Designation: D7979 – 20

Standard Test Method for Determination of Per- and Polyfluoroalkyl Substances in Water, Sludge, Influent, Effluent, and Wastewater by Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS)[1]

This standard is issued under the fixed designation D7979; 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 procedure covers the determination of selected per- and polyfluoroalkyl substances (PFASs) in a water matrix using liquid chromatography (LC) and detection with tandem mass spectrometry (MS/MS). These analytes are qualitatively and quantitatively determined by this test method. This test method adheres to a technique known as selected reaction monitoring (SRM) or sometimes referred to as multiple reaction monitoring (MRM). This is not a drinking water method; performance of this test method has not been evaluated on drinking water matrices.

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 was 10 ng/L for most of the target analytes at the time of development.

1.2.1 The reporting limit in this test method is the minimum value below which data are documented as non-detects. The reporting limit may be lowered providing your lab meets the minimum performance requirements of this test method at the lower concentrations, this test method is performance based and modifications are allowed to improve performance. Analyte detections between the method detection limit and the reporting limit are estimated concentrations and are not reported following this test method. In most cases, the reporting limit is the concentration of the Level 1 calibration standard as shown in Table 4 for the PFASs after taking into account the 50 % dilution with methanol. It is above the Level 1 calibration concentration for FHEA and FOEA, these compounds can be identified at the Level 1 concentration but the standard deviation among replicates at this lower spike level resulted in a higher reporting limit.

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
D3694 Practices for Preparation of Sample Containers and for Preservation of Organic Constituents
D4841 Practice for Estimation of Holding Time for Water Samples Containing Organic and Inorganic Constituents
D5847 Practice for Writing Quality Control Specifications for Standard Test Methods for Water Analysis
E2554 Practice for Estimating and Monitoring the Uncertainty of Test Results of a Test Method Using Control Chart Techniques

[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. Five-mL sample of water was utilized. A detailed process determining the MDL is explained in the reference and is beyond the scope of this test method to be explained here.

[3] Reporting range concentration is calculated from Table 4 concentrations assuming a 30-µL injection of the Level 1 calibration standard for PFASs, and the highest level calibration standard with a 10-mL final extract volume of a 5-mL water sample. Volume variations 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.

TABLE 1 Method Detection Limit and Reporting Range

Analyte[A]	MDL (ng/L)	Reporting Ranges (ng/L)
PFTreA[B]	1.2	10 – 400
PFTriA[B]	0.7	10 – 400
PFDoA[B]	1.2	10 – 400
PFUnA[B]	1.2	10 – 400
PFDA[B]	1.4	10 – 400
PFOS[B]	2.2	10 – 400
PFNA[B]	1.1	10 – 400
PFecHS[B]	1.9	10 – 400
PFOA[B]	1.7	10 – 400
PFHxS[B]	1.2	10 – 400
PFHpA[B]	1.0	10 – 400
PFHxA[B]	2.0	10 – 400
PFBS[B]	0.8	10 – 400
PFPeA[B]	4.6	50 – 2000
PFBA[B]	4.6	50 – 2000
FHEA	92.9	300 – 8000
FOEA	106.8	300 – 8000
FDEA	47.2	200 – 8000
FOUEA	2.3	10 – 400
FHpPA	3.3	10 – 400
FHUEA	1.5	10 – 400

[A] Acronyms are defined in 3.3.
[B] New MDL study was reported in August 2016, which resulted in a reporting limit and range update.

2.2 *Other Standards:*[5]
EPA Publication SW-846 Test Methods for Evaluating Solid Waste, Physical/Chemical Methods
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 *per- and polyfluoroalkyl substances, n—in this test method*, 11 perfluoroalkyl carboxylic acids, 3 perfluoroalkylsulfonates, Decafluoro-4-(pentafluoroethyl)cyclohexanesulfonate and 6 fluorotelomer acids listed in Table 1 collectively (not including any mass labeled surrogates).

3.2.2 *reporting limit, n*—the minimum concentration below which data are documented as non-detects.

3.3 *Acronyms:*

3.3.1 *CCC, n*—Continuing Calibration Check

3.3.2 *FTAs and FTUAs, n*—Fluorotelomer and Unsaturated Fluorotelomer Acids

3.3.2.1 *FDEA, n*—2-perfluorodecyl ethanoic acid

3.3.2.2 *FHEA, n*—2-perfluorohexyl ethanoic acid

3.3.2.3 *FHpPA, n*—3-perfluoroheptyl propanoic acid

3.3.2.4 *FHUEA, n*—2H-perfluoro-2-octenoic acid

3.3.2.5 *FOEA, n*—2-perfluorooctyl ethanoic acid

3.3.2.6 *FOUEA, n*—2H-perfluoro-2-decenoic acid

3.3.3 *IC, n*—Initial Calibration

3.3.4 *LC, n*—Liquid Chromatography

3.3.5 *LCS/LCSD, n*—Laboratory Control Sample/Laboratory Control Sample Duplicate

3.3.6 *MDL, n*—Method Detection Limit

3.3.7 *MeOH, n*—Methanol

3.3.8 *mM, n*—millimolar, 1×10^{-3} moles/L

3.3.9 *MPFAC, n*—Isotopically labeled Perfluoroalkylcarboxylates

3.3.9.1 *MPFBA, n*—$^{13}C_4$-Perfluorobutanoate

3.3.9.2 *MPFDA, n*—$^{13}C_2$-Perfluorodecanoate

3.3.9.3 *MPFDoA, n*—$^{13}C_2$-Perfluorododecanoate

3.3.9.4 *MPFHxA, n*—$^{13}C_2$-Perfluorohexanoate

3.3.9.5 *MPFNA, n*—$^{13}C_5$-Perfluorononanoate

3.3.9.6 *MPFOA, n*—$^{13}C_4$-Perfluorooctanoate

3.3.9.7 *MPFUnA, n*—$^{13}C_2$-Perfluoroundecanoate

3.3.10 *MPFAlS, n*—Isotopically labeled Perfluoroalkylsulfonates

3.3.10.1 *MPFHxS, n*—$^{18}O_2$-Perfluorohexylsulfonate

3.3.10.2 *MPFOS, n*—$^{13}C_4$-Perfluorooctylsulfonate

3.3.11 *MRM, n*—Multiple Reaction Monitoring

3.3.12 *MS/MSD, n*—Matrix Spike/Matrix Spike Duplicate

3.3.13 *NA, adj*—Not Available

3.3.14 *ND, n*—non-detect

3.3.15 *P&A, n*—Precision and Accuracy

3.3.16 *PFAC, n*—Perfluoroalkyl Carboxylic Acid

3.3.16.1 *PFBA, n*—Perfluorobutanoate

3.3.16.2 *PFDA, n*—Perfluorodecanoate

3.3.16.3 *PFDoA, n*—Perfluorododecanoate

3.3.16.4 *PFHpA, n*—Perfluoroheptanoate

3.3.16.5 *PFHxA, n*—Perfluorohexanoate

3.3.16.6 *PFNA, n*—Perfluorononanoate

3.3.16.7 *PFOA, n*—Perfluorooctanoate

3.3.16.8 *PFPeA, n*—Perfluoropentanoate

3.3.16.9 *PFTreA, n*—Perfluorotetradecanoate

3.3.16.10 *PFTriA, n*—Perfluorotridecanoate

3.3.16.11 *PFUnA, n*—Perfluoroundecanoate

3.3.17 *PFAlS, n*—Perfluoroalkylsulfonate

3.3.17.1 *PFBS, n*—Perfluorobutylsulfonate

3.3.17.2 *PFecHS, n*—Decafluoro-4-(pentafluoroethyl) cyclohexanesulfonate

3.3.17.3 *PFHxS, n*—Perfluorohexylsulfonate

3.3.17.4 *PFOS, n*—Perfluorooctylsulfonate

3.3.18 *PFASs, n*—Per- and Polyfluoroalkyl Substances

3.3.19 *ppt, n*—parts per trillion, ng/L

3.3.20 *QA, adj*—Quality-Assurance

3.3.21 *QC, adj*—Quality-Control

[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

3.3.22 *RL, n*—Reporting Limit

3.3.23 *RLCS, n*—Reporting Limit Check Sample

3.3.24 *RSD, n*—Relative Standard Deviation

3.3.25 *RT, n*—Retention Time

3.3.26 *SRM, n*—Selected Reaction Monitoring

3.3.27 *SS, n*—Surrogate Standard

3.3.28 *TC, n*—Target Compound

4. Summary of Test Method

4.1 The operating conditions presented in this test method have been successfully used in the determination of PFASs in water; however, this test method is intended to be performance based and alternative operating conditions can be used to perform this test method provided data quality objectives are attained.

4.2 For PFASs analysis, 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 in a polypropylene tube in the field and that total sample is processed in order to limit target analyte loss due to sample manipulation and losses to surfaces, spiked with surrogates (all samples) and target PFASs (laboratory control and matrix spike samples) and hand shaken for 2 minutes after adding 5 mL of methanol. The samples are then filtered through a polypropylene filter unit. Acetic acid (~10 µL) is added to all the samples to adjust to pH ~3 and analyzed by LC/MS/MS. For 5-mL sludge samples; 5 mL methanol is added, adjusted to pH ~9 (adding ~20 µL of ammonium hydroxide), hand shaken, filtered, acidified to pH ~3 (~50 µL acetic acid), and then analyzed 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 surface binding of target compounds may bias data, it is best to collect a 5.0-mL sample in a graduated 15-mL polypropylene BD Falcon tube in the field so that the whole sample is processed in the lab (NO ALIQUOTING). Once this 5.0-mL sample is spiked according to this test method and methanol is added, it is then thoroughly shaken and transferred to a new 15-mL polypropylene tube during filtration. In order to have accurate volumes, the weight of the 15-mL polypropylene BD Falcon tube may be taken before and after sampling in order to obtain an exact volume. 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 of the PFASs are identified by comparing the SRM transition and its confirmatory SRM transition if correlated to the known standard SRM transition (Table 3) and quantitated utilizing an external calibration. The surrogates and some PFASs (PFPeA, PFBA, FOUEA, and FHUEA) only utilize one SRM transition due to a less sensitive or non-existent secondary SRM transition. As an additional quality-control measure, isotopically labeled PFASs surrogates (listed in 12.4) recoveries are monitored. There is no correction to the data based upon surrogate recoveries. The final report issued for each sample lists the concentration of PFASs, if detected, or as a non-detect at the RL, if not detected, in ng/L and the surrogate recoveries.

5. Significance and Use

5.1 PFASs are widely used in various industrial and commercial products; they are persistent, bio-accumulative, and ubiquitous in the environment. PFASs have been reported to exhibit developmental toxicity, hepatotoxicity, immunotoxicity, and hormone disturbance. A draft Toxicological Profile for Perfluoroalkyls from the U.S. Department of Health and Human Services is available.[6] PFASs have been detected in soils, sludges, surface, and drinking waters. Hence, there is a need for quick, easy, and robust method to determine these compounds at trace levels in water matrices for understanding of the sources and pathways of exposure.

5.2 This test method has been investigated for use with reagent, surface, sludge and wastewaters for selected PFASs. This test method has not been evaluated on drinking water matrices.

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 problems. The use of PFASs containing caps shall be avoided.

6.3 Matrix interferences may be caused by contaminants in the sample. The extent of matrix interferences can vary considerably depending on variations of the sample matrices.

6.4 Contaminants have been found in reagents, glassware, tubing, glass disposable pipettes, filters, degassers, and other apparatus that release PFASs. All of these materials and supplies are 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.

6.5 The LC system used should consist, as much as practical, of sample solution or eluent contacting components free of PFASs 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 PFASs in the sample from the PFASs in the LC system.

[6] A Draft Toxicological Profile for Perfluoroalkyls can be found at: http://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=1117&tid=237 (2014).

7. Apparatus

7.1 *LC/MS/MS System:*

7.1.1 *Liquid Chromatography System*[7]—A complete LC system is required in order to analyze samples, this should include 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 used a ternary pumping system. At a minimum, a binary pumping system may be used but the LC conditions in Table 2 must be adjusted to account for a binary system. A LC system that is capable of performing at the flows, pressures, controlled temperatures, sample volumes, and requirements of the standard shall be used.

7.1.2 *Analytical Column*[8]—A reverse phase Charged Surface Hybrid Phenyl-Hexyl particle column was used to develop this test method. 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*[9]—A reverse phase C18 column was used in this test method to separate the target analytes in the LC system and solvents from the target analytes in the analytical sample. This column was placed between the solvent mixing chamber and the injector sample loop.

7.2 *Tandem Mass Spectrometer System*[10]—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 shall be used.

7.3 *Filtration Device:*

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

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

7.3.3 *Filter Unit*[11]—Polypropylene filter units were used to filter the samples.

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.[12] 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*—2-mL amber glass autosampler vials or equivalent.

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.

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:*[13]

8.18.1 *Perfluorobutylsulfonate* (PFBS, CAS #29420-49-3).

8.18.2 *Perfluorohexylsulfonate* (PFHxS, CAS #3871-99-6).

8.18.3 *Perfluorooctylsulfonate* (PFOS, CAS #1763-23-1).

8.18.4 *Perfluorobutanoate* (PFBA, CAS #375-22-4).

[7] A Waters Acquity UPLC H-Class System, or equivalent, has been found suitable for use.

[8] A Waters Acquity UPLC CSH Phenyl-Hexyl, 2.1 × 100 mm and 1.7 µm particle size column, or equivalent, has been found suitable for use. It was used to develop this test method and generate the precision and bias data presented in Section 16.

[9] A Waters Acquity UPLC BEH C18, 2.1 × 50 mm and 1.7 µm particle size column, or equivalent, has been found suitable for use. Note: If back pressure is high, a larger particle size may be used (3–3.5 µm).

[10] A Waters Xevo TQ-S triple quadrupole mass spectrometer, or equivalent, has been found suitable for use.

TABLE 2 Gradient Conditions for Liquid Chromatography

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

[11] An Acrodisc GxF/0.2 µm GHP membrane syringe driven filter unit, or equivalent, has been found suitable for use.

[12] *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.

[13] 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.

TABLE 3 Retention Times, SRM Ions, and Analyte-Specific Mass Spectrometer Parameters

Chemical	Primary/ Confirmatory	Retention Times (min)	Cone (V)	Collision (eV)	MRM Transition	Primary/ Confirmatory SRM Area Ratio
PFTreA	Primary	10.63	20	13	712.9→668.9	7.4
	Confirmatory		20	30	712.9→169	
PFTriA	Primary	10.17	25	12	662.9→618.9	7.4
	Confirmatory		25	28	662.9→169	
PFDoA	Primary	9.61	10	12	612.9→568.9	8.2
	Confirmatory		10	25	612.9→169	
PFUnA	Primary	9.05	15	10	562.9→519	7.2
	Confirmatory		15	18	562.9→269	
PFDA	Primary	8.45	20	10	512.9→468.9	6.5
	Confirmatory		20	16	512.9→219	
PFOS	Primary	8.78	10	42	498.9→80.1	1.3
	Confirmatory		10	40	498.9→99.1	
PFNA	Primary	7.78	20	10	462.9→418.9	4.9
	Confirmatory		20	16	462.9→219	
PFecHS	Primary	8.1	10	25	460.9→381	2.2
	Confirmatory		10	25	460.9→99.1	
PFOA	Primary	7.11	20	10	412.9→369	3.6
	Confirmatory		20	16	412.9→169	
PFHxS	Primary	7.39	15	32	398.9→80.1	1
	Confirmatory		15	32	398.9→99.1	
PFHpA	Primary	6.35	15	10	362.9→319	4.1
	Confirmatory		15	15	362.9→169	
PFHxA	Primary	5.54	15	8	312.9→269	24.1
	Confirmatory		15	18	312.9→119.1	
PFBS	Primary	5.66	10	30	298.9→80.1	1.6
	Confirmatory		10	25	298.9→99.1	
PFPeA	Primary	4.68	10	8	263→219	NA
PFBA	Primary	3.67	10	8	212.9→169	NA
FHEA	Primary	6.14	15	20	376.9→293	3.6
	Confirmatory		15	6	376.9→313	
FOEA	Primary	7.54	15	18	476.9→393	4.3
	Confirmatory		15	12	476.9→413	
FDEA	Primary	8.83	15	8	576.8→493	3.2
	Confirmatory		15	15	576.8→513	
FOUEA	Primary	7.54	20	12	456.9→392.9	NA
FHpPA	Primary	7.54	15	12	440.9→337	1.1
	Confirmatory		15	20	440.9→317	
FHUEA	Primary	6.08	10	12	357→293	NA
MPFBA	Primary	3.67	10	7	217→172.1	NA
MPFHxA	Primary	5.54	15	8	315→270	NA
MPFHxS	Primary	7.39	15	34	402.9→84.1	NA
MPFOA	Primary	7.11	15	10	417→372	NA
MPFNA	Primary	7.81	15	9	467.9→423	NA
MPFOS	Primary	8.78	15	40	502.9→80.1	NA
MPFDA	Primary	8.45	15	10	514.9→470	NA
MPFUnA	Primary	9.05	15	10	564.9→519.9	NA
MPFDoA	Primary	9.61	15	12	614.9→569.9	NA

TABLE 4 Concentrations of Calibration Standards (ng/L)

Analyte/Surrogate	LV1	LV2	LV3	LV4	LV5	LV6	LV7	LV8	LV9
PFPeA, PFBA	25	50	100	200	300	400	500	750	1000
PFTreA, PFTriA, PFDoA, PFUnA, PFDA, PFOS, PFNA, PFHxA, PFHpA, PFBS, PFecHS, PFOA, PFHxS, FOUEA, FHUEA, FHpPA, MPFBS, MPFHxA, MPFUnA, MPFOA, MPFDA, MPFOS, MPFNA, MPFHxS, MPFBA	5	10	20	40	60	80	100	150	200
FHEA, FOEA, FDEA	100	200	400	800	1200	1600	2000	3000	4000

8.18.5 *Perfluoropentanoate* (PFPeA, CAS #2706-90-3).
8.18.6 *Perfluorohexanoate* (PFHxA, CAS #307-24-4).
8.18.7 *Perfluoroheptanoate* (PFHpA, CAS #375-85-9).
8.18.8 *Perfluorooctanoate* (PFOA, CAS #335-67-1).
8.18.9 *Perfluorononanoate* (PFNA, CAS #375-95-1).
8.18.10 *Perfluorodecanoate* (PFDA, CAS #335-76-2).
8.18.11 *Perfluoroundecanoate* (PFUnA, CAS #2058-94-8).
8.18.12 *Perfluorododecanoate* (PFDoA, CAS #307-55-1).
8.18.13 *Perfluorotridecanoate* (PFTriA, CAS #72629-94-8).

8.18.14 *Perfluorotetradecanoate* (PFTreA, CAS #376-06-7).

8.18.15 *Decafluoro-4-(pentafluoroethyl)cyclohexanesulfonate* (PFecHS, CAS #67584-42-3).

8.18.16 *3-perfluoropheptyl propanoic acid* (FHpPA, CAS #812-70-4).

8.18.17 *2H-perfluoro-2-decenoic acid* (FOUEA, CAS #70887-84-2).

8.18.18 *2-perfluorodecyl ethanoic acid* (FDEA, CAS # not available).

8.18.19 *2-perfluorooctyl ethanoic acid* (FOEA, CAS #27854-31-5).

8.18.20 *2H-perfluoro-2-octenoic acid* (FHUEA, CAS # not available).

8.18.21 *2-perfluorohexyl ethanoic acid* (FHEA, CAS #53826-12-3).

8.19 *PFAS Surrogates:*[14]

8.19.1 $^{18}O_2$-*Perfluorohexylsulfonate* (MPFHxS).

8.19.2 $^{13}C_4$-*Perfluorooctylsulfonate* (MPFOS).

8.19.3 $^{13}C_4$-*Perfluorobutanoate* (MPFBA).

8.19.4 $^{13}C_2$-*Perfluorohexanoate* (MPFHxA).

8.19.5 $^{13}C_4$-*Perfluorooctanoate* (MPFOA).

8.19.6 $^{13}C_5$-*Perfluorononanoate* (MPFNA).

8.19.7 $^{13}C_2$-*Perfluorodecanoate* (MPFDA).

8.19.8 $^{13}C_2$-*Perfluoroundecanoate* (MPFUnA).

8.19.9 $^{13}C_2$-*Perfluorododecanoate* (MPFDoA).

9. Hazards

9.1 Normal laboratory safety applies to this test method. Analysts should wear safety glasses, gloves, and lab coats when working in the lab. Analysts should review the Safety Data Sheets (SDS) for all reagents used in this test method.

10. Sampling

10.1 *Sampling and Preservation*—Grab samples are collected in polypropylene containers. Sample containers and contact surfaces with PTFE shall be avoided. As part of the overall quality-assurance program for this test method, field blanks exposed to the same field conditions as samples are collected and analyzed according to this test method to assess the potential for field contamination. Surface binding may bias data. This test method is based on a 5-mL sample size per analysis. If different sample sizes are used, spiking solution amounts may need to be modified. 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 PFASs free in order to prevent contamination of the samples. EPA Publication SW-846, Guide D3856, and Practices D3694 may be used as guides. Samples shall be shipped on ice with a trip blank. Once received the sample temperature is taken and 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. No in-depth holding time study has been done on the different water matrices tested in this test method. A holding time study was done on sewage treatment plant influent over 31 days and showed all concentrations over the time period to be within the performance of the test method. This study used the complete sample, NO ALIQUOTING. Another study, where aliquots of sample were taken, resulted in large losses for many of the target analytes. Holding time may vary depending on the matrix and individual laboratories should determine the holding time in their matrix.[15]

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 30-µL volume. Other injection volumes may be used to optimize conditions. Standards 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 carryover of analytes from sample injection to sample injection. The gradient conditions for LC are shown in Table 2.

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: 60 % acetonitrile/40 % 2-propanol; Time: 5 minutes.

11.3 *Mass Spectrometer Parameters:*

11.3.1 To acquire the maximum number of data points per SRM channel while maintaining adequate sensitivity, the tune parameters may be optimized according to your instrument. Each peak requires at least 10 scans per peak for adequate quantitation. This test method contains nine surrogates, which are select isotopically labeled PFASs, and 21 PFASs which were split up into eighteen MRM acquisition functions to optimize sensitivity. Variable parameters regarding retention times, SRM transitions, and cone and collision energies are shown in Table 3. Mass spectrometer parameters used in the development of this test method are listed below:

The instrument is set in the Electrospray negative source setting.
Capillary Voltage: 0.75 kV
Cone: Variable depending on analyte
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
Low Mass Resolution 1: 2.6
High Mass Resolution 1: 14
Ion Energy 1: 1
Entrance Energy: 1
Collision Energy: Variable depending on analyte

[14] PFAS surrogates from Wellington Laboratories Inc. or equivalent, have been found suitable for use.

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

Exit Energy: 1
Low Mass Resolution 2: 2.5
High Mass Resolution 2: 14
Ion Energy 2: 3
Gain: 1.0
Multiplier: 511.1
Inter-Scan Delay: 0.004 seconds

12. Calibration and Standardization

12.1 The mass spectrometer shall be calibrated as in accordance with manufacturer's specifications before analysis. Analytical values satisfying test method criteria have been achieved using the following procedures. Prepare all solutions in the lab using Class A volumetric glassware.

12.2 *Calibration and Standardization*—To calibrate the instrument, analyze nine calibration standards containing the PFASs and surrogates prior to analysis as shown in Table 4. Calibration stock standard solution is prepared from the target and surrogate spike solutions directly to ensure consistency. Stock standard Solution A containing the PFASs and surrogates is prepared at Level 9 concentration and aliquots of that solution are diluted to prepare Levels 1 through 8. The following steps will produce standards with the concentration values shown in Table 4. 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 curve and six calibration levels are required when using a quadratic calibration curve. An initial nine-point curve may be used to allow for the dropping of the lower calibration points if the individual laboratory's instrument can't achieve low detection limits on certain PFASs. This should allow for at least a five or six-point calibration curve to be obtained. No problems were encountered while using the nine-point calibration curve in developing this test method.

12.2.1 Calibration Stock Standard Solution A (Level 9, Table 4) is prepared from the target and surrogate spike solutions directly to ensure consistency. 500 µL of the surrogate spike (20 µg/L), 500 µL of PFASs Target Spike I and 500 µL of PFASs Target Spike II (refer to Table 6) is added to a 50-mL volumetric flask and diluted to 50-mL volume with 50:50 methanol:water containing 0.1 % acetic acid. The preparation of the Level 9 standard can be accomplished using appropriate volumes and concentrations of stock solutions as in accordance with a particular laboratory's standard procedure. It is critical to ensure that the analytes are solubilized in the Level 9 standard.

12.2.2 Aliquots of Solution A are then diluted with 50:50 methanol:water containing 0.1 % acetic acid to prepare the desired calibration levels in 2-mL amber glass LC vials. The calibration vials shall be used within 24 hours to ensure optimum results. The end calibration check shall be prepared in a separate LC vial near the mid-level. All calibration standards

TABLE 5 QC Acceptance Criteria

NOTE 1—Table 5 data is preliminary until a multi-lab validation study is completed.

Analyte/Surrogate	Spike Conc. ng/L	Initial Demonstration of Performance			Laboratory Control Sample	
		Recovery (%)		Precision	Recovery (%)	
		Lower Limit	Upper Limit	Maximum % RSD	Lower Control Limit (LCL) %	Upper Control Limit (UCL) %
PFTreA	160	70	130	30	70	130
PFTriA	160	70	130	30	70	130
PFDoA	160	70	130	30	70	130
PFUnA	160	70	130	30	70	130
PFDA	160	70	130	30	70	130
PFOS	160	70	130	30	70	130
PFNA	160	70	130	30	70	130
PFecHS	160	70	130	30	70	130
PFOA	160	70	130	30	70	130
PFHxS	160	70	130	30	70	130
PFHpA	160	50	130	30	50	130
PFHxA	160	50	130	30	50	130
PFBS	160	70	130	30	70	130
PFPeA	800	70	130	30	70	130
PFBA	800	50	130	30	50	130
FHEA	3200	70	130	30	70	130
FOEA	3200	70	130	30	70	130
FDEA	3200	70	130	30	70	130
FOUEA	160	70	130	30	70	130
FHpPA	160	70	130	30	70	130
FHUEA	160	70	130	30	70	130
MPFBA	160	70	130	30	70	130
MPFHxA	160	70	130	30	70	130
MPFHxS	160	70	130	30	70	130
MPFOA	160	70	130	30	70	130
MPFNA	160	70	130	30	70	130
MPFOS	160	70	130	30	70	130
MPFDA	160	70	130	30	70	130
MPFUnA	160	70	130	30	70	130
MPFDoA	160	70	130	30	70	130

TABLE 6 PFASs Target Spike Solutions (PPB)

Analyte	Concentration of Analyte in PFASs Target Spike Solutions		
	PFASs High Target Spike Solutions		PFASs Reporting Limit Spike Solution
	Target Spike I	Target Spike II	
PFTreA, PFTriA, PFDoA, PFUnA, PFDA, PFOS, PFNA, PFHxA, PFHpA, PFBS, PFecHS, PFOA, PFHxS	20 µg/L	–	2 µg/L
PFBA, PFPeA	100 µg/L	–	10 µg/L
FOUEA, FHUEA, FHpPA	–	20 µg/L	2 µg/L
FHEA, FOEA, FDEA	–	400 µg/L	40 µg/L

should only be used once. The analyte concentration in the vial may change after the vial cap is pierced because the vial caps do not reseal after puncture. Changing the caps immediately after the injection should alleviate this problem. Calibration standards are not filtered.

12.2.3 A second source verification standard should be incorporated into this test method at the discretion of the laboratory or project requirements. A second source standard should be analyzed near the midpoint of the calibration range to determine if the standards used are within ±30 % of the second source concentration. If they are not within ±30 %, the data shall be qualified stating in the narrative that the two different sources of standards did not match the acceptance criteria. Currently, a second source from a different vendor may not be readily available for all twenty-four target analytes. In this case, a second lot number from the same vendor may be used. If a second source for any target analyte is not used it should be clearly stated in a narrative accompanying the data package so that the end user of the data is aware that a second source check standard was not used. At a minimum, a second source for PFOA and PFOS is strongly suggested when using this test method.

12.2.4 Inject each standard and obtain its chromatogram. An external calibration technique is used to monitor the primary and confirmatory SRM transitions of each analyte. Calibration software is utilized to conduct the quantitation of the target analytes and surrogates using the primary SRM transition. The ratios of the primary/confirmatory SRM transition area counts are given in Table 3 and will vary depending on the individual tuning conditions. The primary/confirmatory SRM transition area ratio shall be within 35 % of the individual labs' accepted primary/confirmatory SRM transition area ratio. The primary SRM transition of each analyte is used for quantitation and the confirmatory SRM transition for confirmation. This gives added confirmation by isolating the parent ion, forming two product ions by means of fragmentation, and relating it to the retention time in the calibration standard.

NOTE 3—Isotope dilution may be used instead of external standard calibration for the native analytes that have a labeled isotope only. Acceptance criteria must still be met. If a dilution is required, the isotope correction may not be applicable.

12.2.5 Depending on sensitivity and matrix interference issues dependent on sample type, the confirmatory SRM transition can be used as the primary SRM transition for quantitation during analysis. This shall be explained in a narrative accompanying the generated data. A new primary/confirmatory ion ratio will then be determined if switching the SRM transitions used to quantitate and confirm. The primary/confirmatory SRM transition area ratio shall be required to be within 35 % of the individual labs' new primary/confirmatory SRM transition area ratio.

12.2.6 The calibration software manual should be consulted to use the software correctly. The quantitation method is set as an external calibration using the peak areas in ppt 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 Linear calibration may be used if the coefficient of determination, r^2, is ≥0.98 for the analyte. The point of origin is excluded and a fit weighting of 1/X is used in order to give more emphasis to the lower concentrations. If one of the calibration standards other than the high or low point causes the r^2 of the curve to be <0.98, this point shall be re-injected or a new calibration curve shall be regenerated. Each calibration point used to generate the curve shall have a calculated percent deviation less than 30 % from the generated curve. If the low or high point(s), or both, are excluded, minimally a five-point curve is acceptable but the reporting range shall be modified to reflect this change.

12.2.8 Quadratic calibration may be used if the coefficient of determination, r^2, is ≥0.99 for the analyte. The point of origin is excluded, and a fit weighting of 1/X is used in order to give more emphasis to the lower concentrations. If one of the calibration standards causes the curve to be <0.99, this point shall be re-injected or a new calibration curve shall be regenerated. If the low or high point(s), or both, are excluded, minimally a six-point curve is acceptable but the reporting range shall be modified to reflect this change. Each calibration point used to generate the curve shall have a calculated percent deviation less than 30 % from the generated curve.

12.2.9 The retention time window of the SRM transitions 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 calibration curve to determine if there was a shift in retention time during the analysis and the sample needs to be re-injected. If the retention time is still incorrect in the sample, refer to the analyte as an unknown.

12.2.10 A midpoint calibration check standard shall be analyzed at the end of each batch of 30 samples or within 24 hours after the initial calibration curve was generated, the criteria in the individual labs' quality system may be more restrictive pertaining to the number of samples. This end calibration check, in a new not pierced sealed vial, should come from the same calibration standard solution that was used to generate the initial curve. The results from the end calibration check standard shall have a percent deviation less than 30 % from the calculated concentration for the target analytes and surrogates. If the results are not within these criteria,

corrective action including re-occurrence minimization is performed and either all samples in the batch are re-analyzed against a new calibration curve or the affected results are qualified with an indication that they do not fall within the performance criteria of the test method. If the analyst inspects the vial containing the end calibration check standard and notices that the sample evaporated affecting the concentration or other anomaly, a new end calibration check standard may be made and analyzed. If this new end calibration check standard has a percent deviation less than 30 % from the calculated concentration for the target analytes and surrogates, the results may be reported unqualified.

12.3 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.

12.3.1 Analyze at least four replicates of a spiked water sample containing the PFASs and surrogates at a prepared sample concentration in the calibration range of Levels 4–7. The Level 6 concentration of the nine-point calibration curve was used to set the QC acceptance criteria in this test method. The matrix and chemistry should be similar to the matrix used in this test method. Each replicate shall be taken through the complete analytical test method including any sample manipulation and pretreatment steps.

12.3.2 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 5.

12.3.3 This study should be repeated until the single operator precision and mean recovery are within the limits in Table 5. 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.

12.3.3.1 The QC acceptance criteria for the Initial Demonstration of Performance in Table 5 were generated from the single-laboratory data shown in the Precision and Bias, Section 16. Data from reagent, surface, and wastewater matrices are shown in the Precision and Bias, Section 16. It is recommended that the laboratory 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, or Method 8000 in EPA Publication SW-846.

12.4 *Surrogate Spiking Solution:*

12.4.1 A surrogate spiking solution containing nine isotopically labeled PFASs – MPFBA, MPFHxA, MPFHxS, MPFDA, MPFOA, MPFOS, MPFNA, MPFUnA, and MPFDoA 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. Spiking 40 µL of this spiking solution into a 5-mL water sample results in 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 5. 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.

12.4.1.1 The surrogate spiking solution was prepared by adding 500 µL of a 2-mg/L Surrogate Mix[16] in a 50-mL volumetric and diluted to 50 mL with 95 % acetonitrile: 5 % water. Surrogate spiking solutions are routinely replaced every year if not previously discarded for quality-control failure.

12.5 *Method Blank:*

12.5.1 At least two method blanks for every 30 samples are prepared in water to investigate for contamination during sample preparation and extraction. The concentration of target analytes in either/both blank(s) shall be less than half the reporting limit or the data shall be qualified as having a blank issue and the reporting limit for the affected samples shall be raised to at least 3 times above the blank contamination concentration. PFASs are common in the environment and laboratories requiring continual evaluation to ensure that quality data is produced.

12.6 *Reporting Limit Check Sample (RLCS):*

12.6.1 Each batch or within the 24 hour analysis window, a reporting limit check sample shall be analyzed. The reporting limit check sample is processed like a Laboratory Control Sample just spiked at or near the reporting limit. The concentration of the RLCS may be reported below the reporting limit since the spike is at or near the reporting limit. This sample is to check if the analytes were present at the reporting limit, they would be identified. The recovery limits for the RLCS are 35 to 150 %, if any analytes are outside of these limits the QC failure is explained in a narrative accompanying the data.

12.6.2 Five mL of ASTM Type I water is added to a 15-mL polypropylene centrifuge tube. The sample is spiked with 40 µL of surrogate spiking solution and 25 µL of PFASs Reporting Limit Check solution (Table 6) and then taken through the sample preparation and analyzed.

12.7 *Laboratory Control Sample (LCS):*

12.7.1 To ensure that the test method is in control, analyze at least one LCS with the PFASs at a mid-level concentration. A prepared sample, at the Level 6 calibration concentration, was used in this test method, any mid-level (Levels 4–7) concentration may be chosen using this test method. The LCS is prepared following the analytical method and analyzed with each batch of 30 samples or less. Prepare stock matrix spiking solutions — Target Spike I and II in 95 % acetonitrile: 5 % water containing the 21 PFASs at concentrations listed in Table 6. 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 (PFBA and PFPeA), 3200 ng/L (FHEA, FDEA, and FOEA), and 160 ng/L of remaining 16 PFASs (PFTreA, PFTriA, PFDoA, PFUnA, PFDA, PFOS, PFNA, PFHxA, PFHpA, PFBS, PFecHS, PFOA, PFHxS, FOUEA, FHUEA, and FHpPA) in the sample. The result obtained for the LCS shall fall within the limits in Table 5. Spiking solutions are routinely replaced every year if not previously discarded for quality-control failure.

[16] Surrogate Mix from Wellington Laboratories Inc. has been found suitable for use.

12.7.2 If the result is not within these limits, 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 stating that they do not fall within the performance criteria of this test method.

12.8 *Matrix Spike (MS):*

12.8.1 To check for interferences in the specific matrix being tested, perform a MS on at least one sample from each batch of 30 or fewer samples by spiking the sample with a known concentration of PFASs and following the analytical method. Prepare stock matrix spiking solutions — Target Spike I and II in 95 % acetonitrile: 5 % water containing the 21 PFASs at concentrations listed in Table 6. Spike 40 µL of these stock solutions into 5 mL of the site water sample to yield a concentration of 800 ng/L (PFBA and PFPeA), 3200 ng/L (FHEA, FDEA, and FOEA), and 160 ng/L of remaining 16 PFASs (PFTreA, PFTriA, PFDoA, PFUnA, PFDA, PFOS, PFNA, PFHxA, PFHpA, PFBS, PFecHS, PFOA, PFHxS, FOUEA, FHUEA, and FHpPA) in the sample.

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

12.8.3 Calculate the percent recovery of the spike (P) using Eq 1:

$$P = 100\frac{|A(V_s + V) - BV_s|}{CV} \quad (1)$$

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.

12.8.4 The percent recovery of the spike shall fall within the limits in Table 7. If the percent recovery is not within these limits, a matrix interference may be present. Under these circumstances either all samples in the batch may be analyzed by a test method not affected by the matrix interference, or the results shall be qualified indicating that they do not fall within the performance criteria of the test method. It has been found that in some cases the matrix spike concentration may be minimal compared to the concentration in the native sample. If this is the case, the sample may be spiked at a higher level or the generated data may be reported explaining in the narrative accompanying the data that the spike was negligible compared to the native concentration found in the sample.

12.8.5 The matrix spike/matrix spike duplicate (MS/MSD) limits in Table 7 were generated by a single-laboratory study using the data in the Precision and Bias, Section 16. The limits in Table 7 are preliminary until a multi-lab validation study is completed. The matrix variation between different waters may have a tendency to generate significantly wider control limits than those generated for this test method. It is recommended that each laboratory determine in-house QC acceptance criteria meeting or exceeding the criteria stated in this test method.

TABLE 7 MS/MSD QC Acceptance Criteria

NOTE 1—Table 7 data is preliminary until a multi-lab validation study is completed.

Analyte	Spike Conc. ng/L	MS/MSD Recovery (%)		Precision RPD (%)
		Lower Limit	Upper Limit	
PFTreA	160	70	130	30
PFTriA	160	70	130	30
PFDoA	160	70	130	30
PFUnA	160	70	130	30
PFDA	160	70	130	30
PFOS	160	70	130	30
PFNA	160	70	130	30
PFecHS	160	70	130	30
PFOA	160	70	130	30
PFHxS	160	70	130	30
PFHpA	160	50	130	30
PFHxA	160	50	130	30
PFBS	160	70	130	30
PFPeA	800	70	130	30
PFBA	800	50	130	30
FHEA	3200	70	130	30
FOEA	3200	70	130	30
FDEA	3200	70	130	30
FOUEA	160	70	130	30
FHpPA	160	70	130	30
FHUEA	160	70	130	30
MPFBA	160	70	130	30
MPFHxA	160	70	130	30
MPFHxS	160	70	130	30
MPFOA	160	70	130	30
MPFNA	160	70	130	30
MPFOS	160	70	130	30
MPFDA	160	70	130	30
MPFUnA	160	70	130	30
MPFDoA	160	70	130	30

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

12.9 *Duplicate:*

12.9.1 To check the precision of sample analyses, analyze a sample in duplicate with each batch of 30 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/matrix spike duplicate should be used.

12.9.2 Calculate the relative percent difference (RPD) between the duplicate values (or MS/MSD values) as shown in Eq 2. Compare to the RPD limit in Table 7.

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

where:

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

NOTE 4—If using duplicates to calculate RPD, MSR is the sample concentration and MSDR is the duplicates concentration.

12.9.3 If the result exceeds the precision limit (Table 7 RPD %), the batch shall be re-analyzed or the results shall be qualified with an indication that they do not fall within the performance criteria of the test method.

13. Procedure

13.1 This test method is based upon a 5-mL sample size per analysis. The samples shall be analyzed within 28 days of collection. If the samples are received or stored above 6°C, or are not analyzed within 28 days of collection, it is noted in the case narrative that accompanies the data.

13.2 Each batch of samples (30 or less) shall contain at least two method blanks, laboratory control sample, matrix spike, duplicate, and a reporting limit check sample at a minimum.

13.3 The entire collected 5.0-mL sample shall be used without transferring to another sample container. In order to have accurate volumes, the weight of the 15-mL polypropylene BD Falcon tube may be taken before and after sampling in order to obtain an exact volume. 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. Some of these target analytes adhere to the surfaces of the sampling container over time. The entire sample shall be processed in the original container it was collected, otherwise biased low data will result. The entire collected sample is appropriately spiked.

13.4 To all samples, 5 mL of methanol is added and hand shaken/vortexed for ~2 minute (refer to 13.5 for additional steps for sludge samples).

13.5 After vortexing, pH of the sludge sample is adjusted to pH ~9 with ammonium hydroxide (~20 µL) and hand shaken/vortexed again for ~2 minute. This step is not required for water, wastewater, influent, and effluent unless high percent solids (≥0.1 %) are present or low recoveries were observed historically when no base was added.

13.6 All the samples are filtered through the filter unit using a lock tip glass syringe (refer to 13.7 and 13.8 before use) to remove particulates in the samples. Acetic acid (~10 µL for water samples and ~50 µL for ammonium hydroxide prepared samples) is added to all samples to adjust the pH ~3 after filtration. An aliquot of the solution is transferred to a LC vial and a polyethylene cap is applied. The final volume of the solution is estimated to be 10 mL for quantitation purposes since 5 mL of methanol was added to 5 mL of sample.

13.7 The filters shall be washed with two 10-mL volumes of acetonitrile followed by two 10-mL volumes of methanol prior to use to ensure removal of possible PFASs.

NOTE 5—If the filter units were manufactured in a facility that produces or uses PFASs containing products there is a good chance they may be contaminated with PFASs that need to be removed by rinsing.

13.8 The syringe shall be cleaned between each filtration. It is the analyst's responsibility to ensure that the syringe is clean. A suggested method for cleaning the syringe between filtrations is to first rinse with at least 5 syringe volumes of water, followed by at least 3 volumes of acetonitrile, then 3 volumes of methanol, and a final rinse with water.

13.9 Once a passing calibration curve is generated the analysis of samples may begin. An order of analysis may be method blank(s), reporting limit check, laboratory control sample(s), sample(s), duplicate(s), and matrix spike sample(s) followed by an end calibration check standard.

14. Calculation or Interpretation of Results

14.1 For quantitative analysis of the PFASs and surrogates, the SRM transitions are identified by comparison of retention times in the sample to those of the standards. The target compounds are identified by comparing the sample primary SRM transition and its confirmatory SRM transition if correlated to the known standard SRM transition. Confirmatory transitions are available for most of the target analytes (Table 3). The primary/confirmatory SRM ion ratio shall meet the criteria set in the quantitation method by ±35 %. The primary/confirmatory SRM ion ratio is the average of the individual levels primary /confirmatory SRM ion ratios in the calibration curve on the day of analysis. This ratio will vary depending on the instrumental acquisition parameters and shall be checked for every sample batch. External calibration curves are used to calculate the amounts of PFASs and surrogates. Calculate the concentration in ng/L (ppt) for each analyte. The individual PFASs may be reported if present at or above the reporting limit. If the concentration of the analyte is determined to be above the calibration range, the sample is diluted with a solution of 50 % Water/ 50 % MeOH containing 0.1 % acetic acid to obtain a concentration near the mid-point of the calibration range and re-analyzed. This test method uses nine surrogates, MPFBA, MPFHxA, MPFHxS, MPFDA, MPFOA, MPFOS, MPFNA, MPFUnA, and MPFDoA, to monitor performance. The surrogate recoveries are provided with all data generated from this test method.

14.2 If there is no confirmatory transition for the analyte (refer to Table 3), and the presence of the analyte in the sample can't be confirmed with the primary transition and retention time, the analyte is listed as a non-detect or as having a matrix interference present.

14.3 *Example Calculation of Sample Concentration Reported*—The concentration of sample is calculated using Eq 3.

$$\frac{V_f}{V_i}(C_u) = C_f \tag{3}$$

where:

V_f = final volume,
V_i = initial volume,
C_u = uncorrected concentration, and
C_f = final concentration (corrected for dilution).

14.4 There are nine labeled surrogates for this analysis. The labeled analyte represents the unlabeled native analytes. PFTreA and PFTriA are represented by MPFDoA. PFHpA is represented by MPFHxA, PFecHS, and PFBS are represented by MPFHxS and PFPeA is represented by MPFBA. The six fluorotelomer acids do not have associated labeled surrogates. The recoveries of the nearest labeled surrogate should be monitored but does not represent the native compound. No qualifications based on surrogate recovery will be made for the

six fluorotelomer acids. It is a user's judgment to qualify data based upon no-representative surrogates.

14.5 Some of the analytes are comprised of isomeric mixtures, this is the case for PFOS, PFecHS, and PFHxS in this test method. The entire isomeric group shall be quantitated. This is one reason why a secondary transition is required and allows easier determinations to be made by the analyst by comparing the two transitions. If there are parts of the isomeric mixture in the sample that do not match the retention times of the standard they may not be included in the integration and this shall be explained in the narrative accompanying the data.

14.6 The confirmatory ion ratios in "weathered samples" may not match the ion ratios in the calibration standards for the target analytes that may contain isomeric mixtures. Figs. X1.1-X1.4 in Appendix X1 are examples of this for PFHxS and PFOS, these differences in isomer mixtures may be observed with analytes that have the possibility of containing isomeric mixtures. These differences for PFHxS and PFOS were found in groundwater samples and may either be the cause of different compositions used, weathering or degradation or the affinity of the branched isomers to be more soluble than the linear in water and may leach into the water from the soil at a higher rate than the linear. If the ion ratios do not match the ion ratio criteria, document in the case narrative and the affected data should be qualified and explained in the narrative accompanying the data.

15. Report

15.1 Determine the results in units of ng/L (ppt) in a water sample. Calculate the concentration in the sample using the linear or quadratic calibration curve generated. All data that does not meet the specifications in the test method shall be appropriately qualified.

16. Precision and Bias

16.1 The determination of precision and bias was conducted by U.S. EPA Region 5 Chicago Regional Laboratory (CRL) and generated applicable data to determine the precision and bias as described in Practice D2777 for a single laboratory validation study.

16.2 This test method was tested by CRL on reagent water. The samples were spiked with the PFASs to obtain a 800 ng/L (PFBA and PFPeA), 3200 ng/L (FHEA, FDEA, and FOEA) and 160 ng/L of the remaining (PFTreA, PFTriA, PFDoA, PFUnA, PFDA, PFOS, PFNA, PFHxA, PFHpA, PFBS, PFecHS, PFOA, PFHxS, FOUEA, FHUEA, and FHpPA) and a

TABLE 8 Single-Laboratory Recovery Data in Reagent Water

	Measured ng/L from ASTM Type I Water — 160 ng/L spike for PFTreA, PFTriA, PFDoA, PFUnA, PFDA, PFOS, PFNA, PFHxA, PFHpA, PFBS, PFecHS, PFOA, PFHxS, FOUEA, FHUEA, FHpPA, 800 ng/L for PFBA and PFPeA and 3200 ng/L spike for FHEA, FDEA, and FOEA										
Sample	PFTreA	PFTriA	PFDoA	PFUnA	PFDA	PFNA	PFOA	PFHpA	PFHxA	PFPeA	PFBA
Unspiked 1	<RL	<RL	<RL	<RL	<RL	<RL	<RL	<RL	<RL	<RL	<RL
Unspiked 2	<RL	<RL	<RL	<RL	<RL	<RL	<RL	<RL	<RL	<RL	<RL
P&A 1	134.3	138.6	140.7	144.9	147.2	148.6	144.5	98.8	92.0	695.7	550.5
P&A 2	146.5	148.3	148.5	147.8	150.3	151.3	145.2	119.8	93.8	708.2	574.2
P&A 3	152.9	147.7	148.1	149.3	150.8	148.6	145.4	100.5	89.7	692.7	559.6
P&A 4	144.1	147.8	151.7	150.4	150.3	152.0	149.4	102.0	94.1	719.2	562.2
P&A 5	157.5	149.4	149.1	151.5	151.1	153.3	146.4	103.3	92.9	708.6	573.7
P&A 6	146.8	148.3	146.6	148.2	149.9	152.7	143.3	101.0	94.3	703.5	557.4
Average Recovery (ng/L)	147.0	146.7	147.5	148.7	149.9	151.1	145.7	104.2	92.8	704.6	562.9
Average % Recovery	91.9	91.7	92.2	92.9	93.7	94.4	91.1	65.2	58.0	88.1	70.4
Standard Deviation	7.9	4.0	3.7	2.3	1.4	2.0	2.1	7.8	1.7	9.6	9.4
RSD (%)	8.6	4.4	4.0	2.5	1.5	2.2	2.3	7.4	1.9	1.4	1.7
Sample	PFBS	PFHxS	PFOS	PFecHS	FOUEA	FHpPA	FHUEA	FHEA	FOEA	FDEA	
Unspiked 1	<RL	<RL	<RL	<RL	<RL	<RL	<RL	<RL	<RL	<RL	
Unspiked 2	<RL	<RL	<RL	<RL	<RL	<RL	<RL	<RL	<RL	<RL	
P&A 1	150.0	151.0	151.0	143.3	150.0	150.7	145.5	3252.1	3037.1	3183.8	
P&A 2	216.2	150.1	155.6	147.2	148.7	136.1	143.0	3069.8	2776.8	3048.4	
P&A 3	146.2	151.2	153.3	145.0	148.0	144.8	147.0	3161.0	3055.6	3346.8	
P&A 4	149.6	144.5	152.3	145.8	147.5	147.0	151.6	3235.8	3007.3	3243.3	
P&A 5	145.7	147.4	147.1	146.9	146.2	146.9	145.8	3099.8	2853.9	3233.2	
P&A 6	142.8	139.7	146.4	145.6	145.9	133.1	142.4	2977.0	3197.8	3152.9	
Average Recovery (ng/L)	158.4	147.3	150.9	145.6	147.7	143.1	145.9	3132.6	2988.1	3201.4	
Average % Recovery	99.0	92.1	94.3	91.0	92.3	89.4	91.2	97.9	93.4	100.0	
Standard Deviation	28.4	4.5	3.6	1.4	1.5	6.9	3.3	104.8	151.0	100.0	
RSD (%)	17.9	3.1	3.8	1.6	1.0	4.8	2.3	3.3	5.1	3.1	