Designation: ~~D7979 – 17~~ D7979 – 19

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,~~method; performance of this test method has not been evaluated on drinking water matrices.

1.2 The ~~Method Detection Limit~~method detection limit (MDL)[2] and ~~Reporting Range~~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~~ 10 ng/L for most of the target analytes at the time of development.

[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. ~~5 mL~~ 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~~ 30-µL injection of the Level 1 calibration standard for PFASs, and the highest level calibration standard with a ~~10 mL~~ 10-mL final extract volume of a ~~5 mL~~ 5-mL water sample. Volume variations will change the reporting limit and ranges.

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.

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 %~~ 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

2.2 *Other Standards:*[5]

EPA Publication ~~SW-846,~~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

[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.

[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

3.3.5 *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
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~~ 5-mL sludge samples; ~~5 mL~~ 5 mL methanol is added, adjusted to pH ~9 (adding ~20 ~~µl~~µL of ammonium hydroxide), hand shaken, filtered, acidified to pH ~3 (~50 µL acetic ~~acid)~~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%~~ 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~~ 5.0-mL sample in a graduated ~~15 mL~~ 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~~ 5.0-mL sample is spiked according to this ~~Standard~~ test method and methanol is added, it is then thoroughly shaken and transferred to a new ~~15 mL~~ 15-mL polypropylene tube during filtration. In order to have accurate volumes, the weight of the ~~15 mL~~ 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)~~ >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 ~~Liquid Chromatography~~ 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] A Draft Toxicological Profile for Perfluoroalkyls can be found at: http://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=1117&tid=237 (2014).

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.

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

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~~ 25-mL filter-adaptable glass syringe with luer lock.

[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.

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

[12] *Reagent Chemicals, American Chemical Society Specifications*, 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.

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

8.7 *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).
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~~ 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

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

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~~ 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 ~~liquid chromatography~~ LC are shown in Table 2.

11.2 *LC Sample Manager Conditions:*

11.2.1 *Needle Wash Solvent*—60 % acetonitrile/40 % 2-propanol. ~~8~~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
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

[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.

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

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~~ 50-mL volumetric flask and diluted to ~~50 ml~~ 50-mL volume with 50:50 methanol:water containing 0.1 % acetic acid. The preparation of the ~~Level 9~~ 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~~ 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 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

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

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)~~ (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 %~~ 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