2.7 µm particle size (Shimadzu part no. 227-32003-04)

6.1.3 Isolator column:

6.1.3.1 XBridge BEH C18, 2.1 x 50 mm and 3.5 µm particle size (Waters part no. 186003021)

6.1.3.2 ZORBAX RRHD Eclipse Plus C18, 50 × 3.0 mm, 1.8 µm (Agilent part no. 959757-302)

6.1.3.3 BDS Hypersil™C18, 2.1 x 50 mm and 5 µm particle size (Thermo part no. 28105-052130)

6.1.3.4 Shim-pack XR-ODS II, 2 x 75mm and 2.2 µm particle size (Shimadzu part no. 228-41605-93)

6.1.4 Mass Spectrometer (MS) System: An MS capable of multiple reaction monitoring (MRM) analysis with fast enough cycle time to obtain at least ten scans over a peak is needed with adequate sensitivity.

6.2 Support Equipment and Supplies

6.2.1 Adjustable volume pipettes, 10-µL, 20-µL, 100-µL, 200-µL, and 1000-µL, 5-mL, and 10-mL.

6.2.2 Analytical balance, capable of weighing to 0.01g

6.2.3 Sample containers and miscellaneous supplies; all supplies should meet blank criteria in Sec. 9.5 where practical.

6.2.3.1 Vials: 2-mL autosampler vials, HDPE, polypropylene or silanized glass

6.2.3.2 Polyethylene autosampler vial caps (Waters Catalog # 186004169)

6.2.3.3 10- to 25-mL filter-adaptable HDPE, polypropylene or glass syringes with luer lock adapters (rubber surfaces should not be used).

6.2.3.4 50-mL polypropylene tubes (BD Falcon, Catalog # 352098)

6.2.3.5 15-mL polypropylene tubes (BD Falcon, Catalog # 352097); use pre-weighed tubes for collection of field samples and field QC

6.2.3.6 Polyethylene disposable pipettes (SEDI-PETTM PIPET, Source - Samco Scientific, part no. 252 or equivalent)

6.2.3.7 Pipette tips: polypropylene pipette of various sizes (Eppendorf, Catalog #s 022491997, 022492080, 022491954, 022491946, and 022491512)

6.2.3.8 Acrodisc GxF/0.2µm GHP or equivalent membrane syringe driven filter unit. Filters must be cleaned prior to use. A suggested protocol is to rinse each filter with 2 x10 mL acetonitrile and then 2 x10 mL methanol prior to use. Other protocols may be appropriate if PFAS contamination is removed or reduced to levels appropriate for the project.

6.2.4 Reusable labware cleaning instructions – If labware is re-used it should be washed in hot water with detergent such as powdered Alconox, Deto-Jet, Luminox, or Citrojet, rinsed in hot water and rinsed with distilled water. All glassware is subsequently rinsed with organic solvent(s) such as acetone, methanol, and acetonitrile.

7.0 REAGENTS AND STANDARDS

7.1 Reagent-grade or pesticide grade chemicals, at a minimum, should be used in all tests. Unless otherwise indicated, all reagents should conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where specifications are available. Other grades may be used, provided the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination. All reagents should be verified prior to use to ensure the MB criteria in Sec. 9.5 can be met.

7.2 Reagent water: All references to water in this method refer to reagent water unless otherwise specified. Reagent water from in-house DI systems will likely require additional polishing with a point-of-use water purification system to meet blank requirements (Sec. 9.5). The user is cautioned to check for PFAS addition from these systems (free from fluoropolymers). Some bottled HPLC-grade water has been shown to contain PFAS.

7.3 Reagents and Gases: Items shown are for informational purpose only; equivalent reagents and standards may be used. All reagents and solvents should be of pesticide residue purity or higher to minimize interference problems, preferably LC/MS grade or equivalent.

7.3.1 Acetonitrile, C_2H_3N (CAS RN 75-05-8)

7.3.2 Ultrapure argon and nitrogen

7.3.3 Methanol, CH_3OH (CAS RN 67-56-1)

7.3.4 Isopropyl alcohol, C_3H_8O (CAS RN 67-63-0)

7.3.5 Ammonium hydroxide, NH_4OH (CAS RN 1336-21-6), 28-30%

7.3.6 Ammonium acetate, $C_2H_7NO_2$ (CAS RN 631-61-8), neat

7.3.7 Glacial acetic acid, CH_3COOH (CAS RN 64-19-7)

7.3.8 PFAS target compounds stock standards:

Solutions may be purchased as certified solutions or prepared from pure standard materials. Commercially prepared stock standards may be used at any concentration if they are certified by an accredited supplier or third party. For standards prepared from neat materials, the weight may be used without correction to calculate the concentration of the stock standard when standard compound purity is assayed to be 98% or greater. Use manufacturer's expiration date for purchased prepared standards.

For the multi-laboratory validation study, a mixture of all 24 target analytes listed in Table 1 was obtained from Wellington Laboratories (Catalog # PFAC-24PAR, containing each target analyte at a nominal concentration of 2000 ng/mL in MeOH). Sulfonic acids in this mixture were prepared from salts, and some had certified concentrations of both straight-chain and branched isomers.

7.3.9 PFAS Surrogates: An isotopically-labeled surrogate is recommended to be included for each target analyte. If an isotopically labeled surrogate of sufficient purity cannot be obtained, target analytes should be associated with surrogates that are as chemically similar as possible. For the multi-laboratory validation study, a mixture of 19 isotopically labeled surrogates was obtained from Wellington Laboratories (Catalog # MPFAC-24ES), containing each surrogate at a nominal concentration of 1000 ng/mL in methanol. See Table 6 for the surrogate list and the suggested target associations.

7.4 Standard solutions - All standards must be kept away from PFAS-containing packaging and materials used in preparation and storage. To prevent standard solutions from degrading, all standard solutions should be stored at ≤6°C in the refrigerator. Standards must be brought to room temperature and vortexed prior to use. Expiration date for standards prepared from neat materials is one year from the time prepared or manufacturer's expiration date,

whichever is shorter. The spiking standards and surrogates can be used for more than one year if they fall within ±20% of the expected concentration compared to a freshly opened stock.

The suggested instructions for the preparation of calibration standard solutions and surrogates and target compounds spiking solutions are listed below. Different concentrations may be used depending on the sensitivity of the instrument, response of quantifier/qualifier, calibration range used, or needs of the project. All intermediate stocks and spiking solutions should be prepared in 95:5 acetonitrile-water using calibrated automatic pipettes with polypropylene tips. Standard solutions should be prepared and stored in HDPE or polypropylene containers. Alternate solvents (e.g. 96:4 MeOH-water) may be used provided that method performance is not adversely affected (esterification in MeOH solutions is known to occur; binding of longer chain PFAS to surfaces can occur at lower proportions of organic co-solvent).

The following sections have suggested spiking concentrations for 5 mL water samples from Method 3512 (Appendix B). Spiking amounts and concentrations should be adjusted as needed if other volumes/weights or other preparatory methods are used.

7.4.1 PFAS surrogates spiking solution - Isotopically-labeled PFAS surrogates (Sec. 7.3.9) are added to samples prior to preparation. Addition of 40 µL of a 20 ng/mL surrogates spiking solution to a 5 mL water sample would result in surrogate concentrations of 160 ng/L.

Example preparation of surrogates spiking solution: A 200 µL aliquot of a stock PFAS surrogate mix at 1000 ng/mL concentration brought to 10 mL with 95:5 acetonitrile- water produces a solution at 20 ng/mL concentration.

7.4.2 Matrix spike/matrix spike duplicate (MS/MSD) and laboratory control sample (LCS) spiking solution – PFAS target analytes (Sec. 7.3.8) are added to MS/MSD and LCS samples prior to preparation, at a concentration near the mid-point calibration standard after all preparation steps are completed. Addition of 40 µL of a 20 ng/mL (nom.) MS/MSD and LCS target compounds spiking solution to a 5 mL water sample would result in target analyte concentrations of 160 ng/L.

Example preparation of a MS/MSD and LCS target compounds spiking solution: A 100 µL aliquot of a stock PFAS target mix at 2000 ng/mL concentration brought to 10 mL with 95:5 acetonitrile-water produces a solution at 20 ng/mL concentration (nom.).

7.4.3 LLOQ verification spiking solution – PFAS target analytes (Sec. 7.3.8) are added to LLOQ verification QC samples prior to preparation, at a concentration near the LLOQ (i.e., ½ to 2X the laboratory's established LLOQ; See Sec. 9.9). For example, an LLOQ verification QC sample can be prepared by spiking 25 µL of target compounds spiking solution at 2 ng/mL (nom.) into a 5 mL reagent water sample, resulting in target analyte concentrations of 10 ng/L.

Example preparation of an LLOQ verification spiking solution: A 10 µL aliquot of a stock PFAS target mix at 2000 ng/mL concentration diluted to a final volume of 10 mL with 95:5 acetonitrile-water produces a solution at 2 ng/mL concentration (nom.).

7.4.4 Calibration standards - Two types of calibration standards are used for this method: standards made from the primary source for ICAL and continuing calibration verification (CCV), and standards made from a second source for initial calibration verification (ICV). When using premixed certified solutions, store according to the manufacturer's documented holding time and storage temperature recommendations.

NOTE: Some PFAS analytes have both linear and branched isomers present in commercially available standards (e.g., PFHxS, PFOS, N-MeFOSAA and N-EtFOSAA).

7.4.4.1 ICAL: ICAL standards must be prepared at a minimum of five different concentrations and are recommended to be prepared using the target and surrogates spiking solutions in Secs. 7.4.1 and 7.4.2, or from dilutions of the calibration standards stock described below. Include a minimum of five different concentrations in the calibration for a linear (first-order) calibration model and six different concentrations for a quadratic (second-order) model with the low standard at or below the LLOQ (see Sec. 9.9 and Method 8000). At least one of the calibration standards should correspond to a sample concentration at or below that necessary to meet the DQOs of the project. The remaining standards should correspond to the range of concentrations found in actual samples but should not exceed the working range of the LC/MS/MS system. Each standard and/or series of calibration standards prepared at a given concentration should contain all the desired project-specific target analytes for which qualitative and quantitative results are to be reported by this method.

Table 4 contains suggested calibration levels that were used during validation of this method. Other concentrations or fewer standards may be used depending on the needs of the project or sensitivity of the LC/MS/MS system. A primary dilution standard (PDS) at 200 ng/L can be prepared by adding 100 µL of the surrogate spike at 20 ng/mL (Sec. 7.4.1) and 100 µL of the PFAS target compounds spike at 20 ng/mL (Sec. 7.4.2) and bringing to 10 mL with a 50:50 methanol-water solution containing 0.1% acetic acid (*e.g.*, 10 uL glacial acetic acid into 10 mL). The PDS can be used to prepare lower concentration ICAL standards, e.g., 5 ng/L – 150 ng/L (Table 4) by diluting aliquots of the PDS with appropriate volumes of 50:50 methanol-water containing 0.1% acetic acid.

NOTE: Calibration standards should not be reused once the cap is pierced unless the vial is immediately recapped. Volatile losses can occur rapidly because punctures of polyethylene caps leave large holes, and there is no septum to mitigate losses.

7.4.4.2 ICV: Second source standards for ICV must be prepared using source materials from a second manufacturer or from a manufacturer's batch prepared independently from the batch used for calibration. A second lot number from the same manufacturer may be adequate to meet this requirement. Target analytes in the ICV are recommended to be prepared at concentrations near the mid-point of the calibration range. The standard must contain all calibrated target analytes that will be reported for the project, if readily available.

7.4.4.3 Continuing calibration verification (CCV): CCV standards should be prepared in the same manner as ICAL standards at concentrations near the middle of the calibration range.

NOTE: It may be useful to prepare CCV standards at larger volumes and aliquot into multiple autosampler vials so individual autosampler vials do not have to be reused.

8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

Sample collection, preservation, and storage requirements may vary by EPA program and may be specified in a regulation or project planning document that requires compliance monitoring for a given contaminant. Where such requirements are specified in the regulation, follow those requirements. In the absence of specific regulatory requirements, use the following information as guidance in determining the sample collection, preservation, and storage requirements.

8.1 Sample collection criteria - Grab samples are collected in high density polyethylene (HDPE) or polypropylene containers. PTFE containers and contact surfaces with PTFE must be avoided. Depending on the needs of the project, field blanks may be required and must follow recommended PFAS sampling practices, where available. The samplers should acquire pre-verified reagent water and bottles from the analytical laboratory for preparing field blanks, where practical.

Surface binding of target compounds in water to collection containers is known to occur. If possible, volumes collected for water samples should match volumes consumed in the laboratory's preparation procedure (e.g., for Method 3512, collect 5 mL of sample in 15 mL container to allow volume for the 1:1 dilution in the original container). The laboratory must prepare the entire sample. Each field sample and QC sample must be collected in its own container, including field blanks, MS/MSDs, and duplicates.

NOTE: REMOVING AN ALIQUOT OF WATER FROM A CONTAINER PRIOR TO ADDITION OF ORGANIC SOLVENT IS NOT RECOMMENDED AND CAN RESULT IN SIGNIFICANT LOSS OF LONGER-CHAIN PFAS TARGETS (e.g., carboxylic acids $\geq C_9$, sulfonic acids $\geq C_7$). Water samples and sample extracts containing significant amounts of water may only be transferred to additional containers if 50% organic co-solvent content is achieved prior to transfer. Otherwise, quantitative transfer can be achieved

by solvent-rinsing the empty container with methanol. Refer to Sec. 11.1.1 for more details.

Conventional laboratory practices involving chain of custody, field sampling, lab custody beginning with receipt and transfer custody, and sampling protocols should be followed. Extra samples must be collected in order to analyze duplicate and matrix spike samples for quality assurance (QA) and QC purposes.

8.2 Sample preservation and storage - All samples are iced or refrigerated at ≤6 °C from the time of collection until sample analysis. In the laboratory, samples and sample extracts should be stored in the refrigerator at ≤6 °C while not being analyzed. Formal holding times have not yet been established for these analytes in various matrices. Based on an EPA preliminary holding time study, a 28-day limit from sample collection to preparation of solids or waters, and a 30-day limit from preparation to analysis of sample extracts may be used as a guide until a more formal study is completed.

NOTE: Freezing samples can prevent losses and degradation of some target and non-target PFAS into other PFAS target analytes. See Sec. 16.0, Reference 7.

9.0 QUALITY CONTROL

9.1 General guidance - Refer to SW-846 Chapter One for guidance on quality assurance (QA) and QC protocols. When inconsistencies exist between QC guidelines, method-specific QC criteria take precedence over both technique-specific criteria and Chapter One criteria; technique-specific QC criteria take precedence over Chapter One criteria. Any effort involving collection of analytical data should include development of a structured and systematic planning document, such as a quality assurance project plan (QAPP) or a sampling and analysis plan (SAP), which translates project objectives and specifications into directions for those implementing the project and assess the results.

Each laboratory should maintain a formal QA program. The laboratory should also maintain records to document the quality of the data generated. Development of in-house QC limits for each method is encouraged. All data sheets and QC data should be maintained for reference or inspection.

9.2 Refer to Method 8000 for specific determinative method QC procedures. Refer to Method 3500 and 3600 for QC procedures to ensure the proper operation of the various sample preparation and cleanup techniques. Any more specific QC procedures provided in this method will supersede those noted in Methods 3500, 3600 or 8000.

9.3 QC procedures necessary to evaluate the LC system operation are found in Method 8000 and include evaluation of RT windows, calibration verification, and chromatographic analysis of samples.

9.4 Initial demonstration of proficiency (IDP) – An IDP must be performed by the laboratory prior to independently running an analytical method and should be repeated if other changes occur (e.g., significant change in procedure, change in personnel). Refer to Method 8000 Sec. 9.0 for additional information regarding instrument, procedure, and analyst IDPs. An IDP must consist of replicate reference samples from each sample preparation and determinative method combination utilized by generating data of acceptable precision and bias for target analytes in a clean reference matrix taken through the entire preparation and analysis. If an autosampler is used to perform sample dilutions, prior to use, the laboratory should demonstrate that those dilutions are equivalent to that achieved by an experienced analyst performing manual dilutions.

For an IDP study, at least 4 samples containing all the PFAS and surrogates at or near the midpoint of the calibration range must be analyzed as replicates. These samples are then analyzed according to the method described in Method 8000 Sec. 9.0. Preliminary precision and bias (P&B) acceptance criteria are 30% (RSD) and 70-130% (recovery).

9.5 Blanks

9.5.1 Before processing any samples, the analyst must demonstrate through the analysis of a MB or RB that equipment and reagents are free from contaminants and interferences. If a peak is found in the blank that would prevent the identification or bias the measurement of an analyte, the analyst should determine the source of the contaminant peak and eliminate it, if possible. As a continuing check, each time a batch of samples is prepared and analyzed, and when there is a change in reagents, an additional MB must be prepared and analyzed for the compounds of interest as a safeguard against chronic laboratory contamination. MBs and field blanks must be carried through all stages of sample preparation and analysis. At least one MB or RB must be analyzed on every instrument after calibration standard(s) and prior to the analysis of any samples.

9.5.2 Blanks are generally considered to be acceptable if target analyte concentrations are less than one half the LLOQ or are less than project-specific requirements. Blanks may contain analyte concentrations greater than acceptance limits if the associated samples in the batch are unaffected (i.e., targets are not present in samples or sample concentrations/responses are ≥10X the blank). Other criteria may be used depending on the needs of the project.

9.5.3 If an analyte of interest is found in a sample in the batch near a concentration confirmed in the blank (refer to Sec. 9.5.2), the presence and/or concentration of that analyte should be considered suspect and may require qualification. Samples may require re-extraction and/or re-analysis if the blanks do not meet laboratory-established or project-specific criteria. Re-extraction and/or re-analysis is *not* necessary if the analyte concentration falls well below the action or regulatory limit or if the analyte is deemed not important for the project.

9.5.4 When new reagents or chemicals are received, the laboratory should monitor the blanks associated with samples for any signs of contamination. It may be necessary to test every new batch of reagents or chemicals prior to sample preparation as PFAS contamination is common. If reagents are changed during a preparation batch, separate blanks should be prepared for each set of reagents.

9.5.5 The laboratory should not subtract the results of the MB from those of any associated samples. Such "blank subtraction" may lead to negative sample results. If the MB results do not meet the project-specific acceptance criteria and reanalysis is not practical, then the data user should be provided with the sample results, the MB results, and a discussion of the corrective actions undertaken by the laboratory.

9.5.6 At least one MB for every 20 field samples must be prepared in reagent water to investigate for PFAS contamination throughout sample preparation, extraction, and analysis.

Note: More than one MB or RB may be needed to evaluate for commonly observed laboratory contaminants (e.g., 6:2 FTS) or for applications in which very low levels (i.e., at or near the LLOQ) are of interest.

9.5.7 One RB is prepared for each day of analysis with a 50:50 methanol-water solution containing 0.1% acetic to investigate for system/laboratory contamination. PFAS contamination at low levels is common in laboratory supplies and equipment. The 50:50 methanol-water solution containing 0.1% acetic acid is analyzed to help determine the source of contamination, if present.

9.6 Sample QC for preparation and analysis - The laboratory must also have procedures for documenting the effect of the matrix on method performance (precision, bias, sensitivity). At a minimum, this must include the analysis of a MB and LCS, and where practical, an MS/MSD or MS/duplicate in each preparation batch, as well as monitoring the recovery of surrogates. Any MBs, LCSs, MS/MSDs, and duplicate samples should be subjected to the same analytical procedures (Sec. 11.0) as those used on actual samples.

9.6.1 Matrix Spikes/Duplicates - Documenting the effect of the matrix should include the analysis of at least one MS and one duplicate unspiked sample or one MS/MSD pair. The decision on whether to prepare and analyze duplicate samples or a MS/MSD must be based on knowledge of the samples in the sample batch. If samples are expected to contain target analytes, laboratories may use an MS and a duplicate analysis of an unspiked field sample. If samples are not expected to contain target analytes, then laboratories should use a MS/MSD pair. The preliminary acceptance criteria are 70-130%. Statistically-derived acceptance limits or project defined acceptance limits may be necessary for targets as 70-130% default limits may be too narrow in some matrices. Consult Method 8000 for information on developing acceptance criteria for the MS/MSD.

9.6.1.1 When required and sufficient sample is available, an MS/MSD (Sec. 11.1.5) are prepared for each matrix at a frequency of at least one MS/MSD pair for every 20 field samples to investigate for matrix interferences.

9.6.1.2 As part of a QC program, spike accuracy for each matrix is monitored. Bias is estimated from the recovery of spiked analytes from the matrix of interest. Laboratory performance in a clean matrix is estimated from the recovery of analytes in the LCS. Calculate the recovery of each spiked analyte in the MS, MSD (if performed), and LCS according to the following formula.

$$Recovery = \%R = \frac{(C_s - C_u)}{C_n} \quad x \quad 100$$

where:

C_s = Measured concentration of spiked sample aliquot
C_u = Measured concentration of unspiked sample aliquot (use 0 for LCS)
C_n = Nominal (theoretical) concentration increase that results from spiking the sample, or the nominal concentration of the spiked aliquot (for LCS).

NOTE: MS/MSD recoveries may not be meaningful if the amount of analyte in the sample is large relative to the amount spiked.

9.6.2 LCS - At least one LCS must be prepared with each batch of 20 or fewer field samples. The LCS consists of an aliquot of a clean (control) matrix similar to the sample matrix and of the same weight or volume, like the MB. The LCS is spiked with the same analytes and at the same concentrations as the MS/MSD, when appropriate, and is taken through all sample preparation steps. When the results of the MS/MSD analysis indicate a potential problem due to the sample matrix itself, the LCS results are used to verify that the laboratory can perform the analysis in a clean matrix. The preliminary acceptance criteria are 70-130%. Statistically-derived acceptance limits or project defined acceptance limits may be necessary for some targets, including PFTriA, PFBA, and 6:2 FTS, as 70-130% default limits may be too narrow. See Sec. 9.6.1.2 for recovery calculation. Consult Method 8000 for information on developing acceptance criteria for the LCS.

9.6.3 A duplicate sample or MSD is analyzed with every batch of 20 field samples, where available. The relative percent difference (RPD) between the sample and duplicate or MS and MSD should be less than 30%. A laboratory control sample duplicate (LCSD) may be used to demonstrate precision in the batch if extra field sample containers are not received for performing duplicates or MSD.

Calculate the relative percent difference (%RPD) between the duplicates using the following equation:

$$RPD = \frac{|C_1 - C_2|}{\left(\frac{C_1 + C_2}{2}\right)} \quad X \quad 100$$

where:

C1 =Measured concentration of first sample aliquot
C2 =Measured concentration of second sample aliquot.

9.6.4 Surrogate recoveries - Surrogates are added to all field samples and associated QC samples as described in Sec. 11. The isotopically-labeled surrogates should represent the unlabeled native analytes where available. See Method 8000 for information on evaluating surrogate data and developing and updating surrogate limits. Procedures for evaluating recovery of multiple surrogates and associated corrective actions should be defined in the laboratory's SOP or in project planning documents (e.g., QAPP, SAP). Preliminary acceptance criteria are 70-130% recovery. Statistically-derived acceptance limits or project defined acceptance limits may be necessary for some surrogates, including M2PFTeDA, MPFDoA, MPFBA, M2-8:2FTS, M2-6:2FTS, M2-4:2FTS, d5-N-EtFOSAA, and d3-N-MeFOSAA, as 70-130% default limits may be too narrow. Consult Method 8000 for information on developing acceptance criteria for surrogate recovery.

9.7 Initial Calibration Acceptance Criteria (ICAL) - There must be an ICAL of the LC/MS/MS system as described in Sec. 11. Prior to analyzing samples, verify the ICAL standards using a second source ICV standard, if readily available (See Sec. 7.4.4.2).

9.8 CCV - The LC/MS/MS system must be verified using the procedure in Sec. 11.

See Method 8000 for the details on carrying out sample QC procedures for preparation and analysis. In-house method performance criteria for evaluating method performance should be developed using the guidance found in Method 8000.

9.9 Lower limit of quantitation (LLOQ)

General guidance for LLOQ is provided in this section and in Method 8000. The LLOQ is the lowest concentration at which the laboratory has demonstrated target analytes can be reliably measured and reported with a certain degree of confidence. The LLOQ must be greater than or equal to the lowest point in the calibration curve. The laboratory shall establish the LLOQ at concentrations where both quantitative and qualitative requirements can consistently be met (see Sec. 11.6.4). The laboratory shall verify the LLOQ at least annually, and whenever significant changes are made to the preparation and/or analytical procedure, to demonstrate quantitation capability at lower analyte concentration levels. The verification is performed by the extraction and/or analysis of an LCS (or MS) at 0.5 - 2 times the established LLOQ. Additional LLOQ verifications may be useful on a project-specific basis if a matrix is expected to contain significant interferences at the LLOQ. The verification may be accomplished with either clean control material (e.g. reagent water) or a representative sample matrix, free of target compounds. Optimally, the LLOQ should be less than the desired decision level or regulatory action level based on the stated DQOs.

NOTE: LLOQs should be established at concentrations where both quantitative and qualitative requirements can be consistently and reliably met. Target analyte peaks in the calibration standard at the LLOQ should be visually inspected to ensure that peak signal is adequately distinguishable from background and where the signal/noise ratio for all quantitative peaks is ≥3. Additional LLOQ verification samples at higher concentrations may be useful for target analytes with low or no qualifier transitions (e.g., PFBA, PFPeA, PFHxA) in order to provide better signal to noise and confidence in confirmation. Additional LLOQ verification samples at higher concentrations may be needed for target analytes with higher variability, lower response and/or high background (e.g., PFTeDA, PFTriA, PFDoA, PFUnDA, PF8:2 FTS, 6:2 FTS, N-EtFOSAA, and N-MeFOSAA).

NOTE: If project required levels are sufficiently high, the LCS may be used to meet requirements of LLOQ verification.

9.9.1 LLOQ Verification

9.9.1.1 The verification of LLOQs using spiked clean control material represents a best-case scenario because it does not evaluate the potential matrix effects of real-world samples. For the application of LLOQs on a project-specific basis, with established DQOs, a representative matrix-specific LLOQ verification may provide a more reliable estimate of the lower quantitation limit capabilities.

9.9.1.2 The LLOQ verification is prepared by spiking a clean control material with the analyte(s) of interest at 0.5 - 2 times the LLOQ concentration level(s). Alternatively, a representative sample matrix free of targets may be spiked with the analytes of interest at 0.5 - 2 times the LLOQ concentration levels. The LLOQ check is carried through the same preparation and analytical procedures as environmental samples and other QC samples.

9.9.1.3 Recovery of target analytes in the LLOQ verification should be within established in-house limits or within other such project-specific acceptance limits to demonstrate acceptable method performance at the LLOQ. Preliminary acceptance criteria for the LLOQ verification are 50-150%. This practice acknowledges the potential for greater uncertainty at the low end of the calibration curve. Practical, historically based LLOQ acceptance criteria should be determined once sufficient data points have been acquired.

9.9.1.4 It is recommended to analyze an LLOQ verification with every batch of 20 or fewer field samples. In cases where compounds fail low, they may be reported as non-detects if it can be demonstrated that there was adequate sensitivity to detect the compound at the LLOQ or project specific level of interest (e.g., by calibrating below the established LLOQ to confirm the non-detect, or by analyzing a standard near that level to confirm the analyte could be qualitatively identified if it were present [See Sec. 11.7 of Method 8000]). Alternatively, the non-detect could be qualified or the LLOQ raised to a higher level. In cases where compounds fail high in the LLOQ and are not found in the associated field samples, they may be reported without qualification.

9.9.1.5 Reporting concentrations below LLOQ – Concentrations that are below the established LLOQ may still be reported; however, these analytes must be qualified as estimated. The procedure for reporting analytes below the LLOQ should be documented in the laboratory's SOP or in a project-specific plan. Analytes below the LLOQ that are reported should meet most or all of the qualitative identification criteria in Sec. 11.6.4.

9.10 It is recommended that the laboratory adopt additional QA practices for use with this method. Specific practices that are most productive depend upon the needs of the laboratory, the nature of the samples, and project-specific requirements. Field duplicates may be analyzed to assess precision of the environmental measurements. Whenever possible, the laboratory should analyze standard reference materials and participate in relevant performance evaluation studies.

10.0 CALIBRATION AND STANDARDIZATION

See Sec. 11.0 for information on calibration and standardization.

11.0 PROCEDURE

11.1 Samples are normally prepared by one of the following methods prior to LC/MS/MS analysis:

Matrix	Methods
Water	Method 3512 (Appendix B)
Soil/sediment	TBD
Biota (fish, plant)	TBD

11.2 Sample cleanup – Cleanup procedures should not be necessary for relatively clean sample matrices. Extracts from highly contaminated environmental, waste or biota samples may require additional cleanup steps prior to analysis. The specific cleanup procedure used will depend upon the analytes of interest, the nature of the interferences, and the DQOs for the project.

11.3 ICAL

11.3.1 Calibrate the mass spectrometer according to manufacturer’s specifications after any changes to the system and when mass shifts of more than 0.2 Dalton are noticed by the analyst. Acceptable system performance may be demonstrated by meeting manufacturer specifications for mass resolution, mass accuracy, and sensitivity using an internal calibrant. Tuning the instrument should only be performed prior to initial calibration. System calibration must not begin until manufacturer’s performance criteria are met, and calibration standards and samples must be analyzed under the same conditions.

NOTE: Prior to running this method and performing the IDP in Sec. 9.4, the laboratory must optimize instrument settings to obtain acceptable responses for each parent to product ion transition for every target analyte and surrogate (e.g., cone voltage, collision energy).

11.3.2 Analyze a consistent volume of each calibration standard (i.e., containing the compounds for quantitation and the appropriate surrogates). LC conditions and MS/MS conditions used in method development are listed in Table 5. A set of at least five calibration standards must be analyzed with the low standard at or below the LLOQ (see Sec. 9.9 and Method 8000). Quantitation is based on external standard calibration models, and a minimum of five standards must be used for average calibration factor or linear (first-order) calibration models. Six or more standards must be used for a quadratic (second-order) model. See Sec. 11.4 in Method 8000 for additional information. The injection volume must be the same for the analysis of all standards and samples.

NOTE: Concentrations for salt forms of sulfonates are typically corrected to anion concentration for reporting purposes. For example, in the Wellington standard (PFAC-24PAR), PFBS salt has a concentration of 2 ug/mL and the anion concentration is 1.77 ug/mL.

NOTE: LLOQs should be established at concentrations where both quantitative and qualitative requirements can be consistently and reliably met (see Secs. 9.9 and 11.6). Target analyte peaks in the calibration standard at the LLOQ should be visually inspected to ensure that peak signal is adequately distinguishable from background and meets the qualitative requirements outlined in 11.6.

11.3.3 Identify the target compounds using optimized multiple reaction monitoring (MRM) transitions from Sec. 11.3.1. A qualifier transition is available for most of the analytes (Table 3).

NOTE: PFAS targets can be calibrated using a summation of the responses for all of the branched and linear peaks if present in quantitative standards (for example, sum or integrate all of the C6 sulfonic acid linear and branched isomers as one calibration point) or by calibrating with only the linear isomer. If a quantitative standard containing both linear and branched isomers is not available, a separate technical grade standard may be used to identify retention times of isomer peaks. Regardless of which calibration procedure is used, quantitation of targets in samples must include both branched and linear isomers either summed or integrated together. The data should be reported such that the calibration choice is clear to the data user. See Figures 1-4, Sec. 17.

11.3.4 Initial calibration calculations

Average calibration factor, linear or quadratic regression models may be used with this analysis. Calculation of average calibration factors is given below. For linear and quadratic calculations see Sec. 11.5 in Method 8000.

11.3.5 Tabulate the response of the quantifier transition (see Table 3 for suggested ions) against the concentration for each target analyte. Calculate Calibration factors (CFs) for each target analyte as follows:

$$\mathrm{CF} = \frac{peak\ response\ of\ the\ standard\ compound}{concentration\ of\ the\ compound\ (ng/L)}$$

11.3.5.1 Calculate the mean CF and the relative standard deviation (RSD) of the CFs for each target analyte using the following equations:

$$SD = \sqrt{\frac{\sum_{i=1}^{n}\left(CF_i - \overline{CF}\right)^2}{n-1}} \quad mean\ CF = \overline{CF} = \frac{\sum_{i=1}^{n} CF_i}{n} \quad RSD = \frac{SD}{\overline{CF}}\ x\ 100$$

where:

CF_i = CF for each of the calibration standards
$\overline{CF}$ = Mean CF for each compound from the ICAL
n = Number of calibration standards (e.g., 5)
SD = Standard deviation

Where *n* is the number of calibration standards and RSD is expressed as a percentage (%).

11.3.6 Linearity of target analytes – If the RSD of any target analyte is 20% or less, then the CF is assumed to be constant over the calibration range, and the average CF may be used for quantitation (Sec. 11.3.5). The average CF should not be used for compounds that have an RSD greater than 20%. If a regression model is used for quantitative purposes, *r* (correlation coefficient) or r^2 (coefficient of determination) should be ≥ 0.995 or 0.99, respectively. Relative standard error (%RSE) may also be used for acceptance of the calibration model. See Sec. 11.5.4 of Method 8000 for additional information. Example RSE calculations can be found in Reference 8. Forcing linear and quadratic models through the origin may be appropriate when background PFAS are present to better estimate background concentrations.

11.3.7 When the calibration does not meet the acceptance criteria, the plotting and visual inspection of a calibration curve can be a useful diagnostic tool. The inspection may indicate analytical problems, including errors in standard preparation, the presence of active sites in the chromatographic system, analytes that exhibit poor chromatographic behavior, etc.

NOTE: It is considered inappropriate once the calibration models have been finalized to select an alternate fit solely to pass the recommended QC criteria for samples and associated QC on a case-by-case basis.

11.3.8 If more than 10% of the compounds included with the ICAL (or more than 10% of those that will be reported) exceed the 20% RSD limit and do not meet the minimum criteria ($r^2 \geq 0.99$ or relative standard error (RSE) ≤20%) for alternate curve fits, then the system is considered unacceptable for analysis to begin. Correct the source of the problem; then repeat the calibration procedure beginning with Sec. 11.3. If compounds fail to meet these criteria, the associated concentrations may still be determined but they must be reported as estimated. To report non-detects, it must be demonstrated that there is sufficient accuracy to detect the failed compounds at the applicable LLOQ (see Secs. 11.3.9 for refitting standards and 11.4 for CCV).

11.3.9 Calibration, especially when using regression models, has the potential for a significant bias at the lower portion of the calibration curve. All calibration points should be recalculated (not reanalyzed) using the final calibration curve in which this standard is used (i.e., re-fitting the response from the calibration standard back into the curve and determining % error). See Sec. 11.5.4 of Method 8000 for additional details. The recalculated concentration of the low calibration point should be within ±50% of the standard's true concentration, and the recalculated concentrations of any other calibration standards (above the LLOQ) should be within ±30%. Alternate criteria may be applied depending on the needs of the project; however, those criteria should be clearly defined in a laboratory SOP or a project-specific QAPP. Analytes which do not meet the re-fitting criteria should be evaluated for corrective action (choosing an alternative model or weighting). If a failure occurs in the low point and it is equivalent to the LLOQ, the analyte should be reported as estimated near that concentration, or the LLOQ should be reestablished at a higher concentration.

11.3.10 ICV - Prior to analyzing samples, verify the ICAL using a standard obtained from a second source to the calibration standard. Suggested acceptance criteria for the analyte concentrations in this standard are 70 - 130% of the expected analyte concentration(s). Alternative criteria may be appropriate based on project-specific DQOs. Quantitative sample analyses should not proceed for those analytes that do not meet the ICV criteria. However, analyses may continue for those analytes that do not meet the criteria with an understanding that these results could be used for screening purposes and would be considered estimated values.

11.4 Continuing Calibration Verification (CCV)

11.4.1 Verify the initial calibration by analyzing a mid-level CCV standard prior to any samples, after every 10 field samples (or every 12 hours, whichever is shorter), and at the end of the analytical sequence. The CCV is prepared from the same stock solutions or source materials used for the ICAL standards. The results must be compared against the most recent ICAL and should meet the acceptance criteria provided below.

11.4.2 The calculated concentration or amount of each analyte of interest in the CCV standard should fall within ±30% of the expected value. If not, a separately prepared CCV may be prepared and analyzed to meet acceptance criteria.

11.4.3 If the percent difference (%D) or percent drift for a compound is ≤30% in the CCV, then the ICAL for that compound is assumed to be valid. Due to the number of compounds that may be analyzed by this method, it is expected that some compounds may fail to meet the criterion. The analyst should strive to place more emphasis on meeting the CCV criteria for those compounds that are critical to the project. If the criterion is not met (i.e., greater than ±30%D or drift) for more than 10% of the compounds included in the ICAL (or more than 10% of those that will be reported), then corrective action must be taken prior to the analysis of samples. Target analytes that do not meet the CCV criteria and are reported in the associated samples must be qualified to indicate the reported concentrations are potentially estimated or biased values. In cases where compounds fail low, they may be reported as non-detects if it can be demonstrated that there was adequate sensitivity to detect the compound at the LLOQ or project specific level of interest (e.g., analysis of an LLOQ verification in every batch, or by analyzing a standard near that level to confirm the analyte could be qualitatively identified if it were present [See Sec. 11.7 of Method 8000]). Alternatively, the non-detect could be qualified or the LLOQ raised to a higher level. In cases where compounds fail high in the CCV and are not found in the associated field samples, they may be reported without qualification.

NOTE: For the $\overline{CF}$ calibration model, %D between the calculated CF of an analyte in the CCV and the $\overline{CF}$ of that analyte from the ICAL is the same value as % drift for calculated versus expected concentration. Refer to Method 8000 for guidance on calculating %D and % drift.

NOTE: The analyst must closely monitor responses and chromatography in the CCV for signs that the system is unacceptable for analysis to continue (e.g., unusual tailing, loss of resolution). If significant losses of target analytes/surrogates occur (<50% recovery) or if significant degradation of the chromatography occurs, system maintenance must be performed or the analyst must demonstrate there is adequate sensitivity at the LLOQ.

11.4.4 A MB or RB must be analyzed after the CCV and prior to samples to ensure that the system (i.e., introduction device, transfer lines and LC/MS system) is free from levels of contaminants that would bias the results. If the blank indicates contamination, then it may be appropriate to analyze additional blanks to help determine the source of contamination (See Sec. 9.5). A MB or RB is not required after a CCV at the end of an analytical sequence. Refer to Sec. 9.5.2 regarding qualification of data and/or corrective actions related to MB or RB contamination.

NOTE: Background of PFAS target analytes may increase in some LC systems while they are held under initial conditions or while idle; re-started sequences should typically begin with at least one blank to bleed out any accumulated background and to provide information about the potential for any carryover in the system. Refer to Sec. 9.5 for associated acceptance criteria.

11.5 Sample analysis procedure

11.5.1 Inject samples using the same LC and MS/MS conditions as used to generate the ICAL.

A suggested sequence order is:
RB
ICAL standards and ICV or opening CCV
MB
LLOQ verification
LCS
Field samples (with a CCV every 10 field samples)
Duplicates
Matrix spike/matrix spike duplicate
Closing CCV

11.5.2 The laboratory should monitor recoveries of the isotopically-labeled surrogates (listed in Sec. 7.3.11). The percent recovery of each surrogate should fall within the acceptance criteria, especially for those QC samples prepared in clean matrices or reagent water (e.g., MB, LCS, LLOQ verification). If multiple surrogates fail to meet the acceptance criteria and/or the target analytes associated with the failing surrogate(s) are important to the project, reanalysis and/or repreparation of samples may be warranted. Otherwise, the associated target analytes may be reported with appropriate data qualifiers. See additional guidance in Sec. 9.6 of Method 8000.

11.5.3 If the concentration of any analyte exceeds the ICAL range of the system, the sample extract should be diluted with 50:50 methanol-water with 0.1% acetic acid and reanalyzed. If dilutions cannot be performed, concentrations that exceed the calibration range and are reported must be qualified as estimated. When the response of a compound in the sample exceeds the calibration range, analysis of a RB can help determine the extent of any carryover that may occur under the conditions used at the laboratory.

NOTE: The laboratory is cautioned against subsampling of aqueous samples prior to adding sufficient MeOH, as larger chain PFAS are known to adhere to surfaces unless the sample contains at least 50% organic cosolvent by volume. See Sec. 8.1.

11.6 Target Identification - MRM analysis provides qualitative identification by isolating the precursor ion and fragmenting it into the product ions, which are then used to calculate ion ratios that can be compared between samples and standards to confirm the identification of the analyte. RTs of target analytes in samples are also compared to those in standards, and RT shifts of target analytes are compared to associated surrogates in the same samples to further confirm the identification.

11.6.1 Identify the target compounds by comparing the quantifier MRM transitions and applicable qualifier MRM transitions in the sample to the MRM transitions in the standard. Qualifier transitions are available for most of the analytes (Table 3). The quantifier/qualifier MRM ion ratio should be within ±30% of the average of the quantifier/qualifier MRM ion ratios calculated from the calibration levels on the day of analysis or ±30% of the ion ratios calculated from a mid-level ICAL point or from the CCV. Some ion ratios may not meet the ±30% criterion at the lower concentrations. The analyst should use professional judgment when interferences are observed or ion ratios are not met to prevent reporting false positive or false negative results.

NOTE: Depending on sensitivity and matrix interference issues, a qualifier MRM transition might be used as a quantifier MRM transition for quantitation during the analysis. This must be clearly documented if these changes are made.

NOTE: The qualifier ion ratios in samples may not match the ion ratios in the calibration standards for the target analytes that contain branched and linear isomers. Figures 1 - 4 (Sec. 17.0) show how branched isomers in samples can be significantly larger compared to calibration standards for PFHxS and PFOS, which may cause the ion ratio difference. The complete isomer grouping must be integrated consistently for all standards and samples.

11.6.2 The RT of the MRM transitions should be within ±10 seconds of the RT for this analyte in a mid-level ICAL standard, the CCV run at the beginning of the analytical sequence or the CCV analyzed just prior to the sample (delta RT 0.17 minute). Alternatively, a relative deviation (in %) may be used for confirmation of target compounds. The delta RT of the mass labelled analog (surrogate) should also be considered to confirm target analytes. RT shifts may result in the compound eluting outside the analytical time segment, which could produce false negative results. Time segments and RT windows for analytes must include branched chain isomers.

11.7 Analyte quantitation - Once a target compound has been identified, the quantitation of that compound will be based on the integrated abundance of the quantifier transition. It is highly recommended to use the integration produced by the software if the integration is correct because the software should produce more consistent integrations than an analyst will manually. However, manual integrations may be necessary when the software does not produce proper integrations because baseline selection is improper; the correct peak is missed; a co-elution is integrated; the peak is partially integrated; etc. Manual integrations will be required on most chromatography data systems to include branched and straight chain isomers where certified individual standards are not available. The analyst is responsible for ensuring that the integration is correct whether performed by the software or done manually. Manual integrations should not be substituted for proper maintenance of the instrument or setup of the method (e.g., RT updates, integration parameter files, etc.). The analyst should seek to minimize manual integration where practical by properly maintaining the instrument, updating RTs, and configuring peak integration parameters.

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 Calculations and documentation – Sample concentrations are quantitated using the following equations:

$$Concentration\ in\ \frac{ng}{L} = \frac{(X_s)(V_t)(D)}{(V_s)}$$

$$Concentration\ in\ \frac{ng}{g} = \frac{(X_s)(V_t)(D)}{(W_s)}$$

where:

V_t = Total volume of extract or diluted sample (in L).
V_s = Volume of aqueous sample prior to preparation (in L).
D = Dilution factor, if sample or extract was diluted prior to analysis. If no dilution, D=1. This value is always dimensionless.
W_s = Weight of sample extracted (in grams). If kg units are used for this term, multiply results by 1000 g/kg.
X_s = Calculated concentration of analyte (ng/L) from the analysis. Type of calibration model used determines derivation of X_s. See Secs. 11.5.1.3, 11.5.2.3, and 11.5.3 of Method 8000.

12.2 See Secs. 11.5 and 11.10 of Method 8000 for additional information and formulas for quantitating results.

13.0 METHOD PERFORMANCE

Please refer to Tables 2A-2C for a summary of method performance from a multi-laboratory validation study of aqueous samples prepared by the method in Appendix B (draft Method 3512).

14.0 POLLUTION PREVENTION

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

14.2 For information about pollution prevention that may be applicable to laboratories and research institutions consult Less is Better: Laboratory Chemical Management for Waste Reduction, a free publication available from the American Chemical Society (ACS), Committee on Chemical Safety: https://www.acs.org/content/dam/acsorg/about/governance/committees/chemicalsafety/publications/less-is-better.pdf.

15.0 WASTE MANAGEMENT

The EPA requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management, consult *The Waste Management Manual for Laboratory Personnel* available at: http://www.labsafetyinstitute.org/FreeDocs/WasteMgmt.pdf.

16.0 REFERENCES

1. ASTM Standard D7979-15, “Standard Test Method for Determination of Perfluorinated Compounds in Water, Sludge, Influent, Effluent and Wastewater by Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS)”, 18pp., 2015.
2. J.A. Shoemaker, P.E. Grimmett, B.K. Boutin, “EPA Method 537- Determination Of Selected Perfluorinated Alkyl Acids In Drinking Water By Solid Phase Extraction And Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) - Research Summary “, U.S. EPA, National Exposure Research Laboratory, Office of Research and Development, US EPA, Cincinnati, OH, 22pp.
3. U.S. EPA 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)”, National Exposure Research Laboratory, Office of Research and Development, US EPA, Cincinnati, OH, Version 1.0, 2018.
4. Occupational Safety and Health Administration, OSHA Safety and Health Standards, 29 CFR 1910.120, “Hazardous Waste Operations and Emergency Response” and 29 CFR 1910.1200, “Hazard Communication”.
5. Standard Practices for Sampling Water, American Society for Testing and Materials, Philadelphia. ASTM Annual Book Standards, Part 31, D3370-76.
6. R. Burrows, Basic RSE calculator v2 and instructions, December 2016. Available at: http://nelac-institute.org/docs/comm/emmec/Calculating%20RSE.pdf.
7. Million B. Woudneh, Bharat Chandramouli, M.C. Hamilton, Richard Grace, John R. Cosgrove, “EFFECT OF SAMPLE STORAGE ON THE QUANTITATIVE

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17.0 TABLES, DIAGRAMS, FLOW CHARTS, AND VALIDATION DATA

The following pages contain the tables, figures, and appendices referenced by this method.

TABLE 1

SUGGESTED LLOQ AND CALIBRATION RANGE

Analyte	Suggested LLOQ	Calibration Ranges (ng/L)
PFTeDA	40	10 - 400
PFTrDA	40	10 - 400
PFDoA	40	10 - 400
PFUdA	40	10 - 400
PFDA	10	10 - 400
PFDS	10	10 - 400
PFOS	10	10 - 400
PFNA	10	10 - 400
PFNS	10	10 - 400
PFOA	10	10 - 400
PFHpS	40	10 - 400
PFHxS	40	10 - 400
PFHpA	40	10 - 400
PFHxA	40	10 - 400
PFBS	10	10 - 400
PFPeS	10	10 - 400
PFPeA	50	10 - 400
PFBA	50	10 - 400
FOSA	10	10 - 400
4:2 FTS	10	10 - 400
6:2 FTS	40	10 - 400
8:2 FTS	40	10 - 400
N-EtFOSAA	40	10 - 400
N-MeFOSAA	40	10 – 400

NOTE: Calibration ranges listed in this table account for the 2-fold dilution factor of samples with methanol during preparation, so these LLOQs and the calibration ranges are a factor of two higher than the calibration standard concentrations provided in Table 4.

TABLE 2A

LCS PERFORMANCE SUMMARY FROM MULTI-LABORATORY VALIDATION STUDY

Analyte	LCS Recovery (n=48, 160 ng/L, nom.)			LCS/LCSD Relative % Difference (RPD, n=24 pairs)		
	Average recovery (%)	Standard Deviation of Recovery	% recovered within 70-130%	Average RPD (%)	Standard Deviation RPD (%)	# of LCS/LCSD pairs with RPD >30%
PFTreA	103	18.9	89.6	7.1	4.9	100
PFTriA	107	22.7	83.3	8.9	8.0	95.8
PFDoA	104	16.7	91.7	10.3	9.7	95.8
PFUnA	101	12.1	100	9.0	8.7	100
PFDA	102	11.5	97.9	8.9	7.4	95.8
PFNA	103	12.3	95.8	6.9	7.2	100
PFOA	101	12.1	97.9	6.8	6.6	95.8
PFHpA	96.4	8.7	100	5.4	5.3	100
PFHxA	95.8	10.5	100	8.1	7.0	100
PFPeA	94.1	10.1	100	4.1	3.3	100
PFBA	91.5	15.1	87.5	4.4	4.4	100
PFDS	100	10.2	100	5.5	4.8	100
PFNS	105	12.6	100	6.9	6.3	100
PFOS	99.9	8.9	100	5.1	4.9	100
PFHpS	101	9.1	100	5.2	5.2	100
PFHxS	97.9	8.1	100	4.5	4.7	100
PFPeS	98.0	7.2	100	5.5	5.2	100
PFBS	93.2	9.6	100	3.3	5.5	100
PFOSA	98.7	8.2	100	3.6	2.7	100
FtS 8:2	104	15.0	93.8	8.2	6.9	100
FtS 6:2	91.1	33.0	64.6	10.2	8.4	100
FtS 4:2	98.0	12.0	95.8	8.8	8.9	95.8
N-EtFOSAA	102	15.6	93.8	9.0	8.6	95.8
N-MeFOSAA	102	15.2	93.8	9.2	7.7	100
Surrogates:						
M2PFTeDA	106	20.4	85.4	9.6	11.8	95.8

Analyte	LCS Recovery (n=48, 160 ng/L, nom.)			LCS/LCSD Relative % Difference (RPD, n=24 pairs)		
	Average recovery (%)	Standard Deviation of Recovery	% recovered within 70-130%	Average RPD (%)	Standard Deviation RPD (%)	# of LCS/LCSD pairs with RPD >30%
MPFDoA	105	16.6	89.6	11.5	15.5	87.5
M7PFUdA	103	11.3	97.9	8.3	12.2	95.8
M6PFDA	103	13.1	95.8	9.6	14.1	95.8
M9PFNA	102	13.5	97.9	10.0	13.9	91.7
M8PFOA	103	11.9	97.9	9.1	13.1	91.7
M4PFHpA	98.9	12.7	97.9	8.6	13.5	91.7
M5PFHxA	98.7	13.4	97.9	7.3	11.8	95.8
M5PFPeA	97.3	11.6	97.9	5.3	10.4	95.8
MPFBA	95.2	14.7	87.5	5.4	11.8	95.8
M8PFOS	103	13.1	97.9	10.8	12.7	95.8
M3PFHxS	101	12.3	97.9	7.4	11.8	95.8
M3PFBS	95.6	13.5	97.9	7.2	12.2	95.8
M8FOSA-I	103	13.9	95.8	7.1	12.0	95.8
M2-8:2FTS	108	14.4	95.8	8.3	11.3	95.8
M2-6:2FTS	107	17.3	91.7	10.8	11.8	95.8
M2-4:2FTS	103	23.5	87.5	15.7	20.7	87.5
d5-N-EtFOSAA	106	15.3	91.7	11.2	12.9	91.7
d3-N-MeFOSAA	103	13.6	95.8	8.7	10.1	95.8

TABLE 2B

LLOQ VERIFICATION PERFORMANCE FROM MULTI-LABORATORY VALIDATION STUDY

Analyte:	10 ng/L (nom., in 5 mL water) LLOQ verification (n=21)			20 ng/L (nom., in 5 mL water) LLOQ verification (n=18)		
	Average Recovery (%, non-zero)	Standard Deviation of Recovery (%, non-zero)	% within 50-150% recovery	Average recovery (%, non-zero)	Standard Deviation of Recovery (%, non-zero)	% within 50-150% recovery
PFTreA	111.6	25.1	81.0	110.2	30.0	94.4
PFTriA	125.6	53.0	71.4	118.2	32.4	83.3
PFDoA	102.9	29.9	81.0	109.3	20.5	94.4
PFUnA	107.6	27.4	81.0	99.9	16.0	100
PFDA	96.8	26.3	85.7	103.1	19.5	94.4
PFNA	100.1	26.1	90.5	99.7	14.1	100
PFOA	100.7	26.8	85.7	99.5	16.3	100
PFHpA	93.2	15.7	100	99.5	13.8	100
PFHxA	99.1	41.6	85.7	94.9	17.6	100
PFPeA	102.7	36.2	85.7	99.1	13.2	100
PFBA	89.1	27.4	85.7	95.2	20.6	94.4
PFDS	104.9	24.6	81.0	100.4	24.2	100
PFNS	102.3	28.5	95.2	105.7	18.0	100
PFOS	111.8	23.0	85.7	106.3	16.8	100
PFHpS	89.1	41.8	90.5	105.1	14.5	100
PFHxS	99.0	17.7	100	99.3	12.7	100
PFPeS	95.8	12.4	100	99.7	12.3	100
PFBS	93.1	17.2	95.2	91.6	12.5	100
PFOSA	100.6	15.0	100	99.7	8.5	100
FtS 8:2	112.4	36.5	66.7	129.0	57.9	72.2
FtS 6:2	1471.4	5540.8	57.1	124.9	152.3	50.0
FtS 4:2	101.6	16.4	90.5	96.2	14.3	100
NEtFOSAA	121.8	33.6	71.4	111.0	18.9	77.8
NMeFOSAA	109.2	52.8	71.4	104.4	34.1	83.3

TABLE 2C. RECOVERY AND PRECISION OF TARGET ANALYTES AND SURROGATES IN MULTI-LABORATORY STUDY MATRICES PREPARED BY METHOD 3512[1]

Target Analyte or Surrogate name (abbrev)	All matrices (n=479)				Reagent water (n=120)				Groundwater (n=120)				Surface water (n=120)				Wastewater (n=119)			
	low spike (60 ng/L nom.)		high spike (200 ng/L, nom.)		low spike (60 ng/L nom.)		high spike (200 ng/L, nom.)		low spike (60 ng/L nom.)		high spike (200 ng/L, nom.)		low spike (60 ng/L nom.)		high spike (200 ng/L, nom.)		low spike (60 ng/L nom.)		high spike (200 ng/L, nom.)	
	Mean % Rec.	% RSD	Mean % Rec.	% RSD	Mean % Rec.	% RSD	Mean % Rec.	% RSD	Mean % Rec.	% RSD	Mean % Rec.	% RSD	Mean % Rec.	% RSD	Mean % Rec.	% RSD	Mean % Rec.	% RSD	Mean % Rec.	% RSD
PFTreA	89.5	27.3	95.0	18.3	95.5	26.6	101.2	18.5	88.2	24.6	92.9	17.4	83.8	23.4	94.1	16.8	90.7	32.4	91.8	19.3
PFTriA	95.7	25.2	99.8	18.2	97.7	22.8	102.5	17.6	95.8	25.4	99.3	17.6	92.3	24.7	99.4	17.8	97.2	28.2	98.2	20.4
PFDoA	95.8	25.2	102.1	18.0	95.8	24.3	103.0	14.5	98.0	26.2	102.8	19.5	93.9	20.9	101.2	19.0	95.6	29.3	101.2	19.2
PFUnA	97.3	18.9	104.2	16.1	98.9	18.6	104.5	15.2	96.0	17.4	103.3	16.8	96.3	20.1	103.0	13.2	98.2	19.7	106.0	18.7
PFDA	99.4	18.8	104.4	12.7	102.1	19.9	105.5	14.8	98.2	20.3	102.0	10.7	97.2	17.1	103.5	12.1	100.0	18.0	106.4	12.7
PFNA	96.8	14.3	102.1	11.4	97.0	12.5	103.5	11.1	96.5	16.2	100.6	10.5	95.1	15.4	100.9	12.8	98.5	13.0	103.3	11.4
PFOA	99.9	13.9	103.2	10.6	100.0	13.8	104.4	10.8	98.8	13.9	102.0	11.3	100.6	15.8	102.6	9.7	100.2	12.5	103.8	10.7
PFHpA	97.9	13.3	100.0	9.2	98.5	13.0	101.3	9.8	96.2	13.3	98.8	8.5	95.6	15.7	100.0	8.1	101.3	10.5	100.1	10.4
PFHxA	97.2	18.4	98.5	11.2	97.4	13.5	99.5	11.0	95.0	14.8	96.9	10.5	98.2	22.3	98.9	9.9	98.2	21.7	98.7	13.5
PFPeA	106.5	24.7	100.3	12.6	104.0	17.2	100.1	13.1	112.7	38.4	100.2	11.7	102.5	17.8	99.5	11.8	107.0	14.1	101.5	14.0
PFBA	93.6	24.1	94.8	17.5	93.1	23.6	96.4	18.6	98.0	22.5	97.2	12.0	86.7	29.0	90.2	19.4	96.6	20.5	95.4	18.9
PFDS	95.7	20.4	100.3	17.6	97.7	18.1	103.5	15.2	95.6	20.2	102.2	16.0	94.4	22.1	100.1	16.0	95.1	21.7	95.5	22.5
PFNS	100.4	17.8	105.6	13.9	101.7	16.5	106.1	14.0	99.8	18.6	106.1	13.7	99.4	19.5	105.6	12.7	100.6	17.2	104.4	15.6
PFOS	103.5	17.8	108.0	27.9	100.0	19.5	104.1	11.4	102.5	17.9	105.1	11.5	103.5	15.6	103.3	10.3	108.1	18.0	119.5	46.7
PFHpS	98.9	14.2	102.4	10.2	101.2	13.6	103.9	10.1	97.5	15.2	101.6	9.4	96.5	14.5	100.2	10.5	100.2	13.4	103.9	10.8
PFHxS	97.2	15.9	102.1	10.5	95.3	16.4	101.1	9.9	94.9	18.7	100.5	7.6	96.9	14.0	101.3	8.1	101.7	14.1	105.6	14.3
PFPeS	96.0	10.8	99.3	8.8	96.8	9.8	99.0	9.1	95.8	10.2	99.2	8.2	95.3	14.0	98.7	8.9	96.3	8.6	100.1	9.1
PFBS	96.7	14.4	99.8	11.1	92.7	12.1	100.0	12.3	99.1	15.9	100.5	9.9	94.9	15.1	99.2	10.9	100.0	13.0	99.5	11.7
PFOSA	88.7	14.1	95.5	10.6	87.7	12.4	92.1	11.1	89.6	13.9	97.3	10.4	85.2	18.3	94.2	7.9	92.4	10.3	98.6	11.4
FtS82	102.6	20.1	109.7	16.1	105.1	13.9	108.8	13.4	100.9	20.8	105.1	12.9	93.9	20.0	106.7	15.3	110.6	21.8	118.2	18.9
FtS62	95.0	192	92.4	51.8	85.5	49.7	92.3	29.3	75.6	34.1	97.1	84.4	130.3	275.5	86.2	33.8	88.4	37.0	93.9	33.5
FtS42	95.1	19.7	102.1	14.4	98.5	15.9	104.0	9.5	91.0	21.5	97.6	12.5	92.2	18.6	101.1	14.2	98.7	21.7	105.7	18.8

Target Analyte or Surrogate name (abbrev)	All matrices (n=479)				Reagent water (n=120)				Groundwater (n=120)				Surface water (n=120)				Wastewater (n=119)			
	low spike (60 ng/L nom.)		high spike (200 ng/L, nom.)		low spike (60 ng/L nom.)		high spike (200 ng/L, nom.)		low spike (60 ng/L nom.)		high spike (200 ng/L, nom.)		low spike (60 ng/L nom.)		high spike (200 ng/L, nom.)		low spike (60 ng/L nom.)		high spike (200 ng/L, nom.)	
	Mean % Rec.	% RSD	Mean % Rec.	% RSD	Mean % Rec.	% RSD	Mean % Rec.	% RSD	Mean % Rec.	% RSD	Mean % Rec.	% RSD	Mean % Rec.	% RSD	Mean % Rec.	% RSD	Mean % Rec.	% RSD	Mean % Rec.	% RSD
NEtFOSAA	99.3	26.4	106.6	20.1	96.5	24.1	104.0	21.6	97.6	29.2	107.7	21.3	99.0	26.3	105.4	18.7	104.4	26.2	109.4	19.1
NMeFOSAA	98.2	22.7	101.7	16.6	98.6	18.4	100.4	13.6	96.0	20.1	103.3	17.2	98.5	28.9	103.0	18.0	99.8	22.5	100.0	17.7
M2PFTeDA	95.2	21.3	99.8	18.2	99.9	22.2	100.8	18.1	91.6	20.1	97.0	17.3	93.2	21.4	98.5	14.9	96.0	21.1	102.9	21.7
MPFDoA	100.3	15.1	101.2	15.1	100.4	14.0	101.9	8.9	98.1	12.9	98.7	14.2	99.6	17.5	98.6	13.0	103.2	15.6	105.6	20.8
M7PFUdA	103.0	11.8	104.3	12.6	102.8	11.2	104.3	10.0	101.9	13.3	101.9	11.3	101.8	11.6	103.1	10.4	105.6	11.3	107.8	16.8
M6PFDA	104.5	11.7	105.0	13.2	103.1	9.8	105.6	9.5	103.8	12.9	104.8	9.4	105.2	12.5	102.3	11.0	105.8	11.6	107.3	19.6
M9PFNA	101.9	12.0	101.8	13.0	102.0	11.4	102.7	10.3	101.1	14.7	100.6	11.6	103.5	11.4	101.1	11.5	101.1	10.4	102.8	17.6
M8PFOA	100.6	10.5	101.2	11.5	100.9	10.6	101.2	7.8	99.4	11.5	99.6	9.6	101.6	10.9	101.0	8.2	100.4	9.0	102.9	17.4
M4PFHpA	98.5	12.0	98.8	12.8	99.8	10.7	99.6	8.7	99.0	13.1	97.1	8.1	98.8	11.0	98.8	8.6	96.2	13.3	99.9	21.1
M5PFHxA	96.6	12.8	97.7	13.6	98.2	11.5	99.0	10.1	96.4	13.5	97.9	10.7	96.3	14.4	96.7	11.2	95.4	12.0	97.4	20.3
M5PFPeA	98.3	7.8	99.0	11.2	97.9	8.2	99.3	7.9	96.9	8.2	99.3	6.1	99.2	7.6	97.2	7.2	99.2	7.2	100.4	18.5
MPFBA	95.1	12.6	96.5	12.2	93.0	15.0	95.9	11.4	97.3	9.6	97.0	7.1	94.1	12.9	94.2	10.2	96.1	12.4	98.9	17.4
M8PFOS	98.9	11.5	100.2	13.1	97.8	9.1	99.6	9.9	100.3	9.5	100.5	9.4	98.9	13.1	98.8	9.7	98.7	13.7	101.8	20.0
M3PFHxS	95.4	7.8	96.2	11.0	94.4	7.4	96.6	5.9	95.6	8.7	94.8	6.6	95.7	8.3	95.1	8.9	95.8	6.9	98.3	17.9
M3PFBS	89.8	12.3	89.9	15.7	89.4	10.6	91.0	10.3	90.2	11.2	91.2	9.2	90.1	13.7	86.7	19.2	89.5	13.7	90.8	20.9
M8FOSAI	108.3	9.1	108.7	12.3	108.6	7.6	107.5	7.2	107.3	9.1	108.6	8.7	108.5	10.0	107.8	12.3	108.8	9.6	110.8	18.0
M282FTS	106.8	12.7	114.0	15.0	104.4	14.1	112.3	11.8	104.8	14.0	110.3	11.7	107.8	12.6	111.4	12.9	110.3	9.3	122.0	19.3
M262FTS	99.1	18.5	105.1	15.2	98.0	13.7	105.8	11.2	95.2	10.9	100.6	10.6	104.4	29.2	103.9	14.1	98.9	10.0	110.0	20.9
M242FTS	93.4	19.2	99.3	22.4	97.0	18.2	98.7	16.5	87.6	22.8	94.9	20.6	93.3	13.9	100.3	27.0	95.9	20.4	103.3	23.8
d5NEtFOSAA	110.8	14.9	109.3	16.3	107.6	13.3	107.9	14.2	108.0	13.1	106.1	16.7	112.7	16.5	109.0	15.9	115.2	15.7	114.2	17.7
d3NMeFOSAA	107.8	15.7	106.2	17.5	106.4	13.4	103.9	13.5	105.2	14.4	108.2	17.7	110.8	19.5	105.2	14.6	108.9	14.6	107.4	22.8

[1]% Recovery of each replicate sample was calculated after subtracting average unspiked concentration by matrix determined at each laboratory if the average unspiked concentration was > 5 ng/L.

TABLE 3

RETENTION TIME (RT) AND MRM IONS

Chemical	Quantifier/Qualifier	MRM Transition	RT (minutes)
PFTeDA	Quantifier	713→669	10.6
	Qualifier	713→169	
PFTrDA	Quantifier	663→619	10.2
	Qualifier	663→169	
PFDoA	Quantifier	613→569	9.6
	Qualifier	613→169	
PFUdA	Quantifier	563→519	9.0
	Qualifier	563→269	
PFDA	Quantifier	513→469	8.4
	Qualifier	513→219	
PFDS	Quantifier	599→80	9.8
	Qualifier	599→99	
PFOS	Quantifier	499→80	8.8
	Qualifier	499→99	
PFNA	Quantifier	463→419	7.8
	Qualifier	463→219	
PFNS	Quantifier	549→80	9.2
	Qualifier	549→99	
PFOA	Quantifier	413→369	7.1
	Qualifier	413→169	
PFHpS	Quantifier	449→80	7.9
	Qualifier	449→99	
PFHxS	Quantifier	399→80	7.4
	Qualifier	399→99	
PFHpA	Quantifier	363→319	6.3
	Qualifier	363→169	
PFHxA	Quantifier	313→269	5.5
	Qualifier	313→119	

Chemical	Quantifier/Qualifier	MRM Transition	RT (minutes)
PFBS	Quantifier	299→80	5.7
	Qualifier	299→99	
PFPeA	Quantifier	263→219	4.7
PFPeS	Quantifier	349→80	6.4
	Qualifier	349→99	
PFBA	Quantifier	213→169	3.7
4:2 FTS	Quantifier	327→307	5.2
	Qualifier	327→81	
6:2 FTS	Quantifier	427→407	6.7
	Qualifier	427→81	
8:2 FTS	Quantifier	527→507	8
	Qualifier	527→81	
N-MeFOSAA	Quantifier	570→419	8.4
	Qualifier	570→483	
N-EtFOSAA	Quantifier	584→419	8.7
	Qualifier	584→483	
FOSA	Quantifier	498→78	9.8
M4PFBA	Quantifier	217→172	3.7
M5PFHxA	Quantifier	318→273	5.5
M3PFHxS	Quantifier	402→80	7.4
M8PFOA	Quantifier	421→376	7.1
M9PFNA	Quantifier	472→427	7.8
M8PFOS	Quantifier	507→80	8.8
M6PFDA	Quantifier	519→474	8.4
M7PFUdA	Quantifier	570→525	9.0
M2PFDoA	Quantifier	615→570	9.6
M2-4:2 FTS	Quantifier	329→309	5.2
M2-6:2 FTS	Quantifier	429→409	6.7
M2-8:2 FTS	Quantifier	529→509	8.0
d3-N-MeFOSAA	Quantifier	573→419	8.4
d5-N-EtFOSAA	Quantifier	589→419	8.7

Chemical	Quantifier/Qualifier	MRM Transition	RT (minutes)
M3PFBS	Quantifier	302→80	5.7
M5PFPeA	Quantifier	268→223	4.7
M4PFHpA	Quantifier	367→322	6.3
M2PFTeDA	Quantifier	715→670	10.6
M8FOSA	Quantifier	506→78	9.8

Note: Acceptable qualifier ions were not identified for PFBA and PFPeA during method development; qualifier ions are not identified for the surrogates

TABLE 4

PREPARATION OF CALIBRATION STANDARDS*

ICAL Levels	5 ng/L	10 ng/L	20 ng/L	40 ng/L	60 ng/L	80 ng/L	100 ng/L	150 ng/L	200 ng/L
PDS	25 µL	50 µL	100 µL	200 µL	300 µL	400 µL	500 µL	750 µL	1000 µL
Solution B	975 µL	950 µL	900 µL	800 µL	700 µL	600 µL	500 µL	250 µL	0 µL

PDS: 200 ng/L stock solution prepared according to Section 7.4.4

Solvent: 50:50 methanol-water with 0.1% acetic acid.

* These values are the nominal concentrations in the calibration standards. The concentration obtained from the instrument is then corrected for the 2-fold dilution made during the sample preparation process producing the reporting ranges in Table 1. Salt concentrations may also require conversion to the anion for reporting purposes. For example in the Wellington standard (PFAC-24PAR), PFBS salt has a concentration of 2 ug/mL and the anion is 1.77 ug/mL.

TABLE 5A

EXAMPLE OF TERNARY GRADIENT CONDITIONS FOR LIQUID CHROMATOGRAPHY

To prepare 1 liter of solvent C, dissolve 30.8 g of ammonium acetate in 950 mL of reagent water and add 50 mL of acetonitrile.

Time (min)	Flow (mL/min)	% Solvent Line A 95% water: 5% acetonitrile	% Solvent Line B Acetonitrile	% Solvent Line C 400mM ammonium acetate (95% water: 5% acetonitrile)
0	0.3	95	0	5
1	0.3	75	20	5
6	0.3	50	45	5
13	0.3	15	80	5
14	0.4	0	95	5
17	0.4	0	95	5
18	0.4	95	0	5
21	0.4	95	0	5

TABLE 5B

EXAMPLE OF BINARY GRADIENT CONDITIONS FOR LIQUID CHROMATOGRAPHY

To prepare 1 liter of solvent A, dissolve 1.54 g of ammonium acetate in 950 mL of reagent water and add 50 mL of acetonitrile.

To prepare 1 liter of solvent B, dissolve 0.771 g of ammonium acetate in 50 mL of reagent water and add 950 mL of acetonitrile

Time (min)	Flow (mL / min)	% Solvent Line A 20mM ammonium acetate in 95% water: 5% acetonitrile	% Solvent Line B 10mM* ammonium acetate in 95% acetonitrile: 5% water
0	0.3	100	0
1	0.3	80	20
6	0.3	50	50
13	0.3	15	85
14	0.4	0	100
17	0.4	0	100
18	0.4	100	0
21	0.4	100	0

* 20 mM ammonium acetate may not be soluble in 95:5 acetonitrile-water

TABLE 5C

INSTRUMENT CONDITIONS USED IN METHOD DEVELOPMENT

Analytical column: See 6.1.2

Isolator Column: See 6.1.3

Column temperature: 35-50ºC.

Injection volume: 10-30µL

Needle wash: 60% acetonitrile / 40% 2-propanol

Instrument: Waters Xevo TQ-S

Capillary voltage: 0.75 kV

Source temperature: 150°C

Desolvation gas temperature: 450°C

Desolvation gas flow: 800 L/hr

Cone gas flow: 200 L/hr

Collision gas flow: 0.15 mL/min

TABLE 6

EXAMPLES OF SURROGATES AND RECOMMENDED TARGET ANALYTE ASSOCIATIONS

Examples of Isotopically Labeled PFAS Surrogates	Recommended target analyte association(s)
Sulfonic Acid Surrogates	
Perfluoro-1-[2,3,4-$^{13}C_3$]butanesulfonic acid (M3PFBS)	PFBS, PFPeS
Perfluoro-1-[1,2,3-$^{13}C_3$]hexanesulfonic acid (M3PFHxS)	PFHxS, PFHpS
Perfluoro-1-[$^{13}C_8$]octanesulfonic acid (M8PFOS)	PFOS, PFNS, PFDS
1H, 1H, 2H, 2H-perfluoro-1-[1,2-$^{13}C_2$] hexanesulfonic acid (M2-4:2 FTS)	4:2FTS
1H, 1H, 2H, 2H-perfluoro-1-[1,2-$^{13}C_2$] octanesulfonic acid (M2-6:2 FTS)	6:2FTS
1H, 1H, 2H, 2H-perfluoro-1-[1,2-$^{13}C_2$] decanesulfonic acid (M2-8:2 FTS)	8:2FTS
Carboxylic Acid Surrogates	
Perfluoro-n-[$^{13}C_4$]butanoic acid (M4PFBA)	PFBA
Perfluoro-n-[$^{13}C_5$]pentanoic acid (M5PFPeA)	PFPeA
Perfluoro-n-[1,2,3,4,6-$^{13}C_5$]hexanoic acid (M5PFHxA)	PFHxA
Perfluoro-n-[1,2,3,4-$^{13}C_4$]heptanoic acid (M4PFHpA)	PFHpA
Perfluoro-n-[$^{13}C_8$]octanoic acid (M8PFOA)	PFOA
Perfluoro-n-[$^{13}C_9$]nonanoic acid (M9PFNA)	PFNA
Perfluoro-n-[1,2,3,4,5,6-$^{13}C_6$]decanoic acid (M6PFDA)	PFDA
Perfluoro-n-[1,2,3,4,5,6,7-$^{13}C_7$]undecanoic acid (M7PFUdA)	PFUdA
Perfluoro-n-[1,2-$^{13}C_2$]dodecanoic acid (M2PFDoA)	PFDoA, PFTrDA
Perfluoro-n-[1,2-$^{13}C_2$]tetradecanoic acid (M2PFTeDA)	PFTeDA
Sulfonamide and Sulfonamidoacetic acid Surrogates:	
Perfluoro-1-[$^{13}C_8$]octanesulfonamide (M8FOSA)	PFOSA
N-methyl-d3-perfluoro-1-octanesulfonamidoacetic acid (d3-N-MeFOSAA)	N-MeFOSAA
N-ethyl-d5-perfluoro-1-octanesulfonamidoacetic acid (d5-N-EtFOSAA)	N-EtFOSAA

TABLE 7

QC SUMMARY

Quality control type	**Specification and minimum frequency**	**Acceptance criteria**
Initial demonstration of proficiency (IDP) (Sec. 9.4)	4 replicates at mid calibration level initially and after major changes	Meet %recovery / %RSD requirements
Sample holding time (Sec. 8.2)	Samples: 28 days Extracts: 30 days	TBD (pending holding time study)
Initial Calibration (ICAL) (Sec. 9.7, 11.3)	Prior to analysis of samples	Mean CF: (%RSD≤20) linear or quadratic regression: r^2 >0.99 %Error: ≤±50% at LLOQ and ≤±30% for all others ≥90% of targets and surrogates meet ICAL criteria
Initial calibration verification (ICV)(Sec. 9.7, 11.3.10)	After initial calibration and prior to analysis of samples	Target analytes are within ±30% of expected concentrations
Continuing calibration verification (CCV) (Sec. 9.8, 11.4)	At beginning, every 10 samples and at end	≥90% of target analytes and surrogates within ±30% of expected concentrations
Reagent Blank (RB) (Sec. 9.5.7)	One per day of analysis	Target analyte concentrations <1/2 LLOQ or <10% of sample concentrations
Method Blank (MB) (Sec. 9.5, 11.4.4)	One per preparation of 20 or fewer samples	Target analytes <1/2 LLOQ or <10% of sample concentration
LLOQ verification Sec. 9.9.1	One per preparation batch of 20 or fewer samples	Target analytes 50-150% recovery
Laboratory Control Sample (LCS) Sec. 9.6.2	One per preparation batch of 20 or fewer samples	Target analytes 70-130% recovery
Surrogates Sec. 9.10	Each sample	Surrogates 70-130% recovery;
Target analytes Section 11.6		Meets qualitative ID criteria (RT in sample is within ±10 sec. of CCV, or RT shift is similar to associated surrogate; qualifier ion ratio within ±30% of expected ratio (midpoint ICAL or CCV), as applicable
Matrix spike/duplicate or matrix spike/matrix spike duplicate (MS/MSD) (Sec. 9.6.1)	One set per preparation of 20 or fewer field samples (if sufficient replicate samples are provided)	MS/MSD targets: 70-130% recovery MSD or duplicate: ≤30% RPD

FIGURE 1

PFOS IN CALIBRATION STANDARD

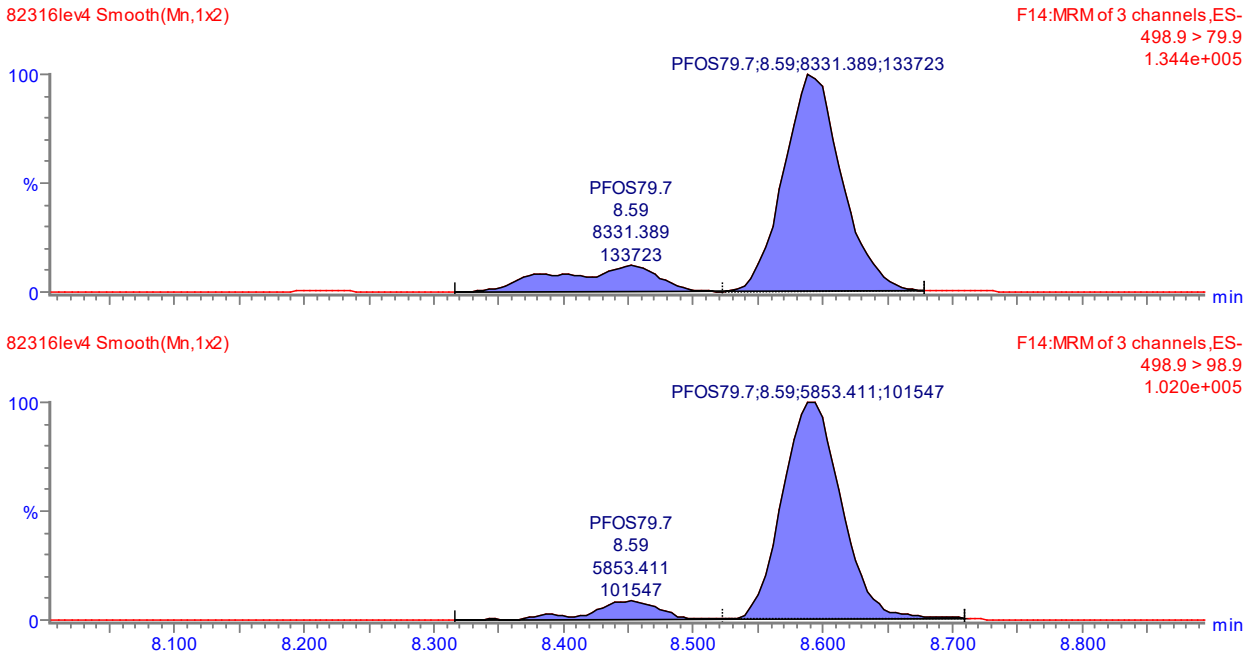


FIGURE 2

PFOS IN GROUNDWATER SAMPLE

Note: The peak at 8.22 min is an example of a structural isomer of PFOS evident in a sample that was not observed in the calibration standard for used for quantitation.

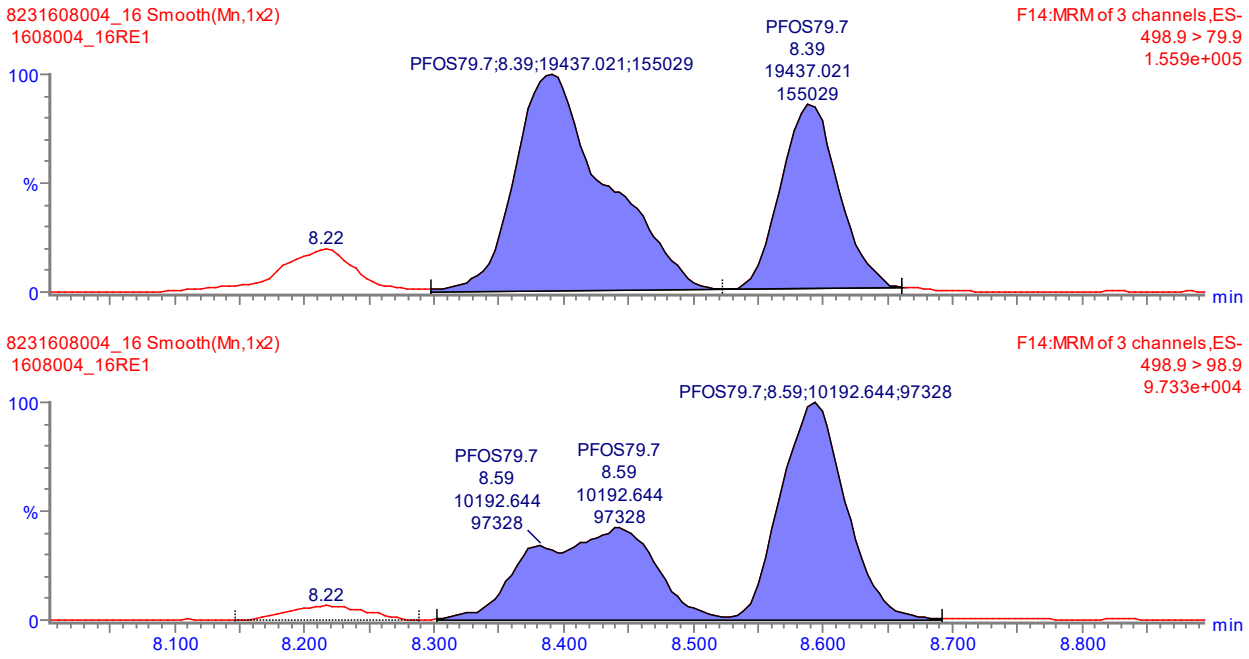


FIGURE 3

PFHxS IN CALIBRATION STANDARD

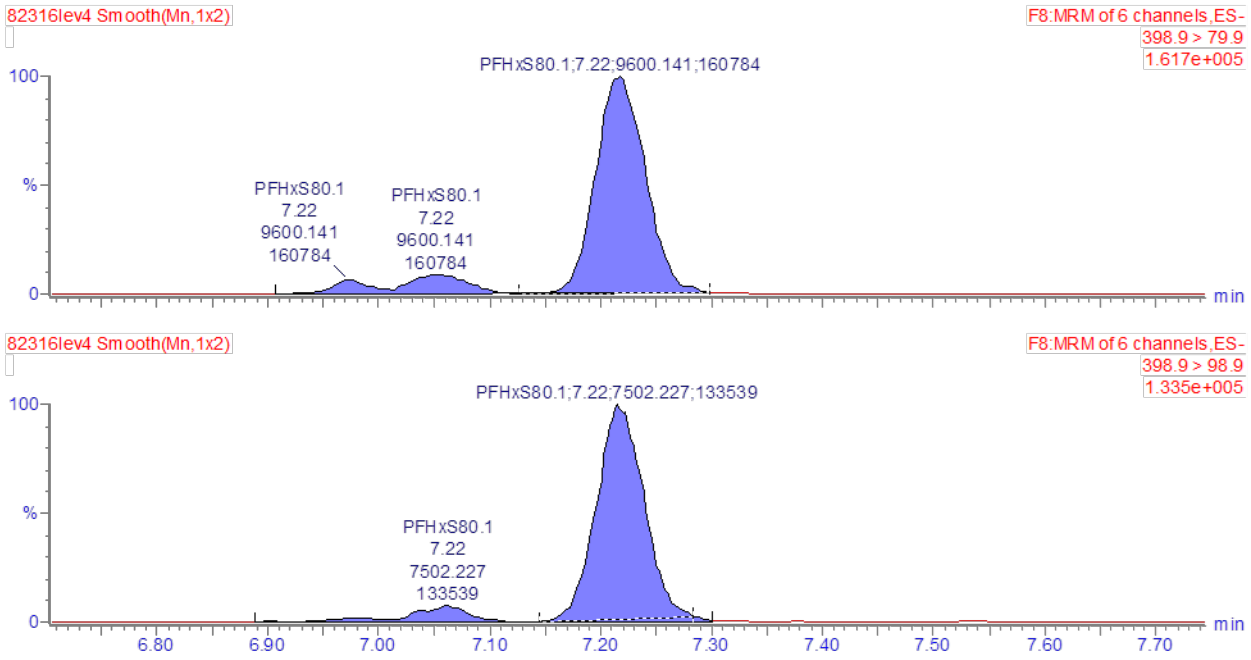
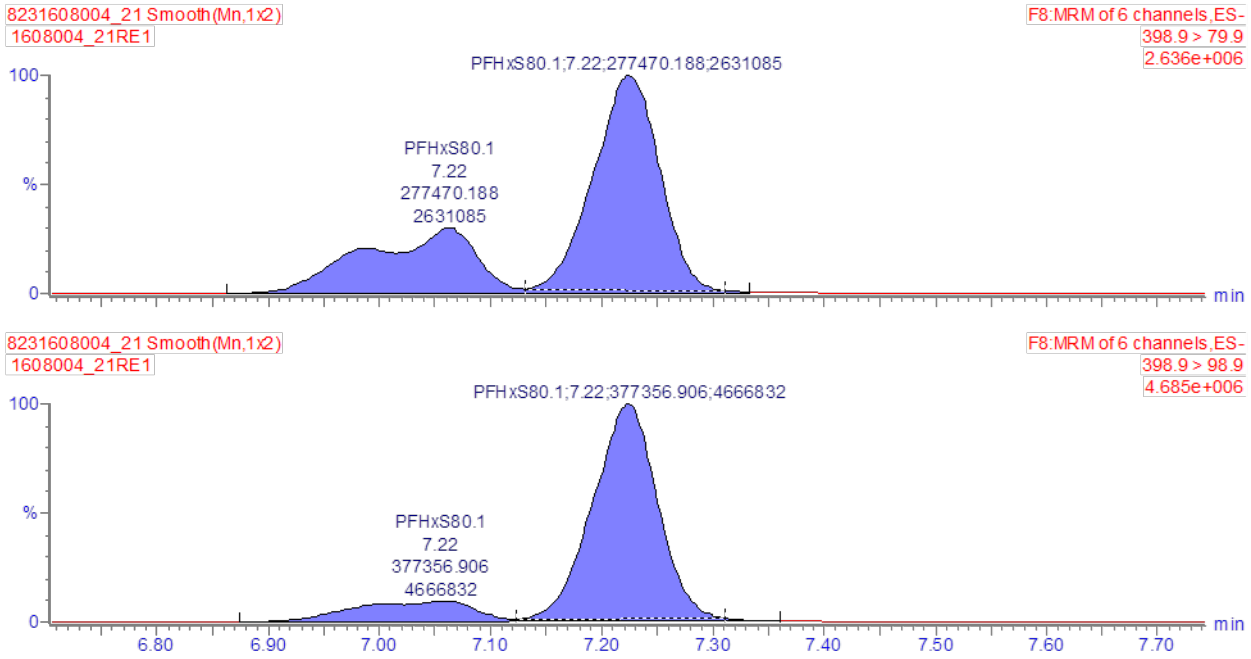


FIGURE 4

PFHxS IN GROUNDWATER SAMPLE



APPENDIX A - GLOSSARY

ASTM	ASTM International, formerly American Society for Testing and Materials
CAS RN	Chemical Abstract Service Registry Number®
CCV	continuing calibration verification
DQOs	Data Quality Objectives
EPA	U.S. Environmental Protection Agency
HDPE	high density polyethylene
ICAL	initial calibration
ICV	initial calibration verification
IDP	initial demonstration of proficiency
LC	liquid chromatography
LC/MS/MS	liquid chromatography/tandem mass spectrometry
LCS	laboratory control sample
LCSD	laboratory control sample duplicate
LLOQ	lower limit of quantitation
MB	method blank
MeOH	methanol
MRM	multiple reaction monitoring
MS	mass spectrometer
MS/MSD	matrix spike / matrix spike duplicate
OSHA	U.S. Occupational Safety and Health Administration
PEEK	Polyetheretherketone
PFAS	per- and polyfluoroalkyl substances
PPE	personal protective equipment
P&B	precision and bias
PTFE	polytetrafluoroethylene
QA	quality assurance
QAPP	quality assurance project plan
QC	quality control
RB	reagent blank
RSD	relative standard deviation

RT	retention time
Delta RT	delta retention time (in minutes or seconds)
SAP	sampling and analysis plan
SDS	safety data sheet
SOP	standard operating procedure
SRM	single reaction monitoring
TBD	to be determined
UPLC	ultraperformance liquid chromatograph

APPENDIX B (future Method 3512) – AQUEOUS SAMPLE PREPARATION

Note: The aqueous sample preparation procedure in this appendix will become a new standalone preparation method (Method 3512) after the validation study is complete and the public comments for the analytical method have been addressed. This follows the typical modular approach for SW-846 methods allowing this preparation procedure to be combined with other analytical methods, once available.

METHOD 3512

PER AND POLYFLUORINATED ALKYL SUBSTANCES (PFAS) IN NON-POTABLE WATER BY SOLVENT DILUTION

SW-846 is not intended to be an analytical training manual. Therefore, method procedures are written based on the assumption that they will be performed by analysts who are formally trained in at least the basic principles of chemical analysis and in the use of the subject technology.

In addition, SW-846 methods, with the exception of required method use for the analysis of method-defined parameters, are intended to be guidance methods which contain general information on how to perform an analytical procedure or technique which a laboratory can use as a basic starting point for generating its own detailed standard operating procedure (SOP), either for its own general use or for a specific project application. The performance data included in this method are for guidance purposes only and are not intended to be and must not be used as absolute QC acceptance criteria or for the purpose of laboratory accreditation.

B1.0 SCOPE AND APPLICATION

B1.1 Method 3512 is a preparation procedure for diluting non-potable water samples with an organic solvent prior to analysis by the appropriate determinative method for PFAS. This microscale approach minimizes sample size and solvent usage, thereby reducing the supply costs, health and safety risks, and waste generated.

B1.2 The 24 PFAS that have been evaluated with this preparation method are provided below. This preparation method was validated in conjunction with determinative Method 8327 and included mass-labelled analogs as surrogates. See Method 8327 for performance data. These surrogates may also be used as isotope dilution internal standards using Method 8328. This method has been tested in reagent water, surface water, groundwater, and wastewater matrices.

Analyte	CAS RN*
PFAS sulfonic acids	
Perfluoro-1-butanesulfonic acid (PFBS)	375-73-5
Perfluoro-1-pentanesulfonic acid (PFPeS)	2706-91-4

Analyte	CAS RN*
Perfluoro-1-hexanesulfonic acid (PFHxS)	355-46-4
Perfluoro-1-heptanesulfonic acid (PFHpS)	375-92-8
Perfluoro-1-octanesulfonic acid (PFOS)	1763-23-1
Perfluoro-1-nonanesulfonic acid (PFNS)	68259-12-1
Perfluoro-1-decanesulfonic acid (PFDS)	335-77-3
1H, 1H, 2H, 2H-perfluorohexane sulfonic acid (4:2 FTS)	757124-72-4
1H, 1H, 2H, 2H-perfluorooctane sulfonic acid (6:2 FTS)	27619-97-2
1H, 1H, 2H, 2H-perfluorodecane sulfonic acid (8:2 FTS)	39108-34-4
PFAS carboxylic acids	
Perfluorobutanoic acid (PFBA)	375-22-4
Perfluoropentanoic acid (PFPeA)	2706-90-3
Perfluorohexanoic acid (PFHxA)	307-24-4
Perfluoroheptanoic acid (PFHpA)	375-85-9
Perfluorooctanoic acid (PFOA)	335-67-1
Perfluorononanoic acid (PFNA)	375-95-1
Perfluorodecanoic acid (PFDA)	335-76-2
Perfluoroundecanoic acid (PFUdA)	2058-94-8
Perfluorododecanoic acid (PFDoA)	307-55-1
Perfluorotridecanoic acid (PFTrDA)	72629-94-8
Perfluorotetradecanoic acid (PFTeDA)	376-06-7
PFAS sulfonamides and sulfonamidoacetic acids	
N-ethylperfluoro-1-octanesulfonamidoacetic acid (N-EtFOSAA)	2991-50-6
N-methylperfluoro-1-octanesulfonamidoacetic acid (N-MeFOSAA)	2355-31-9
Perfluoro-1-octanesulfonamide (FOSA)	754-91-6

*Standards for some target analytes may consist of mixtures of structural isomers; however, the Chemical Abstracts Service (CAS) Registry Number (RN) listed in the table is for the normal-chain isomer. All CAS RNs in the above table are for the acid form. Sulfonic acids in stock standard mixes are typically received as the sodium or potassium salt form. CAS RNs for the salt form are not included.

B1.3 This technique may also be applicable to other PFAS compounds, provided that the analyst demonstrates adequate performance (e.g., recovery of 70 - 130%, or at levels that meet project-specific recovery criteria) using spiked sample matrices and an appropriate determinative method of the type included as an 8000 series method in SW-846. The use of organic-free reagent water alone is not considered sufficient for conducting such performance studies; performance must be supported by data from actual sample matrices.

B1.4 This method may not be appropriate for aqueous samples with high levels of suspended solids. If significant particulate matter is present and the total sample is of concern, then the sample should be treated as a multi-phase sample per SW-846 Chapter Two.

B1.5 Prior to employing this method, analysts are advised to consult the base method for each type of procedure that may be employed in the overall analysis (e.g., Methods 3500, 3600, and 8000) for additional information on quality control procedures, development of QC acceptance criteria, calculations, and general guidance. Analysts also should consult the disclaimer statement at the front of the manual and the information in SW-846 Chapter Two for guidance on the intended flexibility in the choice of methods, apparatus, materials, reagents, and supplies, and on the responsibilities of the analyst for demonstrating that the techniques employed are appropriate for the analytes of interest, in the matrix of interest, and at the levels of concern.

In addition, analysts and data users are advised that, except where explicitly required in a regulation, the use of SW-846 methods is *not* mandatory in response to Federal testing requirements. The information contained in this method is provided by EPA as guidance to be used by the analyst and the regulated community in making judgments necessary to generate results that meet the data quality objectives for the intended application.

B1.6 Use of this method is restricted to use by, or under supervision of, appropriately experienced and trained personnel. Each analyst must demonstrate the ability to generate acceptable results with this method.

B2.0 SUMMARY OF METHOD

B2.1 Samples are prepared by adding mass-labelled PFAS isotopes (as surrogates or as isotope dilution internal standards, depending on determinative method), diluting samples 1:1 with the appropriate organic solvent, filtering and pH adjustment, if necessary.

B2.2 Determinative analysis is performed using the appropriate LC/MS/MS method (e.g., 8327, 8328).

B3.0 DEFINITIONS

Refer to the SW-846 Chapter One for and the manufacturer's instructions for definitions that may be relevant to this procedure.

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B4.0 INTERFERENCES

B4.1 In order to avoid compromising data quality, contamination from preparation procedure must be reduced to the lowest practical level. Method blanks (MBs) and reagent blanks (RBs) are prepared and analyzed with all samples and are used to demonstrate that laboratory supplies and preparation and analysis steps do not introduce interferences or PFAS artifacts at levels that would bias quantitation. Careful selection of reagents and consumables is necessary because even low levels of PFAS contamination may alter the precision and bias of the method, and background introduced by these materials (and variability thereof) is cumulative. Refer to each determinative method to be used for specific guidance on QC procedures and to SW-846 Chapter Four for general guidance on glassware cleaning.

B4.2 Refer to Method 8327 or 8328 for additional information on interferences.

B4.3 Procedures employed to prevent or minimize problems.

B4.3.1 All solvents should be of pesticide residue purity or higher (or preferably LC/MS grade) to minimize interference problems.

B4.3.2 PFAS contamination has been found in reagents, glassware, tubing, polytetrafluoroethylene (PTFE) vial caps, aluminum foil, glass disposable pipettes, filters, and other apparatus that release fluorinated compounds. All supplies and reagents should be verified prior to use. If found, measures should be taken to remove the contamination, if possible, or find other suppliers or materials to use that meet method or project criteria.

B4.3.3 Polyethylene disposable pipettes are recommended. Alternate materials may be used if the blank criteria in the determinative method are met. When a new batch of disposable pipettes is received, at least one should be checked for release of target analytes or interferences.

B4.3.4 If labware is re-used, the procedure described for labware cleaning (Sec. A6.4) should be followed to minimize risk of contamination. The blank criteria in Sec. 9.5 of Method 8327 can be used as a guideline for evaluating cleanliness.

B5.0 SAFETY

B5.1 This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of U.S. Occupational Safety and Health Administration (OSHA) regulations regarding the safe handling of the chemicals specified in this method. A reference file of safety data sheets (SDSs) must be available to all personnel involved in these analyses.

B5.2 Users of this method should operate a formal safety program.

B5.3 The toxicity and carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound is treated as a health hazard. Exposure to these chemicals should be reduced to the lowest possible level and the appropriate personal protective equipment (PPE) should be utilized. Review SDSs for specific physical and health hazards including appropriate PPE to be used. SDSs may be accessed at multiple locations (e.g., www.sigmaaldrich.com, www.well-labs.com, and www.isotope.com).

B6.0 EQUIPMENT AND SUPPLIES

The mention of trade names or commercial products in this method is for illustrative purposes only and does not constitute an EPA endorsement or exclusive recommendation for use. The products and instrument settings cited in SW-846 methods represent those products and settings used during method development or subsequently evaluated by the Agency.

Glassware, reagents, supplies, equipment, and settings other than those listed in this manual may be employed provided that method performance appropriate for the intended application has been demonstrated and documented. This section does not list all common laboratory containers (e.g., beakers and flasks) that might be used.

B6.1 Adjustable volume pipettes, 10-µL, 20-µL, 100-µL, 200-µL, and 1000-µL, 5 mL, and 10 mL.

B6.2 Analytical balance, capable of weighing to 0.01g

B6.3 Miscellaneous Supplies

B6.3.1 10- to 25 mL filter-adaptable HDPE, polypropylene, or glass syringe with luer lock (rubber tipped plungers are not to be used).

B6.3.2 50 mL polypropylene tubes (BD Falcon, Catalog # 352098)

B6.3.3 15 mL polypropylene tubes (BD Falcon, Catalog # 352097); use pre-weighed tubes for collection of field samples and field QC

B6.3.4 Polyethylene disposable pipettes (SEDI-PETTM PIPET, Source - Samco Scientific, part no. 252)

B6.3.5 Pipette tips: polypropylene pipette tips of various sizes (Eppendorf, catalogue #s 022491997, 022492080, 022491954, 022491946, and 022491512)

B6.3.6 Acrodisc GxF/0.2µm GHP or equivalent membrane syringe driven filter unit. Filters must be cleaned prior to use. A suggested protocol is to rinse each filter with 2 x10 mL acetonitrile and then 2 x10 mL methanol prior to use. Other protocols may be appropriate if PFAS contamination is removed or reduced to levels appropriate for the project.

B6.4 Labware cleaning instructions – If labware is reused it should be washed in hot water with detergent such as powdered Alconox, Deto-Jet, Luminox, or Citrojet, rinsed in hot water and rinsed with distilled water. Rinse with organic solvents such as acetone, methanol, and/or acetonitrile.

B7.0 REAGENTS AND STANDARDS

B7.1 Reagent-grade or pesticide grade chemicals, at a minimum, should be used in all tests. Unless otherwise indicated, all reagents should conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where specifications are available. Other grades may be used, provided the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

B7.2 Reagent water. All references to water in this method refer to organic-free reagent water as defined in SW-846 Chapter One. Reagent water from in-house DI systems will likely require additional polishing with a point-of-use water purification system to meet method requirements. The laboratory should check for PFAS contamination coming from the point-of-use system (it should not contain fluoropolymers, where practical). Some bottled HPLC water has been shown to contain PFAS.

B7.3 Reagents: Items shown are for informational purpose only; equivalent reagents and standards may be used. All reagents and solvents should be of pesticide residue purity or higher to minimize interference problems, preferably LC/MS grade or equivalent.

B7.3.1 Methanol, CH3OH (CAS RN 67-56-1)

B7.3.2 Acetic acid, CH3COOH (CAS RN 64-19-7)

B7.3.3 50:50 Methanol-reagent water containing 0.1% acetic acid

B7.3.4 See determinative method for surrogate, internal standard, and target spiking solutions.

B8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

See introductory material to SW-846 Chapter Four, “Organic Analytes”, Method 3500, and the specific determinative method to be used.

B9.0 QUALITY CONTROL

B9.1 Refer to SW-846 Chapter One for guidance on quality assurance (QA) and quality control (QC) protocols. When inconsistencies exist between QC guidelines, method-specific QC criteria take precedence over both technique-specific criteria and the criteria given in Chapter One, and technique-specific QC criteria take precedence over the criteria in Chapter One. Any effort involving the collection of analytical data should include development of a structured and systematic planning document, such as a Quality Assurance Project Plan (QAPP) or a Sampling and Analysis Plan (SAP), which translates project objectives and specifications into directions for those that will implement the project and assess the results. Each laboratory should maintain a formal quality assurance program. The laboratory should also maintain records to document the quality of the data generated. All data sheets and quality control data should be maintained for reference or inspection.

B9.2 See Sec. 9 of Methods 8327 and 8328 for QA/QC requirements specific to that analysis.

B9.3 Initial demonstration of proficiency and lower limit of quantitation (LLOQ)

B9.3.1 Each laboratory must demonstrate initial proficiency with each sample preparation and determinative method combination it utilizes by generating data of acceptable accuracy and precision for target analytes in a clean matrix. The laboratory must also repeat the demonstration of proficiency whenever new staff members are trained or significant changes in instrumentation are made. See Method 8000D, Sec. 9.3 for information on how to accomplish a demonstration of proficiency.

B9.3.2 The laboratory should verify the LLOQ initially and in each preparation batch using clean control material (e.g., reagent water) or a representative sample matrix, free of target compounds. See Sec. 9.9 of Method 8327 or Method 8328 for establishing the LLOQ level and for the acceptance criteria to use.

B9.4 Blanks - Before processing any samples, the analyst should demonstrate that all parts of the equipment in contact with the sample and reagents are interference-free. This is accomplished through the preparation and analysis of method blanks (MBs). Each time samples are prepared, and when there is a change in reagents, an MB should be prepared and analyzed for the compounds of interest as a safeguard against chronic laboratory contamination.

B9.4.1 At least one MB for every 20 field samples must be prepared in reagent water to investigate for PFAS contamination throughout sample preparation and analysis. Method blanks are subjected to all steps in Sec. B11.0.

B9.4.2 Because PFAS contamination is common in reagents, a RB should be prepared with each batch of samples using 50:50 methanol-water solution containing 0.1% acetic acid to investigate for system/laboratory contamination

B9.5 Laboratory Controls - Each preparation batch of twenty or fewer samples should also include a laboratory control sample (LCS), a matrix spike sample (MS), a matrix spike duplicate (MSD) or laboratory duplicate sample (if sufficient volume is available), and an LLOQ verification.

B9.6 Any method blanks, LCSs, MS/MSDs, duplicate samples and LLOQ verifications should be subjected to the same preparation procedures (Sec. B11.0) as those used on actual samples.

B9.7 All field and QC samples should be spiked with an appropriate concentration of mass labelled PFAS isotopes, whether used as surrogates or internal standards, as a check on the preparation procedure or to report recovery-corrected concentrations of target analytes.

B10.0 CALIBRATION AND STANDARDIZATION

There are no calibration or standardization steps directly associated with this preparation procedure.

B11.0 PROCEDURE

B11.1 Sample preparation – Each batch of samples (20 or fewer) should contain a MB, LCS, MS, sample duplicate or MSD (if available), and an LLOQ verification sample. The following sections refer to Method 8327 for suggested standard addition concentrations for PFAS target analytes and associated surrogates by QC sample type. Different concentrations may be spiked depending on the needs of the project, the sensitivity of the instrument, or the determinative method used. The analyst should strive to keep the total spike additions to ≤1% of the final volume (≤100 uL in 10 mL) to minimize errors in the dilution.

B11.1.1 Weigh all field collected samples in the 15 mL polypropylene centrifuge tubes prior to any preparation steps and calculate the difference weight using the value of the pre-weighed container. Use a difference weight, assuming a density of 1.0 g/mL, to determine volume. This volume will be used to determine amount of solvent to add if significantly different than 5 mL.

NOTE: If pre-weighed containers were not used to collect samples, mark the level of the sample on the outside of the container for determination of the volume. Certified graduation marks on sample containers may also be used to estimate sample volume.

B11.1.2 MB – Prepare by adding 5.0 mL of reagent water to a 15 mL polypropylene tube and adding an appropriate volume of the surrogate/internal standard spiking solution (Sec. 7.4.1 of Method 8327).

B11.1.3 LCS - Prepare by adding 5.0 mL of reagent water to a 15 mL polypropylene tube and adding appropriate volumes of the surrogate/internal standard spiking solution (Sec. 7.4.1 of Method 8327) and MS/MSD and LCS target compounds spiking solution (Sec. 7.4.2 of Method 8327).

NOTE: If field samples were collected at a different volume, measure a similar volume for MB and LCS into a similarly sized container.

B11.1.4 LLOQ Verification Sample - Prepare by adding 5 mL of reagent water to a 15 mL polypropylene tube and adding appropriate volumes of the surrogate/internal standard spiking solution (Sec. 7.4.1 of Method 8327) and LLOQ verification spiking solution (Sec. 7.4.3 of Method 8327). Prepare additional LLOQ verification samples at higher concentration as needed (Sec. 7.4.3 of Method 8327).

B11.1.5 Sample - Allow the 5 mL sample collected in a 15 mL polypropylene tube to warm to room and weigh. Spike with an appropriate volume of the surrogate /internal standard spiking solution (Sec. 7.4.1 of Method 8327).

NOTE: Different volumes may be received by the laboratory and the entire volume, as received, must be prepared. Removing aliquots from a sample (volumes less than entire container) prior to solvent addition is not recommended prior to addition of MeOH, because longer chain PFAS are known to sorb to container walls in water samples unless ≥50% organic cosolvent is present.

NOTE: If the original container has inadequate volume to hold a 1:1 dilution (i.e. lacking sufficient volume to add the same volume of methanol), the sample may be transferred to a larger container, but the interior of the original sample container must be solvent rinsed, and this rinse must be incorporated into the solvent dilution of the sample (Sec. B11.2). Hand shake or vortex the rinse solvent in the original sample container for ~2 min to ensure quantitative transfer.

B11.1.6 MS/MSD or MS/sample duplicate - Use separately collected containers for the MS, MSD and/or duplicate QC samples, provided sufficient sample is available. Add an appropriate volume of the surrogate/internal standard spiking solution (Sec. 7.4.1 of Method 8327) to each of these QC samples, and add an appropriate volume of the MS/MSD and LCS target compounds spiking solution (Sec. 7.4.2 of Method 8327) to MS/MSD samples.

B11.2 Sample Dilution

B11.2.1 For field collected samples, matrix spikes, matrix spike duplicates and sample duplicates, add 5 mL methanol to each tube. If sample volumes differ from 5 mL by >5%, (i.e. <4.75 mL or >5.25mL), adjust the methanol volume added according to the volume determined in B11.1.1. This may also require the adjustment of the amount of surrogates/internal standard and the amount of target compounds spikes added, if applicable.

NOTE: Adjusting the surrogates and target compounds standard additions for alternate sample volumes can be accomplished by adding surrogates (and target compounds, as applicable) to the methanol used for dilution. For example, field samples, duplicates, and method blanks can be prepared with methanol spiked with surrogates/internal standards at concentrations of 160 ng/L, which would result in surrogate concentrations of 80 ng/L in the samples after 1:1 dilution with methanol. Similarly, LCS and MS/MSD samples can be prepared with methanol that has been spiked with surrogates/internal standards and targets compounds both at concentrations of 160 ng/L (nom.), which would result in surrogate and target analyte concentrations of 80 ng/L in the samples after 1:1 dilution with methanol. For sample volumes <4.75 mL or >5.25 mL, add the same volume of methanol solution as was calculated for the sample in Sec. B11.1.1.

B11.2.2 For blanks, LCS, and LLOQ verification QC samples prepared in 5 mL reagent water, add 5 mL of methanol. If alternate sample volumes are prepared as described in B11.2.1, add surrogates and target compounds (as applicable) in the same manner as was done for field samples and MS/MSD (as spiking solutions or added to the methanol used as a dilution solvent, as appropriate).

B11.2.3 Hand shake or vortex for ~2 min.

B11.2.4 Filter each diluted field sample and associated QC sample through separate rinsed Acrodisc GxF/0.2µm GHP membrane syringe-driven filters (See Sec. 6.3.8 of Method 8327) to remove particulates in the samples.

B11.2.5 Add acetic acid to all samples to adjust to pH ~3 - 4 after filtration (e.g., add 10µL of glacial acetic acid to 10mL). Transfer approximately 1 mL of that solution to an LC vial and apply a polyethylene cap. The sample is now ready for analysis.

B11.2.6 The final volume of the solution is 10 mL for laboratory-prepared QC samples. The final volume for field samples and QC is calculated summing the sample and added methanol volumes.

NOTE: To minimize PFAS contamination in subsequent samples, it is recommended to soak reusable syringes in hot tap water and then rinse with 5 X 10 mL reagent water, 3 X 10 mL acetonitrile and 3 x 10 mL methanol.

B12.0 DATA ANALYSIS AND CALCULATIONS

There are no data analysis and calculation steps directly associated with this procedure. Follow the directions given in the determinative method.

B13.0 METHOD PERFORMANCE

B13.1 Performance data and related information are provided in SW-846 methods only as examples and guidance. The data do not represent required performance goals for users of the methods. Instead, performance criteria should be developed on a project-specific basis, and the laboratory should establish in-house QC performance criteria for the application of this method.

B13.2 TBD

B14.0 POLLUTION PREVENTION

B14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity and/or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operations. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

B14.2 For information about pollution prevention that may be applicable to laboratories and research institutions consult *Less is Better: Laboratory Chemical Management for Waste Reduction*, a free publication available from the American Chemical Society (ACS), Committee on Chemical Safety, http://portal.acs.org/portal/fileFetch/C/WPCP_012290/pdf/WPCP_012290.pdf.

B15.0 WASTE MANAGEMENT

The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management, consult *The Waste Management Manual for Laboratory Personnel* available from the American Chemical Society at the address listed in Sec. B14.2.

B16.0 REFERENCES

1. U.S. Environmental Protection Agency, Region 5 Laboratory, “Standard Operating Procedure for the Analysis of Polyfluorinated Compounds of Interest to OSRTI in Water, Sludge, Influent, Effluent, and Wastewater by Multiple Reaction Monitoring Liquid Chromatography/Mass Spectrometry (LC/MS/MS),” 75 pp., 2016.

2. Standard Practices for Sampling Water, American Society for Testing and Materials, Philadelphia. ASTM Annual Book Standards, Part 31, D3370-76.

B17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION

DATA TBD