Designation: D8421 – 21

Standard Test Method for Determination of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous Matrices by Co-solvation followed by Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS)[1]

This standard is issued under the fixed designation D8421; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This test method covers the determination of per- and polyfluoroalkyl substances (PFASs) in aqueous matrices using liquid chromatography (LC) and detection with tandem mass spectrometry (MS/MS). These analytes are co-solvated by a 1+1 ratio of sample and methanol then qualitatively and quantitatively determined by this test method. Quantitation is by selected reaction monitoring (SRM) or sometimes referred to as multiple reaction monitoring (MRM).

1.2 The method detection limit (MDL)[2] and reporting range[3] for the target analytes are listed in Table 1. The target concentration for the reporting limit for this test method is an integer value that is calculated from the concentration from the lowest standard from the final volume of the prepared sample. This value may be lower than the calculated MDL due to sporadic PFAS hits due to PFAS contamination in consumables/collection tools used during sample collection and preparation. All samples should be taken at a minimal as duplicates in order to compare the precision between the two prepared samples to help ensure the concentration/positive result is reliable.

1.2.1 Recognizing continual advancements in the sensitivity of instrumentation, advancements in column chromatography and other processes not recognized here, the reporting limit may be lowered assuming the minimum performance requirements of this test method at the lower concentrations are met.

1.2.2 Depending on data usage, you may modify this test method but limit to modifications that improve performance while still meeting or exceeding the method quality acceptance criteria. Modifications to the solvents, ratio of solvent to sample, or shortening the chromatographic run simply to save time are not allowed. Use Practice E2935 or similar statistical tests to confirm that modifications produce equivalent results on non-interfering samples. In addition, use Guide E2857 or equivalent statistics to re-validate the modified test.

1.2.3 Analyte detections between the method detection limit and the reporting limit are estimated concentrations. The reporting limit is based upon the concentration of the Level 1 calibration standard as shown in Table 5.

1.3 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.4 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.5 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[4]

D1129 Terminology Relating to Water

D1193 Specification for Reagent Water

D2777 Practice for Determination of Precision and Bias of Applicable Test Methods of Committee D19 on Water

D3856 Guide for Management Systems in Laboratories Engaged in Analysis of Water

D4841 Practice for Estimation of Holding Time for Water

[1] This test method is under the jurisdiction of ASTM Committee D19 on Water and is the direct responsibility of Subcommittee D19.06 on Methods for Analysis for Organic Substances in Water.

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[2] The MDL is determined following the Code of Federal Regulations (CFR), 40 CFR Part 136, Appendix B utilizing dilution and filtration. A detailed process determining the MDL is explained in the reference and is beyond the scope of this test method.

[3] Injection volume variations, and sensitivity of the instrument used will change the reporting limit and ranges.

[4] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

Samples Containing Organic and Inorganic Constituents
D5847 Practice for Writing Quality Control Specifications for Standard Test Methods for Water Analysis
D8272 Guide for Development and Optimization of D19 Chemical Analysis Methods Intended for EPA Compliance Reporting
E694 Specification for Laboratory Glass Volumetric Apparatus
E2554 Practice for Estimating and Monitoring the Uncertainty of Test Results of a Test Method Using Control Chart Techniques
E2857 Guide for Validating Analytical Methods
E2935 Practice for Evaluating Equivalence of Two Testing Processes

2.2 *Other Standards:*[5]
Code of Federal Regulations 40 CFR Part 136, Appendix B

3. Terminology

3.1 *Definitions:*

3.1.1 For definitions of terms used in this standard, refer to Terminology D1129.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *collision cell, n*—chamber in the ion path between m/z separation elements, or between ion source and the first analyzer, in tandem mass spectrometry in space configurations.

3.2.2 *continuing calibration verification (CCV), n*—a mid-range calibration standard which checks the continued validity of the initial calibration of the instrument.

3.2.3 *mass spectrometry/mass spectrometry (MS/MS), n*—acquisition and study of the spectra of the product ions or precursor ions of m/z selected ions, or of precursor ions of a selected neutral mass loss.

3.2.3.1 *Discussion*—MS/MS can be accomplished using instruments incorporating more than one analyzer (tandem mass spectrometry in space) or in trap instruments (tandem mass spectrometry in time).

3.2.4 *multiple reaction monitoring (MRM), n*—application of selected reaction monitoring to multiple product ions from one or more precursor ions.

3.2.5 *per- and polyfluoroalkyl substances (PFAS), n*—synthetic organofluorine chemical compounds with multiple fluorine atoms that includes PFOA, PFOS, GenX, and many other chemicals.

3.2.5.1 *Discussion*—PFAS have a hydrophobic and oleophobic fluorinated "tail" and a hydrophilic "head" making them surfactants. They include the perfluoro sulfonic acids such as the perfluorooctanesulfonic acid (PFOS) and the perfluoro carboxylic acids, such as the perfluorooctanoic acid (PFOA). PFOS and PFOA are persistent organic pollutants. The definition does not include the mass labeled surrogates or internal standards.

3.2.6 *precursor ion, n*—ion that reacts to form product ions or undergoes specified neutral losses.

3.2.7 *product ion, n*—ion formed as the product of a reaction involving a precursor ion.

3.2.8 *single (or selected) reaction monitoring (SRM), n*—data acquired from one or more specific product ions corresponding to m/z selected precursor ions recorded via two or more stages of mass spectrometry.

3.2.9 *tandem mass spectrometer, n*—mass spectrometer designed for mass spectrometry/mass spectrometry.

3.2.10 *triple quadrupole mass spectrometer (triple quad or QQQ), n*—tandem mass spectrometer comprising two transmission quadrupole mass spectrometers in series, with a (non-selecting) RF-only quadrupole (or other multipole) between them to act as a collision cell.

4. Summary of Test Method

4.1 The operating conditions presented in this test method have been validated for use in the determination of PFASs in aqueous samples. Alternative instrument operating conditions may be used provided data quality objectives are met. Follow the manufacturer's instructions. The preparation process, as summarized in 4.2 and described in Section 14 may be automated, but cannot be modified.

4.2 Samples are shipped to the lab at a temperature between 0°C and 6°C and analyzed within 28 days of collection. A sample (5 mL) is collected and processed in the same collection tube in order to limit analyte loss; extra samples must be collected for duplicates/triplicates and matrix spikes. All samples and associated QC samples are spiked with labeled surrogates (QC samples such as laboratory control and matrix spike samples are additionally spiked with target PFASs) and shaken for 2 minutes after adding 5 mL of methanol. The samples are then filtered through a polypropylene filter. Acetic acid (~10 µL) is added to all the samples to adjust to pH ~4 and analyzed by LC/MS/MS. If samples contain more than about 1.0 g/L suspended or settled solids, (for example, sludge, pretreatment, or wastewater influent) adjust to pH ~9 (adding ~20 µL of ammonium hydroxide), shake for 2 minutes, filter, acidify to pH ~4 (~50 µL acetic acid), and then analyze by LC/MS/MS.

NOTE 1—Sludge in this test method is defined as sewage sample containing between 0.1 and 2 % solids based upon a sample by weight.

NOTE 2—Since contact with surfaces may bias data, collect a 5.0-mL sample in a graduated 15-mL polypropylene tube in the field so that the whole sample is processed in the lab. Once this 5.0-mL sample is spiked according to this test method and methanol is added, the sample is filtered into another 15 ml polypropylene tube without analyte loss.

NOTE 3—For accurate volume, the weight of the 15-mL polypropylene tube may be taken before and after sampling. The density of water is assumed to be 1.0 g/mL unless the exact density of the water sample is known, then that conversion should be used.

4.3 Most analytes are identified by comparing the SRM transition and its confirmatory SRM transition correlated to the known standard SRM transition (Table 3) and quantitated utilizing an external calibration. The retention times and ion ratios are shown in Table 4 for each native analyte and isotope. The surrogates and some analytes only have one SRM transition due to a less sensitive or non-existent secondary SRM transition. As an additional quality-control measure, isotopically labeled surrogate (Table 1, Section 13.3) recoveries are

[5] Available from National Technical Information Service (NTIS), U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA, 22161 or at http://www.epa.gov/epawaste/hazard/testmethods/index.htm

TABLE 1 Analyte List with Method Detection Limit and Reporting Range

Analyte Name	Acronym	CAS Number	MDL (ng/L)	Range (ng/L)
Perfluorotetradecanoic acid	PFTreA	376-06-7	8.2	10-400
Perfluorotridecanoic acid	PFTriA	72629-94-8	17.2	10-400
Perfluorododecanoic acid	PFDoA	307-55-1	6.6	10-400
Perfluoroundecanoic acid	PFUnA	2058-94-8	3.9	10-400
Perfluorodecanoic acid	PFDA	335-76-2	3.4	10-400
Perfluorononanoic acid	PFNA	375-95-1	5.2	10-400
Perfluorooctanoic acid	PFOA	335-67-1	2.5	10-400
Perfluoroheptanoic acid	PFHpA	375-85-9	5.9	10-400
Perfluorohexanoic acid	PFHxA	307-24-4	2.1	10-400
Perfluoropentanoic acid	PFPeA	2706-90-3	13.0	50-1000
Perfluorobutanoic acid	PFBA	375-22-4	17.1	50-1000
Perfluorodecanesulfonic acid	PFDS	335-77-3	1.6	10-400
Perfluorononanesulfonic acid	PFNS	68259-12-1	1.2	10-400
Perfluorooctanesulfonic acid	PFOS	1763-23-1	4.4	10-400
Perfluoroheptanesulfonic acid	PFHpS	375-92-8	2.7	10-400
Perfluorohexanesulfonic acid	PFHxS	355-46-4	2.3	10-400
Perfluoropentanesulfonic acid	PFPeS	2706-91-4	2.7	10-400
Perfluorobutanesulfonic acid	PFBS	375-73-5	3.3	10-400
Perfluorooctanesulfonamide	PFOSA	754-91-6	2.2	10-400
8:2 Fluorotelomer sulfonic acid	8:2 FTS	39108-34-4	4.5	10-400
6:2 Fluorotelomer sulfonic acid	6:2 FTS	27619-97-2	2.7	10-400
4:2 Fluorotelomer sulfonic acid	4:2 FTS	757124-72-4	3.2	10-400
N-Ethylperfluorooctanesulfonamidoacetic acid	NEtFOSAA	2991-50-6	2.6	10-400
N-Methylperfluorooctanesulfonamidoacetic acid	NMeFOSAA	2355-31-9	1.3	10-400
Perfluorododecanesulfonic acid	PFDoS	79780-39-5	2.2	10-400
N-Methylperfluorooctanesulfonamide	NMeFOSA	31506-32-8	2.1	10-400
N-Ethylperfluorooctanesulfonamide	NEtFOSA	4151-50-2	1.8	10-400
N-Methylperfluorooctanesulfonamidoethanol	NMeFOSE	24448-09-7	3.1	10-400
N-Ethylperfluorooctanesulfonamidoethanol	NEtFOSE	1691-99-2	2.7	10-400
Hexafluoropropylene oxide dimer acid	HFPO-DA	13252-13-6	3.7	10-400
4,8-dioxa-3H-perfluorononanoic acid	ADONA	919005-14-4	2.1	10-400
9-chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	9Cl-PF3ONS	756426-58-1	2.7	10-400
11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF3OUdS	763051-92-9	2.2	10-400
Pentafluorpropanoic acid	PFPrA	422-64-0	20.3	50-1000
Perfluoro-3,6-dioxaheptanoic acid	NFDHA	151772-58-6	3.7	10-400
Perfluoro(2-ethoxyethane) sulfonic acid	PFEESA	113507-82-7	2.2	10-400
Perfluoro-3-methoxypropanoic acid	PFMPA	377-73-1	2.6	10-400
Perfluoro-4-methoxybutanoic acid	PFMBA	863090-89-5	2.2	10-400
2H,2H,3H,3H-Perfluorohexanoic Acid	3:3 FTCA	356-02-05	3.7	10-400
2H,2H,3H,3H-Perfluorooctanoic Acid	5:3 FTCA	914637-49-3	3.0	10-400
2H,2H,3H,3H-Perfluorodecanoic acid	7:3 FTCA	812-70-4	1.5	10-400
2H-perfluoro-2-octenoic acid	FHUEA	70887-88-6	2.5	10-400
2H-perfluoro-2-decenoic acid	FOUEA	70887-84-2	2.9	10-400
Lithium Bis(trifluoromethane)sulfonimide[A]	HQ-115	90076-65-6	9.0	10-400
Surrogates				
Perfluoro-n-[$^{13}C_4$]butanoic acid	MPFBA	NA	NA	10-400
Perfluor0-n-[$^{13}C_5$]pentanoic acid	M5PFPeA	NA	NA	10-400
Perfluoro-n-[1,2,3,4,6-$^{13}C_5$]hexanoic acid	M5PFHxA	NA	NA	10-400
Perfluoro-n-[1,2,3,4-$^{13}C_4$]heptanoic acid	M4PFHpA	NA	NA	10-400
Perfluoro-n-[$^{13}C_8$]octanoic acid	M8PFOA	NA	NA	10-400
Perfluoro-n-[$^{13}C_9$]nonanoic acid	M9PFNA	NA	NA	10-400
Perfluoro-n-[1,2,3,4,5,6-$^{13}C_6$]decanoic acid	M6PFDA	NA	NA	10-400
Perfluoro-n-[1,2,3,4,5,6,7-$^{13}C_7$]undecanoic acid	M7PFUnA	NA	NA	10-400
Perfluoro-n-[1,2-$^{13}C_2$]dodecanoic acid	MPFDoA	NA	NA	10-400
Perfluoro-n-[1,2-$^{13}C_2$]tetradecanoic acid	M2PFTreA	NA	NA	10-400
Perfluoro-1-[$^{13}C_8$]octanesulfonamide	M8FOSA	NA	NA	10-400
N-methyl-d_3-perfluoro-1-octanesulfonamidoacetic acid	D3-N-MeFOSAA	NA	NA	10-400
N-ethyl-d_5-perfluoro-1-octanesulfonamidoacetic acid	D5-N-EtFOSAA	NA	NA	10-400
N-methyl-d_3-perfluoro-1-octanesulfanamide	d-N-MeFOSA	NA	NA	10-400
N-ethyl-d_5-perfluoro-1-octanesulfanamide	d-N-EtFOSA	NA	NA	10-400
2-(N-methyl-d_3-perfluoro-1-octanesulfonamido)ethan-d4-ol	d7-N-MeFOSE	NA	NA	10-400
2-(N-ethyl-d_5-perfluoro-1-octanesulfonamido)ethan-d4-ol	D9-N-EtFOSE	NA	NA	10-400
2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy-$^{13}C_3$-propanoic acid	MHFPO-DA	NA	NA	10-400
1H,1H,2H,2H-perfluoro-1-[1,2-$^{13}C_2$]hexane sulfonate	M4:2FTS	NA	NA	10-400
1H,1H,2H,2H-perfluoro-1-[1,2-$^{13}C_2$]-octane sulfonate	M6:2FTS	NA	NA	10-400
1H,1H,2H,2H-perfluoro-1-[1,2-$^{13}C_2$]-decane sulfonate	M8:2FTS	NA	NA	10-400
Perfluoro-1-[$^{13}C_8$]octanesulfonate	M8PFOS	NA	NA	10-400
Perfluoro-1-[2,3,4-$^{13}C_3$]butanesulfonate	MPFBS	NA	NA	10-400
Perfluoro-1-[1,2,3-$^{13}C_3$]hexanesulfonate	M3PFHxS	NA	NA	10-400

[A] The Lithium is just the counter ion, report only Bis(trifluoromethane)sulfonimide.

monitored. With external standard calibrations, there is no correction to the data based upon surrogate recoveries. Alternatively, extract an isotopically labelled analog of each analyte (isotope dilution), if available, and correct for recovery. Only exact isotopes of the native analytes may be used for isotope dilution correction. If a structurally different isotope is used to correct a native analyte this is called surrogate correction and either must be clearly stated as performed in the accompanying data report or not allowed. For isotope dilution, the analog and the native compound concentrations (areas) should be within 30% of each other to obtain more accurate results. The final report issued lists the concentration of PFAS, if detected, or as a non-detect at the RL, if not detected, in ng/L and the surrogate recoveries.

NOTE 4—For greater accuracy in the isotope dilution method, add the isotopes at the time sampling or allow the sample and isotope to equilibrate for at least 48 hours prior to addition of methanol.

5. Significance and Use

5.1 PFAS are widely used in various industrial and commercial products; they are persistent, bio-accumulative, and ubiquitous in the environment. PFAS have been reported to exhibit developmental toxicity, hepatotoxicity, immunotoxicity, and hormone disturbance. PFAS have been detected in soils, sludges, surface, and drinking waters. This is a quick, easy, and robust method to quantitatively determine these compounds at trace levels in water matrices.

5.2 This test method has been validated using reagent water and waters from sites that include landfill leachate, metal finisher, POTW Effluent, Hospital, POTW Influent, Bus washing station, Power Plant and Pulp and paper mill effluent for selected PFAS, refer to the Precision and Bias (Section 17).

6. Interferences

6.1 All glassware is washed in hot water (typically >45ºC) with detergent and rinsed in hot water followed by distilled water. The glassware is then dried and heated in an oven (typically at 105ºC) for 15 to 30 minutes. All glassware is subsequently rinsed with methanol or acetonitrile.

6.2 All reagents and solvents should be pesticide residue purity or higher to minimize interference. Avoid the use of PFAS containing caps.

6.3 Matrix interferences may be caused by contaminants in the sample. The extent of matrix interferences varies considerably depending on variations of the sample matrices. Separation of individual components by the LC is vital in minimization of interferences. Shortening of run times simply to speed analysis should be avoided, unless interferences are known to be absent.

6.4 Contaminants have been found in reagents, glassware, tubing, glass disposable pipettes, filters, degassers, and other apparatus and consumables that release PFAS. All these materials and supplies must be routinely demonstrated to be free from interferences by analyzing laboratory reagent blanks under the same conditions as the samples. If found, measures should be taken to remove the contamination or data should be qualified, background subtraction of blank contamination is not allowed. It has become difficult to ensure consumables are PFAS free at the lower concentrations (approximately at less than 30 ng/L) for the entire lot by testing only a very small sub-sample. At a minimum duplicates/triplicates should be taken of each sample to evaluate precision between the set.

6.5 The LC system used should consist, as much as practical, of sample solution or eluent contacting components free of PFAS of interest.

6.6 Polyethylene LC vial caps or any other target analyte free vial caps should be used.

6.7 Polyethylene disposable pipettes or target analyte free pipettes should be used. All disposable pipettes should be checked for release of target analytes of interest.

6.8 Degassers are important to continuous LC operation and most commonly are made of fluorinated polymers. To enable use, an isolator column should be placed after the degasser and prior to the sample injection valve to separate the PFAS in the sample from the PFAS in the LC system.

6.9 *Electro Spray Ionization (ESI)*—ESI should be heated and optimized for recovery of components analyzed by this test method. Using the suggested mobile phase, gradient, and adequate column separation minimizes, or eliminates, quenching and enhancing of signal. This method was validated using ESI, however other modes of ionization may be used provided the detection limits and quality control acceptance criteria of this method are met.

7. Apparatus

7.1 *LC/MS/MS System:*

7.1.1 *Liquid Chromatography System*—A complete LC system is required to analyze samples, this includes a sample injection system, a solvent pumping system capable of mixing solvents, a sample compartment capable of maintaining required temperature and a temperature-controlled column compartment. This test method was developed using a ternary (Table 2) pumping system. A binary LC system may be used by adapting the ternary gradient to a binary system. A LC system that can perform at the flow rates, pressures, controlled temperatures, sample volumes, and requirements of the standard shall be used.

7.1.2 *Analytical Column*—UHPLC CSH Phenyl-Hexyl, 2.1 × 100 mm and 1.7 µm particle size column, or any column that achieves adequate resolution may be used. The retention times and order of elution may change depending on the column used and needs to be monitored.

7.1.3 *Isolator Column*—A reverse phase C18 column is used to separate the target analytes in the LC system and solvents from the target analytes in the analytical sample. Place the column between the solvent mixing chamber and the injector sample loop.

7.2 *Tandem Mass Spectrometer System*—A MS/MS system capable of multiple reaction monitoring (MRM) analysis or any system that is capable of performing at the requirements in this test method.

7.3 *Filtration Device:*

7.3.1 *Hypodermic Syringe*—A luer-lock tip glass syringe capable of holding a syringe driven filter unit.

TABLE 2 Gradient Conditions for a Ternary Pumping System

Time (min)	Flow (mL/min)	95 % Water: 5 % Acetonitrile %	Acetonitrile %	95 % Water: 5 % Acetonitrile, 400 mM Ammonium Acetate %
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

7.3.1.1 A 10-mL Lock Tip Glass Syringe size is recommended in this test method.

7.3.2 *Filter Unit*—Polypropylene syringe-driven filter units (0.2 µm) or equivalent, demonstrated contaminant free below 1/2 MRL.

8. Reagents and Materials

8.1 *Purity of Reagents*—High Performance Liquid Chromatography (HPLC) pesticide residue analysis and spectrophotometry grade chemicals shall be used in all tests. Unless indicated otherwise, it is intended that all reagents shall conform to the Committee on Analytical Reagents of the American Chemical Society.[6] Other reagent grades may be used provided they are first determined to be of sufficiently high purity to permit their use without affecting the accuracy of the measurements.

8.2 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean reagent water conforming to Type 1 of Specification D1193. It shall be demonstrated that this water does not contain contaminants at concentrations sufficient to interfere with the analysis.

8.3 *Gases*—Ultrapure nitrogen and argon.

8.4 *Vials*—Greater than 1.0 mL Amber glass or polypropylene autosampler vials.

8.5 *Polyethylene autosampler vial caps,* or equivalent.

8.6 *Syringe*—10 or 25-mL filter-adaptable glass syringe with luer lock.

8.7 *Polypropylene Tubes*—15 and 50 mL conical with calibration lines.

8.8 *pH paper* (pH range 1–14).

8.9 *Class A Volumetric Glassware.*

8.10 *Pipette tips*—Polypropylene pipette tips free of release agents or low retention coating of various sizes.

8.11 *Polyethylene Disposable Pipettes.*

8.12 *Acetonitrile* (CAS #75-05-8).

8.13 *Methanol* (CAS #67-56-1).

8.14 *Ammonium acetate* (CAS #631-61-8).

8.15 *Acetic acid* (CAS #64-19-7).

8.16 *2-Propanol* (isopropyl alcohol, CAS #67-63-0).

8.17 *Ammonium hydroxide* (CAS #1336-21-6).

8.18 *PFASs Standards*[7]—Refer to Table 1 for the complete analyte list and CAS numbers. These may be purchased from a commercial supplier individually or some as a mixture.

9. Hazards

9.1 *Precaution*—The toxicity or carcinogenicity of chemicals used in this test method has not been precisely defined; each chemical should be treated as a potential health hazard, and exposure to these chemicals should be minimized. Each laboratory is responsible for maintaining awareness of OSHA regulations regarding safe handling of chemicals used in this test method.

9.2 *Warning*—The compound analytes in this test method have been classified as known or suspected human or mammalian carcinogens. Pure standards and stock solutions should be handled in a hood or glovebox.

10. Sampling

10.1 *Sampling and Preservation*—Avoid sample containers and contact with surfaces of fluorinated polymers or PFAS contaminated items. Collect field blanks that are exposed to the same field conditions as samples and analyze according to this test method to assess the potential for field contamination. Collect 5 ± 0.5 ml samples, duplicates/triplicates, matrix spikes and field blanks in graduated 15 ml polypropylene tubes. For greater accuracy, the tubes should be pre-weighed and weighed after sampling in order to achieve an exact weight of the sample. This weight is then used to calculate volume with the assumed density of the water sample as 1.0 g/mL. Conventional sampling practices should be followed with the caution that PFASs containing products may be present in sampling equipment. All sampling equipment and supplies shall be PFAS free to prevent contamination of the samples. EPA Publication SW-846, Guide D3856, and Practices E694 may be used as guides. Ship samples on ice with a trip blank. The temperature of the samples upon receipt at the laboratory

[6] *ACS Reagent Chemicals, Specifications and Procedures for Reagents and Standard-Grade Reference Materials*, American Chemical Society, Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see *Analar Standards for Laboratory Chemicals*, BDH Ltd., Poole, Dorset, U.K., and the *United States Pharmacopeia and National Formulary*, U.S. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.

[7] PFASs standards may be difficult to find, some sources of PFASs standards that have been found suitable for use were from Aldrich Chemical Company, Wellington Laboratories Inc., and Wako Laboratory. Standards from other vendors may be used.

should be less than 6°C. If the receiving temperature is greater than 6°C, the sample temperature is noted in the case narrative accompanying the data. Samples should be stored refrigerated between 0°C and 6°C from the time of collection until analysis. Analyze the sample within 28 days of collection. Holding time may vary depending on the matrix and individual laboratories should determine the holding time in their matrix.[8]

11. Preparation of LC/MS/MS

11.1 *LC Chromatograph Operating Conditions:*

11.1.1 Injections of all standards and samples are made at a 10–30-µL volume. Other injection volumes may be used to optimize conditions. Calibration Standards, reagent blanks, second source and samples shall be in a 50:50 methanol:water solution containing 0.1 % acetic acid. In the case of extreme concentration differences amongst samples, it is wise to analyze a blank after a concentrated sample and before a dilute sample to eliminate carry-over of analytes from sample injection to sample injection. If a flow through needle design is used carry-over should not be a problem. The gradient conditions for LC are shown in Table 2. To ensure chromatographic separation between the targeted analytes and any unknown non-targeted potentially interfering compounds, avoid shortening the analysis time simply to speed the analysis. Refer to Fig. 1 as an example chromatogram of 24 surrogates showing resolution with limited coelution.

11.2 *LC Sample Manager Conditions:*

11.2.1 *Needle Wash Solvent*—60 % acetonitrile/40 % 2-propanol. Eight second wash time before and after injection. Instrument manufacturer's specifications should be followed in order to eliminate sample carry-over.

11.2.2 *Temperatures*—Column, 35°C; Sample compartment, 15°C.

11.2.3 *Seal Wash*—Solvent: 50% water/50 % methanol; Time: 5 minutes.

[8] Guides to help determine holding times can be found at: http://www.epa.gov/esd/cmb/research/bs_033cmb06.pdf (2014) and Practice D4841.

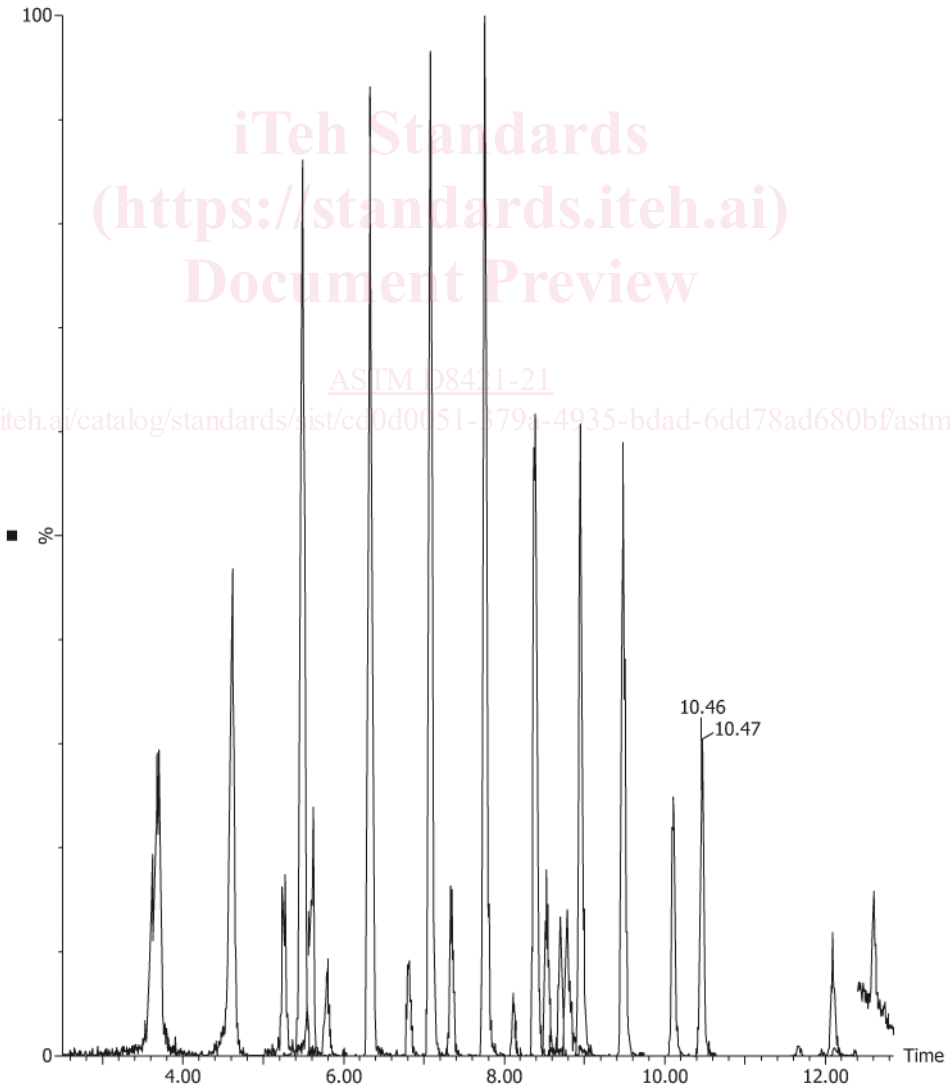

FIG. 1 Example Chromatogram of 24 Surrogates, at the Level 1 Calibration Concentration, Overlayed Showing Resolution with Limited Coelution

11.3 *Mass Spectrometer Parameters:*

11.3.1 To acquire the maximum number of data points per SRM channel while maintaining adequate sensitivity, optimize the tune parameters according to instrument manufacturer instructions. Each peak requires a minimum of 10 scans per peak for adequate quantitation. This test method containing surrogates, which are select isotopically labeled PFAS, and the targeted PFAS may be split into multiple MRM acquisition functions to optimize sensitivity. Retention times, and primary and confirmatory transitions are shown in Table 3. Retention times will vary between columns and gradient used. Each manufacturer may have different terminology to represent various mass spectrometer settings, and different set values depending on the manufacturer and instrument model. Please refer to the manufacturers instructions in optimizing detector settings, including collision energies and cone voltages. Data for this method was collected using electrospray ionization (ESI) operated in negative mode. In recognition of the advancement of LCMSMS instrumentation, other MS operating conditions, including ionization techniques may be used provided the quality control criteria of the method is met.

12. Calibration and Standardization

12.1 The mass spectrometer is calibrated as in accordance with manufacturer's specifications prior to analysis. Prepare all calibration solutions using Class A volumetric glassware (E694).

12.2 *Calibration and Standardization*—Analyze up to nine calibration standards containing the PFAS and surrogates prior to analysis as shown in Table 5. The calibration stock standard solution is prepared from the target and surrogate spike solutions. Stock standard Solution A containing the PFAS and surrogates is prepared at Calibration Level 9 concentration and aliquots of that solution are diluted to prepare Calibration Levels 1 through 8. The following steps will produce standards with the concentration values shown in Table 5. The analyst is responsible for recording initial component weights carefully when working with pure materials and correctly carrying the weights through the dilution calculations. At a minimum, five calibration levels are required when using a linear calibration and six calibration levels are required when using a quadratic calibration curve. An initial nine-points may be used to enable dropping the lower calibration points if the instrument cannot achieve low detection limits on certain PFAS. This will allow at least a five or six-point calibration curve per analyte to be obtained.

12.2.1 Calibration Stock Standard Solution A (Calibration Level 9, Table 5) is prepared from the target and surrogate spike solutions. Transfer 500 µL of the surrogate spike (20 µg/L), 500 µL of PFAS Target Spike I and 500 µL of PFAS Target Spike II (refer to Table 7) to a 50-mL volumetric flask and dilute to 50-mL volume with 50:50 methanol:water containing 0.1 % acetic acid. Ensure that the analytes are solubilized in the Level 9 standard.

12.2.2 Aliquots of Solution A (Calibration Level 9) are then diluted with 50:50 methanol:water containing 0.1 % acetic acid to prepare the desired calibration levels (Table 5) in polypropylene LC vials. For best results, use the calibration standards within 24 hours of preparation. Prepare the end CCV at a mid-level concentration in a separate LC vial. All calibration standards should be used only once because the analyte concentration in the vial may change after the vial cap is pierced. Changing the caps immediately after the injection may alleviate this problem, however, this should be verified in each laboratory. Calibration standards do not need to be filtered.

12.2.3 Incorporate a second source standard, if available. The second source standard should be analyzed near the midpoint of the calibration range to verify that the standards used are within ±30 % of the expected concentration. Currently, a second source from a different vendor may not be readily available for all target analytes. In this case, a second lot number from the same vendor may be used.

12.2.4 Inject each standard and obtain its chromatogram. The instrument software collects the primary and confirmatory SRM transitions of each analyte at the specified retention times. Calibration software conducts the quantitation of the target analytes and surrogates using the primary SRM transition. The ratios of the primary/confirmatory MRM transition area counts will vary depending on the individual tuning conditions. Refer to Table 4 for retention Times and Ion ratios. For confirmation of analyte identity, the primary/confirmatory ratio shall be within 30 % of the individual ratios established during the initial calibration. The average ion ratio is calculated for each batch from the initial calibration levels.

12.2.5 Depending on sensitivity and sample dependent matrix interference, the confirmatory SRM transition may be used as the primary SRM transition for quantitation during analysis.

12.2.6 The calibration software manual or the instrument manufacturer should be consulted to ensure correct software use. The quantitation method is set using the peak areas in ppt (ng/L) units. Concentrations may be calculated using the data system software to generate linear regression or quadratic calibration curves. Forcing the calibration curve through the origin (X = 0, Y = 0) is not recommended.

12.2.7 Either of two procedures may be used to determine calibration function acceptability for linear and non-linear curves. These include refitting the calibration data back to the model. Both % Error and Relative Standard Error (RSE) evaluate the difference between the measured and the true amounts or concentrations used to create the model.

12.2.7.1 Calculation of % Error is shown as Eq 1. Percent error between the calculated and expected amounts should be ≤ 30% for all standards.

$$\%Error = \frac{x_i - x_i'}{x_i} \times 100 \quad (1)$$

where:

x_i' = measured amount of analyte at calibration level i, in mass or concentration units, and

x_i = true amount of analyte at calibration level i, in mass or concentration units.

12.2.7.2 Calculation of Relative Standard Error (RSE – expressed as %) is shown in Eq 2. The RSE acceptance limit criterion for the calibration model is the same as the RSD limit.

TABLE 3 Transitions for Target Analytes and Surrogates

Analyte Name	Acronym	CAS Number	Primary Ion Transition	Confirmation Ion Transition
Perfluorotetradecanoic acid	PFTreA	376-06-7	712.9 → 668.9	712.9 → 168.9
Perfluorotridecanoic acid	PFTriA	72629-94-8	662.9 → 618.9	662.9 → 168.9
Perfluorododecanoic acid	PFDoA	307-55-1	612.9 → 568.9	612.9 → 168.9
Perfluoroundecanoic acid	PFUnA	2058-94-8	562.9 → 519	562.9 → 269
Perfluorodecanoic acid	PFDA	335-76-2	512.9 → 469	512.9 → 218.9
Perfluorononanoic acid	PFNA	375-95-1	462.9 → 419	462.9 → 218.9
Perfluorooctanoic acid	PFOA	335-67-1	412.9 → 369	412.9 → 168.9
Perfluoroheptanoic acid	PFHpA	375-85-9	362.9 → 318.9	362.9 → 168.9
Perfluorohexanoic acid	PFHxA	307-24-4	312.9 → 269	312.9 → 118.9
Perfluoropentanoic acid	PFPeA	2706-90-3	262.9 → 218.9	NA
Perfluorobutanoic acid	PFBA	375-22-4	212.9 →168.9	NA
Perfluorodecanesulfonic acid	PFDS	335-77-3	598.9 → 79.9	598.9 → 98.9
Perfluorononanesulfonic acid	PFNS	68259-12-1	548.9 → 79.9	548.9 → 98.9
Perfluorooctanesulfonic acid	PFOS	1763-23-1	498.9 → 79.9	498.9 → 98.9
Perfluoroheptanesulfonic acid	PFHpS	375-92-8	448.9 → 79.9	448.9 → 98.9
Perfluorohexanesulfonic acid	PFHxS	355-46-4	398.9 → 79.9	398.9 → 98.9
Perfluoropentanesulfonic acid	PFPeS	2706-91-4	348.9 → 79.9	348.9 → 98.9
Perfluorobutanesulfonic acid	PFBS	375-73-5	298.9 → 79.9	298.9 → 98.9
Perfluorooctanesulfonamide	PFOSA	754-91-6	497.9 → 77.9	NA
8:2 Fluorotelomer sulfonic acid	8:2 FTS	39108-34-4	526.9 → 506.9	526.9 → 80.9
6:2 Fluorotelomer sulfonic acid	6:2 FTS	27619-97-2	427 → 407	427 → 80.9
4:2 Fluorotelomer sulfonic acid	4:2 FTS	757124-72-4	326.9 → 306.9	326.9 → 80.9
N-Ethylperfluorooctanesulfonamidoacetic acid	NEtFOSAA	2991-50-6	584 → 419	584 → 482.9
N-Methylperfluorooctanesulfonamidoacetic acid	NMeFOSAA	2355-31-9	569.9 → 419	569.9 → 482.9
Perfluorododecanesulfonic acid	PFDoS	79780-39-5	698.9 → 79.9	698.9 → 98.9
N-Methylperfluorooctanesulfonamide	NMeFOSA	31506-32-8	511.9 → 168.9	511.9 → 218.9
N-Ethylperfluorooctanesulfonamide	NEtFOSA	4151-50-2	525.9 → 168.9	525.9 → 218.9
N-Methylperfluorooctanesulfonamidoethanol	NMeFOSE	24448-09-7	616 → 58.9	NA
N-Ethylperfluorooctanesulfonamidoethanol	NEtFOSE	1691-99-2	630 → 58.9	NA
Hexafluoropropylene oxide dimer acid	HFPO-DA	13252-13-6	285 → 168.9	285 → 184.9
4,8-dioxa-3H-perfluorononanoic acid	ADONA	919005-14-4	376.9 → 251	376.9 → 84.9
9-chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	9Cl-PF3ONS	756426-58-1	530.9 → 350.9	532.9 → 352.9
11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF3OUdS	763051-92-9	630.8 → 450.9	632.8 → 452.9
Pentafluorpropanoic acid	PFPrA	422-64-0	162.9 → 118.9	NA
Perfluoro-3,6-dioxaheptanoic acid	NFDHA	151772-58-6	295 → 200.9	295 → 84.9
Perfluoro(2-ethoxyethane)sulfonic acid	PFEESA	113507-82-7	314.9 → 134.9	314.9→ 82.9
Perfluoro-3-methoxypropanoic acid	PFMPA	377-73-1	228.9 → 84.9	NA
Perfluoro-4-methoxybutanoic acid	PFMBA	863090-89-5	278.9 → 84.9	NA
2H,2H,3H,3H-Perfluorohexanoic Acid	3:3 FTCA	356-02-05	241 → 176.9	241→ 116.9
2H,2H,3H,3H-Perfluorooctanoic Acid	5:3 FTCA	914637-49-3	340.9 → 216.9	340.9 →237
2H,2H,3H,3H-Perfluorodecanoic acid	7:3 FTCA	812-70-4	440.9 → 337	440.9 → 316.9
2H-perfluoro-2-octenoic acid	FHUEA	70887-88-6	356.9 → 292.9	NA
2H-perfluoro-2-decenoic acid	FOUEA	70887-70-4	456.9 → 393	NA
Lithium Bis(trifluoromethane)sulfonimide	HQ-115	90076-65-6	279.9 →146.9	279.9 →210.9
Surrogates				
Perfluoro-n-[$^{13}C_4$]butanoic acid	MPFBA	NA	216.9 → 171.9	NA
Perfluoro-n-[$^{13}C_5$]pentanoic acid	M5PFPeA	NA	267.9 → 222.9	NA
Perfluoro-n-[1,2,3,4,6-$^{13}C_5$]hexanoic acid	M5PFHxA	NA	317.9 → 272.9	NA
Perfluoro-n-[1,2,3,4-$^{13}C_4$]heptanoic acid	M4PFHpA	NA	366.9 → 321.9	NA
Perfluoro-n-[$^{13}C_8$]octanoic acid	M8PFOA	NA	421 → 376	NA
Perfluoro-n-[$^{13}C_9$]nonanoic acid	M9PFNA	NA	471.9 → 426.9	NA
Perfluoro-n-[1,2,3,4,5,6-$^{13}C_6$]decanoic acid	M6PFDA	NA	518.9 → 473.9	NA
Perfluoro-n-[1,2,3,4,5,6,7-^{13}C7]undecanoic acid	M7PFUnA	NA	569.9 → 524.9	NA
Perfluoro-n-[1,2-$^{13}C_2$]dodecanoic acid	MPFDoA	NA	614.9 → 569.9	NA
Perfluoro-n-[1,2-$^{13}C_2$]tetradecanoic acid	M2PFTreA	NA	714.9 → 669.9	NA
Perfluoro-1-[$^{13}C_8$]octanesulfonamide	M8FOSA	NA	505.9 → 77.9	NA
N-methyl-d_3-perfluoro-1-octanesulfonamidoacetic acid	D3-N-MeFOSAA	NA	572.9 → 418.9	NA
N-ethyl-d_5-perfluoro-1-octanesulfonamidoacetic acid	D5-N-EtFOSAA	NA	589 → 418.9	NA
N-methyl-d_3-perfluoro-1-octanesulfanamide	d-N-MeFOSA	NA	514.9 → 168.9	NA
N-ethyl-d_5-perfluoro-1-octanesulfanamide	d-N-EtFOSA	NA	531 →168.9	NA
2-(N-ethyl-d_3-perfluoro-1-octanesulfonamido)ethan-d4-ol	d7-N-MeFOSE	NA	623 → 58.9	NA
2-(N-methyl-d_3-perfluoro-1-octanesulfonamido)ethan-d4-ol	D9-N-EtFOSE	NA	639 → 58.9	NA
2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy-$^{13}C_3$-propanoic acid	MHFPO-DA	NA	287 → 168.9	NA
1H,1H,2H,2H-perfluoro-1-[1,2-$^{13}C_2$]hexane sulfonate	M4:2FTS	NA	328.9 → 308.9 328.9 → 80.9[A]	NA

TABLE 3 *Continued*

Analyte Name	Acronym	CAS Number	Primary Ion Transition	Confirmation Ion Transition
1H,1H,2H,2H-perfluoro-1-[1,2-$^{13}C_2$]-octane sulfonate	M6:2FTS	NA	428.9 → 408.9 428.9 → 80.9[A]	NA
1H,1H,2H,2H-perfluoro-1-[1,2-$^{13}C_2$]-decane sulfonate	M8:2FTS	NA	528.9 → 508.9 528.9 → 80.9[A]	NA
Perfluoro-1-[$^{13}C_8$]octanesulfonate	M8PFOS	NA	506.9 → 79.9	NA
Perfluoro-1-[2,3,4-$^{13}C_3$]butanesulfonate	M3PFBS	NA	301.9 → 79.9	NA
Perfluoro-1-[1,2,3-$^{13}C_3$]hexanesulfonate	M3PFHxS	NA	401.9 → 79.9	NA

[A] If high concentrations of the native FTS interfere with Isotope of the FTSs, this transition should be used. It is not as sensitive, but the interference/high bias is removed.

$$RSE = 100 \times \sqrt{\sum_{i=1}^{n}\left[\frac{x_i' - x_i}{x_i}\right]^2 / \ (n - p)} \qquad (2)$$

where:

x_i = true amount of analyte in calibration level i, in mass or concentration units,

x_i' = measured amount of analyte in calibration level i, in mass or concentration units,

p = number of terms in the fitting equation (average = 1, linear = 2, quadratic = 3, cubic = 4), and

n = number of calibration points.

12.2.8 The retention time window of an unknown shall be within 5 % of the retention time of the analyte in a midpoint calibration standard. If this is not the case, re-analyze the calib ration curve to determine if there was a shift in retention time during the analysis. If the retention time of the known standard is correct, and the retention time of the peak in the sample is still incorrect in the sample, refer to the analyte as an unknown.

12.2.9 Analyze a CCV at the end of each batch of 20 samples, 20 samples does not include QC samples. This interval may be tightened according to the laboratory's QA program or accreditation requirements. This end CCV, in a new never pierced sealed vial, should come from the same stock calibration standard solution that was used to generate the initial calibration curve. The concentration of each analyte and surrogate in the end CCV standard shall be within 30 % of the expected concentration. If the concentration is not within 30 %, corrective action is performed and either all samples in the batch are re-analyzed against a new calibration curve or qualify affected samples. If the analyst inspects the vial containing the end CCV and notices a probable cause for the failure, a new end CCV may be prepared and analyzed. If this new end CCV is within 30 % from the expected concentration for the target analytes and surrogates, the results do not need to be qualified.

13. Quality Control

13.1 Quality control (QC) requirements include the initial demonstration of laboratory capability followed by routine analyses of laboratory reagent blanks, field reagent blanks, and laboratory fortified blanks, and matrix spikes. The laboratory must maintain records to document the quality of the data generated. The criteria in this section were used for, or derived from, the method validation.

13.2 If a laboratory has not performed the test before or if there has been a major change in the measurement system, for example, new analyst, new instrument, etc., an instrument qualification study including method detection limit (MDL), calibration range determination and precision and bias determination shall be performed to demonstrate laboratory capability.

13.2.1 Analyze at least four replicates of a spiked water sample containing the analytes and surrogates at a prepared sample concentration in the range of Calibration Levels 4–7. Calibration Level 6 was used to establish the QC acceptance criteria in this test method. Take each replicate through the complete analytical test method including any sample manipulation and pretreatment steps.

13.2.2 Calculate the mean (average) percent recovery and relative standard deviation (RSD) of the four values and compare to the acceptable ranges of the QC acceptance criteria for the Initial Demonstration of Performance in Table 6.

13.2.3 Repeat until the single operator precision and mean recovery are within the limits in Table 6. If a concentration other than the recommended concentration is used, refer to Practice D5847 for information on applying the F test and t test in evaluating the acceptability of the mean and standard deviation.

13.2.3.1 The QC acceptance criteria for the Initial Demonstration of Performance in Table 6 were generated from the combined single-laboratory data from reagent water as shown in Section 17. Laboratories should generate their own in-house QC acceptance criteria which meet or exceed the criteria in this test method. References on how to generate QC acceptance criteria are Practices D2777, D5847, and E2554.

13.3 *Surrogate Spiking Solution:*

13.3.1 A surrogate spiking solution containing each isotopically labeled PFAS are added to all samples; including method blanks, duplicates, laboratory control samples, matrix spikes, and reporting limit checks. A stock surrogate spiking solution is prepared at 20 µg/L in 95 % acetonitrile: 5 % water. Add 40 µL of this spiking solution into a 5-mL water sample for a concentration of 160 ng/L of the surrogate in the sample. The results obtained for the surrogate recoveries shall fall within the limits of Table 6. If the limits are not met, the affected results shall be qualified with an indication that they do not fall within the performance criteria of the test method.

13.3.1.1 Prepare the surrogate spiking solution mix containing all twenty-four surrogates shown in Table 1 with 95 % acetonitrile: 5 % water. It should be replaced every year if not previously discarded for quality-control failure.

13.4 *Method Blank:*

13.4.1 Prepare at least one method blank for every 20 samples to evaluate contamination during sample preparation and extraction. The concentration of target analytes in the

TABLE 4 Retention Times and Ion Ratios for Target Analytes and Surrogates

Analyte Name	Acronym	Retention Time	Primary/Confirmatory Ion Ratio
Perfluorotetradecanoic acid	PFTreA	10.40	4.7
Perfluorotridecanoic acid	PFTriA	9.94	4.2
Perfluorododecanoic acid	PFDoA	9.43	5.2
Perfluoroundecanoic acid	PFUnA	8.90	5.0
Perfluorodecanoic acid	PFDA	8.33	5.4
Perfluorononanoic acid	PFNA	7.70	4.5
Perfluorooctanoic acid	PFOA	7.03	2.4
Perfluoroheptanoic acid	PFHpA	6.28	3.7
Perfluorohexanoic acid	PFHxA	5.45	20
Perfluoropentanoic acid	PFPeA	4.58	NA
Perfluorobutanoic acid	PFBA	3.67	NA
Perfluorodecanesulfonic acid	PFDS	9.76	1.0
Perfluorononanesulfonic acid	PFNS	9.24	1.0
Perfluorooctanesulfonic acid	PFOS	8.64	1.2
Perfluoroheptanesulfonic acid	PFHpS	8.00	1.0
Perfluorohexanesulfonic acid	PFHxS	7.29	1.1
Perfluoropentanesulfonic acid	PFPeS	6.49	1.2
Perfluorobutanesulfonic acid	PFBS	5.56	1.5
Perfluorooctanesulfonamide	PFOSA	10.08	NA
8:2 Fluorotelomer sulfonic acid	8:2 FTS	8.06	3.1
6:2 Fluorotelomer sulfonic acid	6:2 FTS	6.76	3.1
4:2 Fluorotelomer sulfonic acid	4:2 FTS	5.22	3.3
N-Ethylperfluorooctanesulfonamidoacetic acid	NEtFOSAA	8.73	1.8
N-Methylperfluorooctanesulfonamidoacetic acid	NMeFOSAA	8.47	1.8
Perfluorododecanesulfonic acid	PFDoS	10.74	1.0
N-Methylperfluorooctanesulfonamide	NMeFOSA	12.10	1.6
N-Ethylperfluorooctanesulfonamide	NEtFOSA	12.60	1.6
N-Methylperfluorooctanesulfonamidoethanol	NMeFOSE	11.65	NA
N-Ethylperfluorooctanesulfonamidoethanol	NEtFOSE	12.16	NA
Hexafluoropropylene oxide dimer acid	HFPO-DA	5.75	2.0
4,8-dioxa-3H-perfluorononanoic acid	ADONA	6.61	2.2
9-chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	9Cl-PF3ONS	9.21	3.1
11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF3OUdS	10.29	3.1
Pentafluorpropanoic acid	PFPrA	1.79	NA
Nonafluoro-3,6-dioxaheptanoic acid	NFDHA	5.33	3.1
Perfluoro(2-ethoxyethane)sulfonic acid	PFEESA	5.96	15.5
Perfluoro-3-methoxypropanoic acid	PFMPA	4.05	NA
Perfluoro-4-methoxybutanoic acid	PFMBA	4.84	NA
2H,2H,3H,3H-Perfluorohexanoic Acid	3:3 FTCA	4.27	3.9
2H,2H,3H,3H-Perfluorooctanoic Acid	5:3 FTCA	6.01	1.0
2H,2H,3H,3H-Perfluorodecanoic acid	7:3 FTCA	7.59	1.0
2H-perfluoro-2-octenoic acid	FHUEA	6.00	NA
2H-perfluoro-2-decenoic acid	FOUEA	7.45	NA
Lithium Bis(trifluoromethane)sulfonimide	HQ-115	6.8	6.3
Surrogates			
Perfluoro-n-[$^{13}C_4$]butanoic acid	MPFBA	3.67	NA
Perfluor0-n-[$^{13}C_5$]pentanoic acid	M5PFPeA	4.71	NA
Perfluoro-n-[1,2,3,4,6-$^{13}C_5$]hexanoic acid	M5PFHxA	5.45	NA
Perfluoro-n-[1,2,3,4-$^{13}C_4$]heptanoic acid	M4PFHpA	6.28	NA
Perfluoro-n-[$^{13}C_8$]octanoic acid	M8PFOA	7.03	NA
Perfluoro-n-[$^{13}C_9$]nonanoic acid	M9PFNA	7.70	NA
Perfluoro-n-[1,2,3,4,5,6-$^{13}C_6$]decanoic acid	M6PFDA	8.34	NA
Perfluoro-n-[1,2,3,4,5,6,7-$^{13}C_7$]undecanoic acid	M7PFUnA	8.9	NA
Perfluoro-n-[1,2-$^{13}C_2$]dodecanoic acid	MPFDoA	9.43	NA
Perfluoro-n-[1,2-$^{13}C_2$]tetradecanoic acid	M2PFTreA	10.4	NA
Perfluoro-1-[$^{13}C_8$]octanesulfonamide	M8FOSA	10.09	NA
N-methyl-d_3-perfluoro-1-octanesulfonamidoacetic acid	D3-N-MeFOSAA	8.47	NA
N-ethyl-d_5-perfluoro-1-octanesulfonamidoacetic acid	D5-N-EtFOSAA	8.73	NA
N-methyl-d_3-perfluoro-1-octanesulfanamide	d-N-MeFOSA	12.10	NA
N-ethyl-d_5-perfluoro-1-octanesulfanamide	d-N-EtFOSA	12.60	NA
2-(N-ethyl-d_3-perfluoro-1-octanesulfonamido)ethan-d4-ol	d7-N-MeFOSE	11.65	NA
2-(N-methyl-d_3-perfluoro-1-octanesulfonamido)ethan-d4-ol	D9-N-EtFOSE	12.16	NA
2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy-$^{13}C_3$-propanoic acid	MHFPO-DA	5.75	NA
1H,1H,2H,2H-perfluoro-1-[1,2-$^{13}C_2$]hexane sulfonate	M4:2FTS	5.22	NA
1H,1H,2H,2H-perfluoro-1-[1,2-$^{13}C_2$]-octane sulfonate	M6:2FTS	6.76	NA
1H,1H,2H,2H-perfluoro-1-[1,2-$^{13}C_2$]-decane sulfonate	M8:2FTS	8.06	NA
Perfluoro-1-[$^{13}C_8$]octanesulfonate	M8PFOS	8.64	NA
Perfluoro-1-[2,3,4-$^{13}C_3$]butanesulfonate	M3PFBS	5.56	NA
Perfluoro-1-[1,2,3-$^{13}C_3$]hexanesulfonate	M3PFHxS	7.29	NA

blank(s) shall be less than half the reporting limit or the data qualified and the reporting limit for the affected samples shall be raised. PFAS are common laboratory contamination requiring continual evaluation to ensure that quality data is produced.

TABLE 5 Concentrations of Calibration Standards (ng/L)

Analyte/Surrogate	Cal1	Cal2	Cal3	Cal4	Cal5	Cal6	Cal7	Cal8	Cal9
PFPeA, PFBA, PFPrA	25	50	100	200	300	400	500	750	1000
All Other PFAS and Surrogates (Exclude PFPeA, PFBA and PFPrA)	5	10	20	40	60	80	100	150	200

13.5 *Minimum Reporting Limit Check Sample (MRL):*

13.5.1 For each batch of 20 or fewer samples, or within a 24-hour analysis window, analyze an MRL check sample. The MRL is processed the same as a Laboratory Control Sample except spiked at the reporting limit. The recovery limits for the MRL are 50 to 150 %, if any analytes are outside of these limits with no assignable cause, the data must be flagged with the QC failure.

13.5.1.1 Five mL of ASTM Type I water is added to a 15-mL polypropylene centrifuge tube. Spike with 40 µL of surrogate spiking solution and 25 µL of PFAS MRL Check solution (Table 7) and then take through the sample preparation and analyze.

13.6 *Laboratory Control Sample (LCS):*

13.6.1 Analyze at least one LCS at a mid-level concentration. Calibration Level 6 was used to establish the criteria in Table 5, however any mid-level (Levels 4–7) concentration may be used. The LCS is prepared exactly as a sample and analyzed with each batch of 20 samples or less.

13.6.1.1 Prepare stock matrix spiking solutions — Target Spike I and II in 95 % acetonitrile: 5 % water containing the PFAS at concentrations listed in Table 7.

(1) Spike 40 µL each of Target Spike I and Target Spike II into 5 mL of water to yield a concentration of 800 ng/L (PFPrA, PFBA and PFPeA) and 160 ng/L of remaining 41 PFAS in the sample.

(2) The result obtained for the LCS shall fall within the limits in Table 6. Replace spiking solutions every year if not previously discarded for quality-control failure.

13.6.2 If the result of the LCS is not within the limits in Table 6, sample analysis is halted until corrective action resolving the problem has been performed. Impacted samples in the batch are ether re-analyzed, or the results are flagged with a qualifier.

13.7 *Matrix Spike (MS):*

13.7.1 To check for interferences specific matrices being tested, perform a MS on at least one sample from each batch of 20 or fewer samples by spiking the sample with a known concentration of PFAS and following the analytical method.

13.7.1.1 Prepare stock matrix spiking solutions — Target Spike I and II in 95 % acetonitrile: 5 % water containing the 44 PFAS at concentrations listed in Table 7.

13.7.1.2 Spike 40 µL of these stock solutions into 5 mL of the site water sample to yield a concentration of 800 ng/L (PFPrA, PFBA and PFPeA) and 160 ng/L of remaining 41 PFAS in the sample.

13.7.2 If the spiked concentration plus the background concentration exceeds that of the Level 9 calibration standard, dilute the sample (using 50 % methanol/50 % water with 0.1 % acetic acid) to a level near the midpoint of the calibration curve.

13.7.3 Calculate the percent recovery of the spike (P) using Eq 3:

$$P = 100\,\frac{|A(V_s + V) - BV_s|}{CV} \tag{3}$$

where:

A = concentration found in spiked sample,
B = concentration found in unspiked sample,
C = concentration of analyte in spiking solution,
V_s = volume of sample used,
V = volume of spiking solution added, and
P = percent recovery.

13.7.4 The percent recovery of the spike shall fall within the limits in Table 8. If the percent recovery is not within these limits, a matrix interference may be present.

13.7.5 The matrix spike/matrix spike duplicate (MS/MSD) limits in Table 8 were generated using the data in the Precision and Bias, Section 17.

13.7.5.1 The laboratory should generate its own in-house QC acceptance criteria after the analysis of 15–20 matrix spike samples of representative matrices. References on how to generate QC acceptance criteria are Practices D5847, D2777, and E2554, or Method 8000 in EPA Publication SW-846.

13.8 *Duplicate:*

13.8.1 To check the precision of sample analyses, analyze a sample in duplicate with each batch of 20 or fewer samples. If the sample contains the analyte at a level greater than 5 times the reporting limit of the method, the sample and duplicate may be analyzed unspiked; otherwise, a matrix spike and matrix spike duplicate should be used.

13.8.2 Calculate the relative percent difference (RPD) between the duplicate values (or MS/MSD values) as shown in Eq 4. Compare to the RPD limit in Table 7.

$$RPD = \frac{|MSR - MSDR|}{(MSR + MSDR)\div 2} \times 100 \tag{4}$$

where:

RPD = relative percent difference,
MSR = matrix spike recovery, and
MSDR = matrix spike duplicate recovery.

NOTE 5—If using duplicates to calculate RPD, MSR is the sample concentration and MSDR is the duplicates concentration.

13.8.3 If the result exceeds the precision limit (Table 8, RPD), re-analyze if there is a probable cause, or qualify results with an indication that the matrix duplicate did not fall within the performance criteria of the test method.

14. Procedure

14.1 This test method is based upon a 5-mL sample size per analysis. Analyze within 28 days of collection.