METHOD 3512

SOLVENT DILUTION OF NON-POTABLE WATERS

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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 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 methods used 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.

1.0 SCOPE AND APPLICATION

This method is for preparation of non-potable water samples by dilution with an organic solvent prior to analysis by the appropriate determinative method.

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. See Method 8327 and SW-846 website for performance data. This method has been tested in surface water, groundwater, and wastewater matrices. This preparation method may also be applicable to other target compounds and other aqueous matrices, provided that the laboratory can demonstrate adequate performance (refer to Sec. 9.0 of the applicable determinative method or to project-specific acceptance criteria) using representative sample matrices. Please refer to Method 8000 for additional information.

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 (PFUnDA)	2058-94-8
Perfluorododecanoic acid (PFDoDA)	307-55-1

Analyte	CAS RN*
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 (PFOSA)	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.

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 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.

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 Refer to the appropriate determinative method for more information about method performance and related considerations. Note that 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. Larger sample collection volumes or centrifugation may aid phase separation.

2.0 SUMMARY OF METHOD

2.1 Samples are prepared by adding isotopically labeled analogs of PFAS target analytes (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.

2.2 Determinative analysis is performed using the appropriate liquid chromatography/tandem mass spectrometry (LC/MS/MS) method.

3.0 DEFINITIONS

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

4.0 INTERFERENCES

4.1 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.

4.2 Refer to determinative method for additional information on interferences.

4.3 Procedures employed to prevent or minimize problems (see determinative method for specific requirements and criteria).

4.3.1 All solvents should be of LC/MS grade, or equivalent, to minimize interference problems. Solvents must be checked by lot prior to use.

4.3.2 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.

4.3.3 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.

4.3.4 During method development, loss of some PFAS target analytes was observed during storage of standard solutions in 1:1 methanol-water containing 0.1% acetic acid in glass containers. Polypropylene containers should be used for preparation and storage of samples and standards. Other materials may be used, such as high density polyethylene (HDPE), if it can be shown the target analytes are not adversely affected (i.e., all quality control criteria in Sec. 9.0 of the determinative method can be met). Glass autosampler vials have been successfully used for solutions in 1:1 methanol-water containing 0.1% acetic acid during analysis.

4.3.5 If labware is re-used, the procedure described for labware cleaning (Sec. 6.4) should be followed to minimize risk of contamination. The blank criteria in the appropriate determinative method can be used as a guideline for evaluating cleanliness.

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 may 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 products and settings used during method development or subsequently evaluated by the Agency. Labware, 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, including meeting acceptance criteria for all categories of quality controls listed in Sec. 9.0. This section does not list all common labware (e.g., beakers and flasks) that might be used.

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.

6.1 Adjustable volume pipettes, 10-µL to 10-mL.

6.2 Analytical balance, capable of weighing to 0.01g

6.3 Miscellaneous Supplies

6.3.1 10- to 25-mL filter-adaptable syringe with luer lock: high density polyethylene (HDPE), polypropylene or glass (rubber tipped plungers are not to be used).

6.3.2 50-mL polypropylene tubes (BD Falcon, Catalog # 352098)

6.3.3 15-mL polypropylene tubes (BD Falcon, Catalog # 352097); use pre-weighed tubes for collection of field samples and field QC

6.3.4 Polyethylene disposable pipettes (Samco Thermo Scientific, Catalog # 252)

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

6.3.6 Pall Acrodisc GxF/0.2-µm 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 x 10 mL acetonitrile and then 2 x10 mL methanol prior to use.

6.3.7 Autosampler vials: HDPE, polypropylene or glass

6.3.8 Polyethylene autosampler vial caps (Waters Catalog # 186004169)

6.4 Labware cleaning instructions – If labware is reused it should be washed in hot water with detergent such as powdered Alconox, Detojet, Luminox, or Citrojet, rinsed in hot water and rinsed with distilled water. Rinse with organic solvents such as acetone, methanol, and/or acetonitrile. Traces of target compounds should be reduced to a minimum.

7.0 REAGENTS AND STANDARDS

7.1 Chemicals used in all tests should be LC/MS grade if available, or reagent grade at a minimum. Unless otherwise indicated, all reagents should conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society (ACS), 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 blank acceptance criteria in Sec. 9.5 can be met.

7.2 Reagent water – All references to water in this method refer to reagent water as defined in SW-846 Chapter One. Reagent water from in-house deionized water systems may need additional treatment prior to use (e.g., with a point-of-use water purification system) to meet blank acceptance criteria (Sec. 9.5). The laboratory should check for PFAS contamination coming from the point-of-use system (it should not contain fluoropolymers, where practical). Bottled reagent water should be evaluated in the same manner as reagent water from other sources.

7.3 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.

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

7.3.2 Acetic acid, CH_3COOH (CAS RN 64-19-7)

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

7.4 Standard Solutions

See the relevant determinative method for information about standards used for sample preparation (e.g., surrogates, internal standards, and/or target compounds spiking solutions).

8.0 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.

9.0 QUALITY CONTROL

9.1 General Guidance - 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 who will implement 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. Refer to Method 8000 and to the relevant determinative method for more information and guidance on evaluation and reporting of sample data associated with non-compliant quality controls.

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 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 See Sec. 9.0 of the appropriate determinative method for QA/QC requirements specific to that analysis.

9.4 Initial demonstration of proficiency (IDP) -- 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.

9.5 Blanks – Before processing any samples, the analyst must 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, a MB should be prepared and analyzed for the compounds of interest as a safeguard against chronic laboratory contamination.

9.5.1 At least one MB must be prepared with each batch of 20 or fewer field samples to investigate for PFAS contamination throughout sample preparation and analysis. MBs are reagent water samples that are subjected to all sample preparation steps in Sec. 11.0.

9.5.2 At least one RB should be prepared each day that samples are prepared to investigate for contamination in laboratory reagents and consumables. PFAS contamination at low levels is common in laboratory supplies and equipment. RBs contain the reagents (1:1 methanol-water containing 0.1% acetic acid) used in the preparation batch, but they are not subjected to sample preparation procedures like MBs (e.g., filtration). Surrogates are not required to be added to RBs.

9.6 Sample QC for preparation and analysis

The laboratory must have procedures for documenting the effect of the matrix on method performance (precision, bias, sensitivity). At a minimum, this must include preparation and analysis of a MB and LCS, and where practical, an MS/MSD pair or MS and duplicate in each preparation batch of 20 or fewer samples. An LLOQ verification QC sample is also recommended to be included in each sample preparation batch, as needed for the project. These QC samples are subjected to the same preparation procedures (Sec. 11.0) as those used on actual samples.

9.7 All field samples and QC samples should be spiked with an appropriate concentration of isotopically labeled analogs of PFAS target analytes used as surrogates or internal standards.

10.0 CALIBRATION AND STANDARDIZATION

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

11.0 PROCEDURE

The following sections are written assuming a container size of 15 mL and a 5 mL sample size. Alternate sample volumes and container sizes may be used provided the solvent proportions are maintained and the entire sample is prepared (e.g., 20 mL samples are collected in 50 mL polypropylene tubes and diluted with 20 mL of solvent). Refer to the appropriate determinative method for suggested concentrations for surrogates/internal standards and any target analytes by QC sample type. The analyst should limit standard additions to ≤1% of the final volume (e.g., ≤100 µL in 10 mL) to minimize errors in the dilution.

CAUTION: Surface binding of target compounds from aqueous solution to collection containers is known to occur. Subsampling or transfer of water from a container prior to addition of a sufficient proportion of organic solvent can result in significant loss of longer-chain PFAS target analytes (e.g., carboxylic acids ≥C_9, sulfonic acids ≥C_7). Aqueous samples may only be subsampled or transferred to other containers if 50% organic co-solvent content is achieved beforehand. Quantitative transfer can be achieved by solvent-rinsing the empty container with methanol. If subsampling is performed prior to achieving 50% organic cosolvent content, i.e., when preparing the entire water sample is not possible or practical, the data must be qualified appropriately.

11.1 Initial Sample Volume

11.1.1 Field Samples, MS/MSD, and duplicate QC samples – Use separately collected containers for each field sample and each MS, MSD and/or duplicate QC sample. Allow the samples to warm to room temperature and determine the sample volume using one of the options below. If the sample is transferred to another container prior to diluting 1:1 with methanol, the original container must be solvent rinsed and the rinsate included in the solvent dilution of the sample (Sec. 11.2). Hand-shake or vortex the rinse solvent in the original sample container for ~2 min to ensure quantitative transfer.

11.1.1.1 If sample containers were pre-weighed prior to collection, sample volume may be determined by weighing the container plus sample, calculating the sample mass by difference and converting to volume, assuming a density of 1.0 g/mL.

11.1.1.2 Sample volume may be determined either by mass difference of the original sample and the container after transfer (using assumed density as described above), by marking the original sample volume on the outside of the

container and determining after transfer, or by direct measurement (e.g., using certified graduation marks on sample containers).

11.1.2 MB, LCS, and LLOQ verification QC samples – Add 5.0 mL (or other appropriate volume) of reagent water to separate 15-mL polypropylene tubes.

NOTE: If field samples were collected at a different volume, measure a similar volume for MBs, LLOQ verifications, and LCSs into similarly sized containers.

11.2 Sample Preparation

11.2.1 Spike each field sample and associated QC sample with an appropriate volume of the surrogate/internal standard spiking solution; and spike each LLOQ verification, LCS, or MS/MSD sample with an appropriate volume of a target compounds spiking solution. For external standard calibration determinative methods, the spiking solution volumes should be scaled to the sample volumes if they differ significantly from the nominal expected volume (e.g., 5.0 mL).

11.2.2 Dilute each field sample and associated QC sample 1:1 with methanol by adding a volume equivalent to the initial sample volume (Sec. 11.1) to each tube (e.g., 5.0 mL).

11.2.3 Hand shake or vortex each sample for ~2 min.

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

11.2.5 Add 0.1% (v/v) acetic acid to each field sample and associated QC sample after filtration (e.g., add 10 µL of glacial acetic acid to 10 mL). Transfer an aliquot of that solution to an LC vial and apply a polyethylene cap. The sample is now ready for analysis.

11.2.6 The final volume of each prepared field sample and associated QC sample may be calculated as the sum of the aqueous sample and methanol volumes or measured. The decrease in solution volume upon mixing of equal volumes of methanol and water is small (see Reference 4 in Sec. 16.0) and can be considered insignificant.

NOTE: To minimize PFAS contamination in subsequent samples, a suggested protocol is 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.

12.0 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.

13.0 METHOD PERFORMANCE

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.

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 ACS, Committee on Chemical Safety at: https://www.acs.org/content/dam/acsorg/about/governance/committees/chemicalsafety/publications/less-is-better.pdf.

15.0 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 at: http://www.labsafetyinstitute.org/FreeDocs/WasteMgmt.pdf.

16.0 REFERENCES

1. ASTM Standard D7979-20, “Standard Test Method for Determination of Perfluorinated Compounds in Water, Sludge, Influent, Effluent and Wastewater by Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS)”, ASTM International, West Conshohocken, PA, 2020. Available at www.astm.org.
2. 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.
3. Standard Practices for Sampling Water, American Society for Testing and Materials, Philadelphia. ASTM Annual Book Standards, Part 31, D3370-76.
4. Mikhail, S.Z., & Kimel, W.R. (1961). Densities and Viscosities of Methanol-Water Mixtures. Journal of Chemical and Engineering Data. 6(4), 533-537. https://doi.org/10.1021/je60011a015

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION

Refer to the relevant determinative method for performance data.