PFAS Testing Procedures

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What are PFAS?

"Per- and Poly-Fluoro-Alkyl Substances" (PFAS)

Consist of more than 3,000 chemicals

- Became widely used due to unique chemical properties

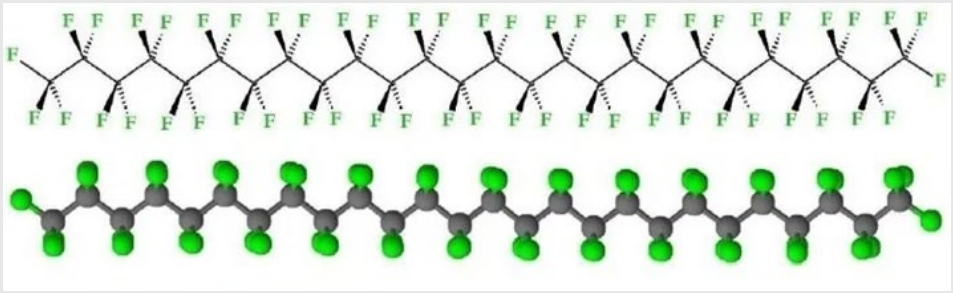
  1) Repel oil and water (coatings)

  2) Temperature resistant (fire-fighting foams)

  3) Reduce friction (lubricants)

First PFAS discovered

Polytetrafluoroethylene (PTFE) was accidentally discovered by Dupont chemist Roy J. Plunkett in 1938



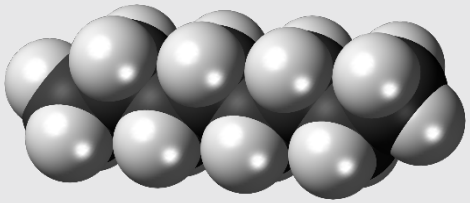
Where are they found?

- Stain-resistant clothing, carpeting, upholstery
- Household products such as non-stick cookware,
- Polishes, waxes, and paints
- Food packaging-popcorn bags, pizza boxes, fast food wrappers/containers
- Metal plating and electronics manufacturing

Unique Properties of PFAS

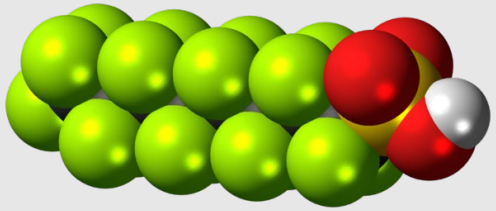
C-H Bond

- 1.09 Å average bond length
- 347-377 kJ/mol bond dissociation energy
- Hydrogen covalent radius = 31 pm



C-F Bond

- 1.35 Å average bond length
- 481 kJ/mol bond dissociation energy
- Fluorine covalent radius = 57 pm



Why does it matter?

- Many PFAS bioaccumulate
- Nearly everyone in the US has been exposed to PFAS
- PFOA, PFNA, PFHxS, and PFOS most common
- Studies suggest that exposure to PFOA and PFOS can lead to adverse health effects
- Studies with laboratory animals have shown PFOA and PFOS can affect reproduction and development, kidney and liver function, and cause immunological effects
- Increased cholesterol levels
- Low birth weights
- Thyroid hormone disruption
- Possible carcinogen

Routes of Exposure

Contaminated drinking water:

- Public and private systems typically with localized release

Food sources:

- Eating plants and meat, including fish that accumulated PFAS
- Consumption of food that came into contact with PFAS containing products

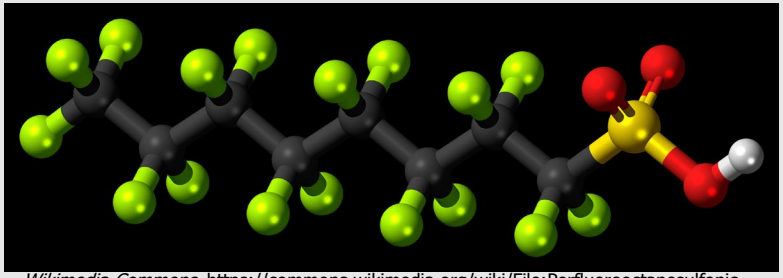
At Home: Household products and indoor dust containing PFAS

In the Workplace: Chemical production facility (Manu. use PFAS.)

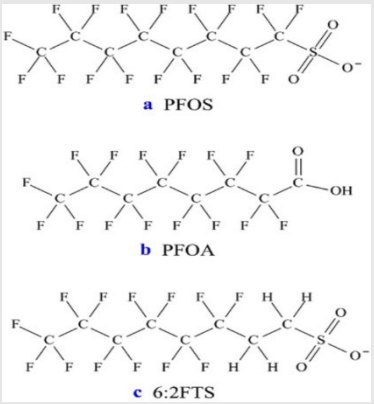
To the environment:

- Aqueous film forming foam (AFFF)
- Manufacturing facilities
- Landfills
- Wastewater effluents

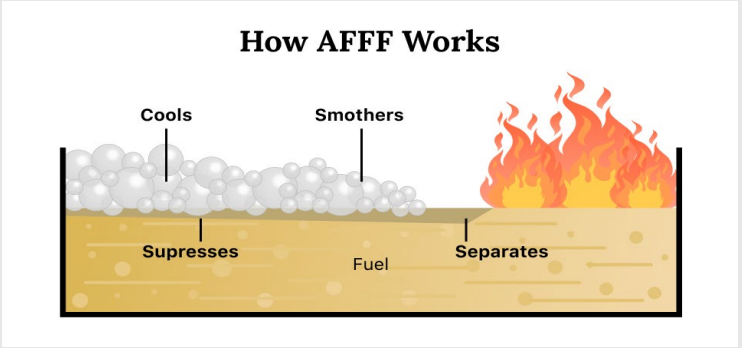
AFFF (Aqueous Film Forming Foam)



Wikimedia Commons. https://commons.wikimedia.org/wiki/File:Perfluorooctanesulfonic-acid-3D-balls.png (Accessed 2023-04-18)



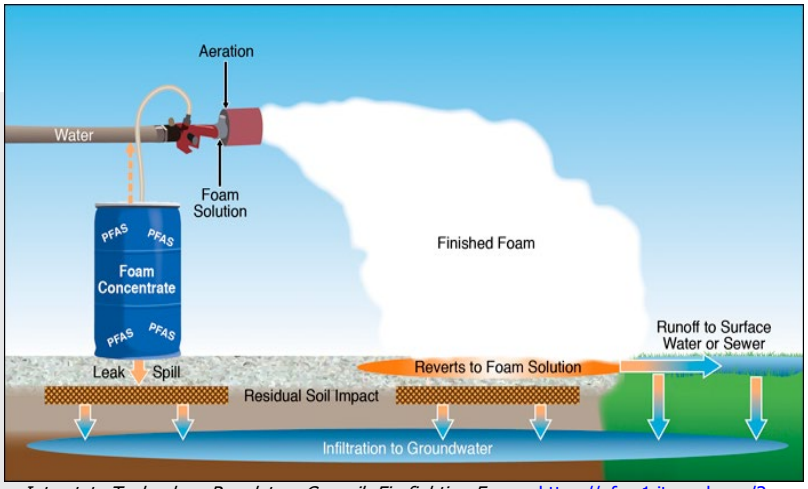
National Center for Biotechnology Information https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5719435/ (Accessed 2023-04-20)



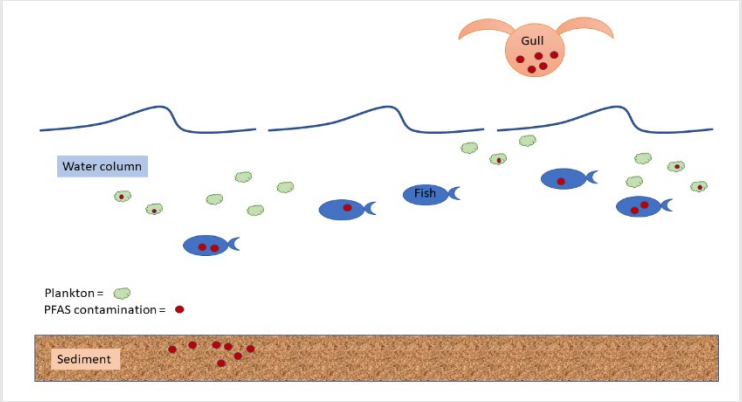
Consumer Notice https://www.consumernotice.org/environmental/afff/ (Accessed 2023-04-18)

AFFF Pollution

Air Combat Command. https://www.acc.af.mil/News/Article-Display/Article/199717/airmen-test-foam-suppression-system/ (Accessed 2023-04-18)



Interstate Technology Regulatory Council Firefighting Foams. https://pfas-1.itrcweb.org/3-firefighting-foams/#3_2 (Accessed 2023-04-18)



Wikipedia. https://en.wikipedia.org/wiki/Per-_and_polyfluoroalkyl_substances (Accessed 2023-04-20)

EPA 537.1

EPA Document #: EPA/600/R-08/092

METHOD 537. DETERMINATION OF SELECTED PERFLUORINATED ALKYL ACIDS IN DRINKING WATER BY SOLID PHASE EXTRACTION AND LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY (LC/MS/MS)

Version 1.1
September 2009

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DETERMINATION OF SELECTED PERFLUORINATED ALKYL ACIDS IN DRINKING WATER BY SOLID PHASE EXTRACTION AND LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY (LC/MS/MS)

1. SCOPE AND APPLICATION

1.1 This is a liquid chromatography/tandem mass spectrometry (LC/MS/MS) method for the determination of selected perfluorinated alkyl acids (PFAAs) in drinking water. Accuracy and precision data have been generated in reagent water, and finished ground and surface waters for the compounds listed in the table below.

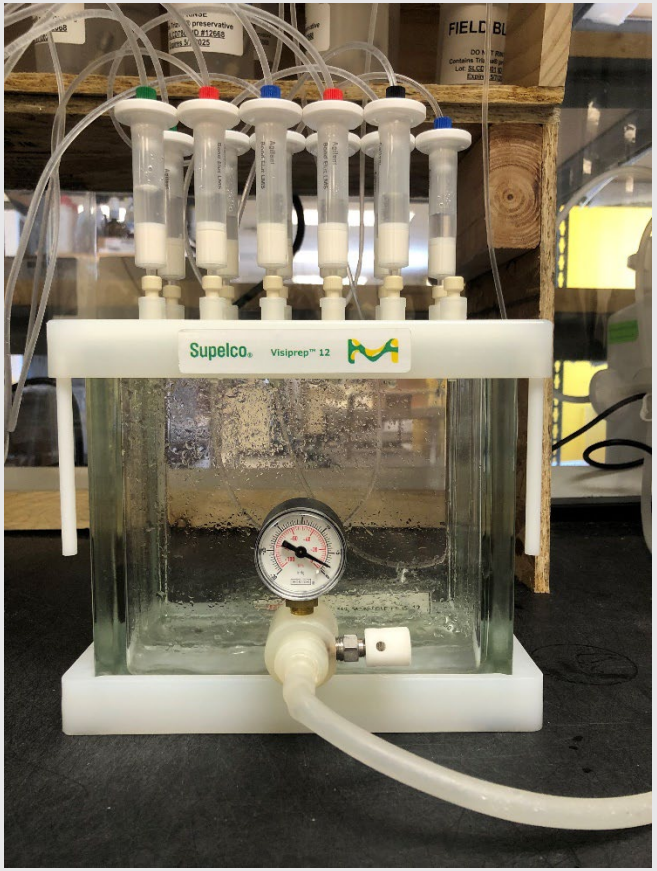
Analyte	Acronym	Chemical Abstract Services Registry Number (CASRN)
N-ethyl perfluorooctanesulfonamidoacetic acid	NEtFOSAA	–
N-methyl perfluorooctanesulfonamidoacetic acid	NMeFOSAA	–
Perfluorobutanesulfonic acid	PFBS	375-73-5
Perfluorodecanoic acid	PFDA	335-76-2
Perfluorododecanoic acid	PFDoA	307-55-1
Perfluoroheptanoic acid	PFHpA	375-85-9
Perfluorohexanesulfonic acid	PFHxS	355-46-4
Perfluorohexanoic acid	PFHxA	307-24-4
Perfluorononanoic acid	PFNA	375-95-1
Perfluorooctanesulfonic acid	PFOS	1763-23-1
Perfluorooctanoic acid	PFOA	335-67-1
Perfluorotetradecanoic acid	PFTA	376-06-7
Perfluorotridecanoic acid	PFTrDA	72629-94-8
Perfluoroundecanoic acid	PFUnA	2058-94-8

1.2 The Minimum Reporting Level (MRL) is the lowest analyte concentration that meets Data Quality Objectives (DQOs) that are developed based on the intended use of this method. The single laboratory lowest concentration MRL (LCMRL) is the lowest true concentration for which the future recovery is predicted to fall, with high confidence (99%), between 50 and 150% recovery. Single laboratory LCMRLs for analytes in this method range from 2.9-14 ng/L, and are listed in Table 5. The procedure used to determine the LCMRL is described elsewhere.[1]

SPE (Solid Phase Extraction)

- Analytes in solution interact with a solid sorbent bed to remove them from solution
- Once the entire sample matrix has passed through the SPE cartridge, the analytes are eluted from the cartridge using a solvent that has stronger interactions with the analytes than the SPE sorbent.

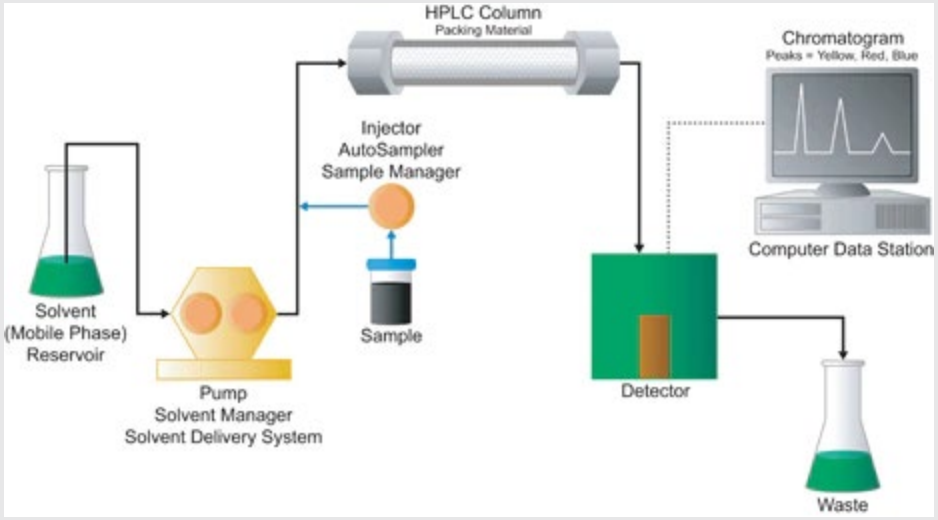
SPE (Solid Phase Extraction)



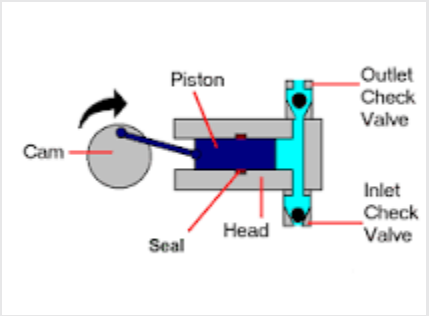
High Performance Liquid Chromatography (HPLC)

- Separation technique where a liquid mobile phase is pumped through a column containing a solid sorbent stationary phase.
- The analytes will separate based on their individual interaction with both the mobile phase and the stationary phase.
- The time it takes for an analyte to travel from the injection site to the detector is called the “retention time”, this can be determined individually for each analyte.

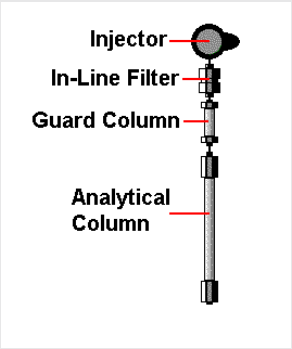
High Performance Liquid Chromatography (HPLC)



Waters. https://www.waters.com/waters/en_US/How-Does-HighPerformance-Liquid-Chromatography-Work%3F/nav.htm?locale=en_US&cid=10049055 (Accessed 2023-04-26)

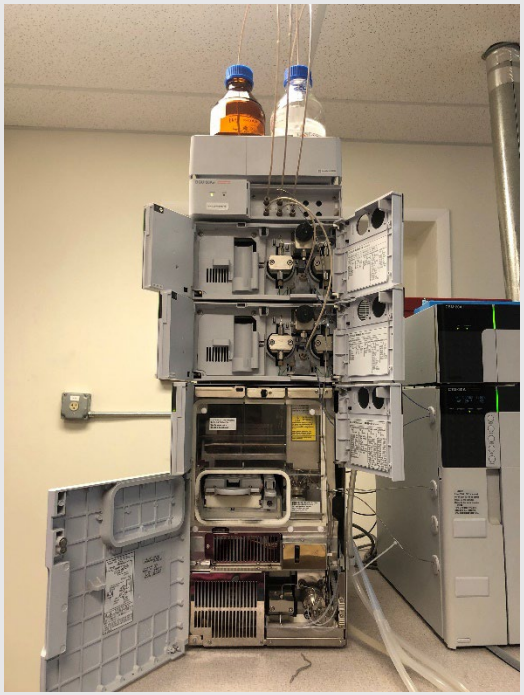


LC Resources. http://www.lcresources.com/resources/getstart/i.htm (Accessed 2023-04-26)



LC Resources. http://www.lcresources.com/resources/getstart/i.htm(Accessed 2023-04-26)

High Performance Liquid Chromatography (HPLC)

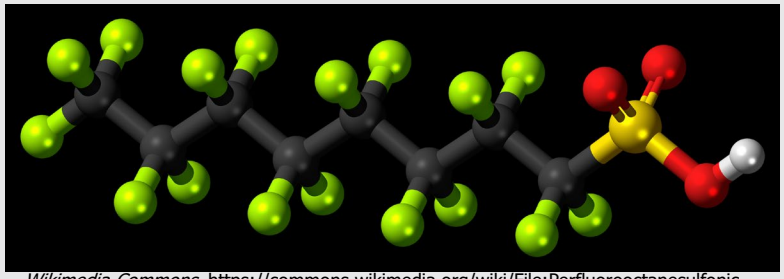


Mass Spectrometry

- Analytical technique where analytes are detected by their mass-to-charge (m/z) ratio
- The analytes must be ionized prior to being analyzed by mass spectrometry
- Mass spectrometry is typically used to determine what products were formed during organic synthesis

Triple Quad Mass Spectrometry

- Analytes are identified by m/z ratio, then fragmented.
- The fragments are then identified by their unique m/z ratio
- Triple quad mass spectrometry is a powerful detection technique, because there are two events where the analyte are identified

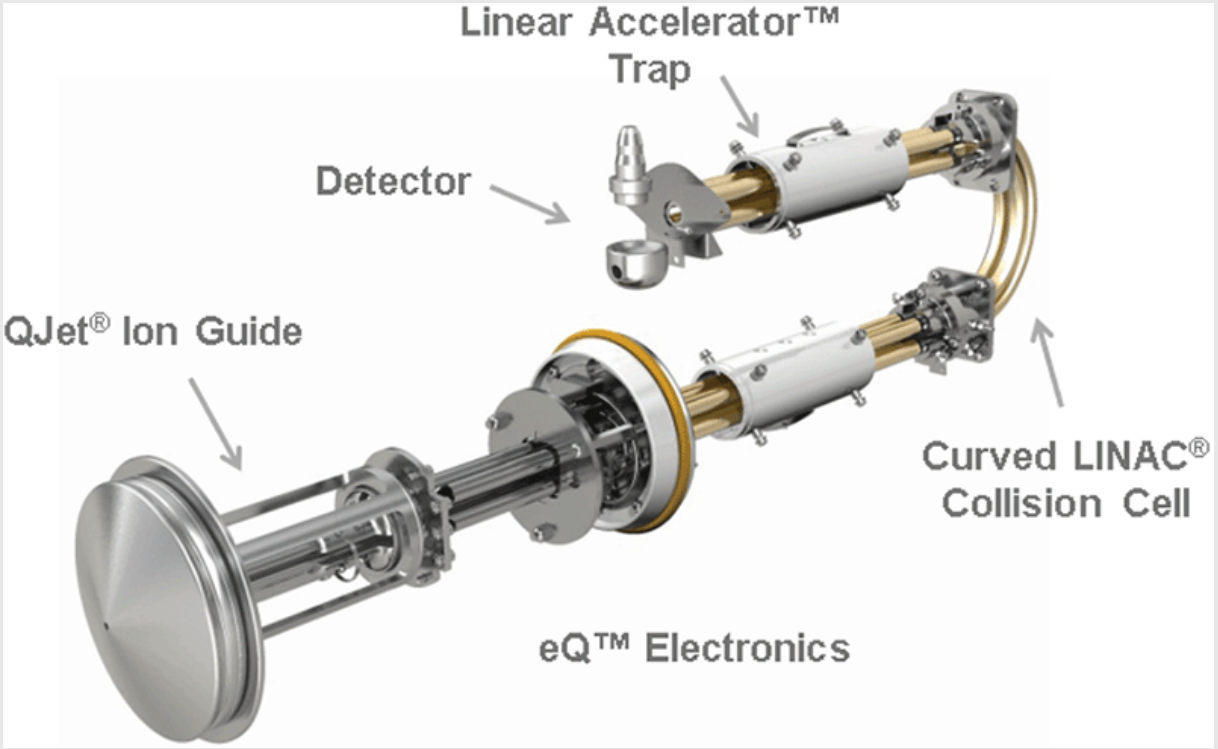


Wikimedia Commons. https://commons.wikimedia.org/wiki/File:Perfluorooctanesulfonic-acid-3D-balls.png (Accessed 2023-04-18)

- PFOS: 500.119 amu
- Fluorinated Carbon Chain: 419.054
- Sulfonate Head: 81.065

Triple Quad Mass Spectrometry

Triple Quad Mass Spectrometry



Sciex. https://www.sciex.com/tech-notes/technology/------powerful-scan-modes-of-qtrap--system-technology- (Accessed 2023-04-26)

EPA 537.1 Implementation Challenges

LC systems typically contain fluoropolymer tubing and components

Therefore…

-Replace factory tubing with polyetheretherketone (PEEK) tubing

-Use delay column: PFAS from mobile phase or LC system elutes after PFAS in sample

Solid phase extraction (SPE) manifold and procedure

-Remove Teflon® guide needles

-Replace Teflon® tubing present in large volume samplers

EPA 537.1 Implementation Challenges

- Polypropylene autosampler vials are only good for one injection
- PEEK plumbing is not always rated for higher pressures

537.1 QA/QC Parameters

TABLE 13. ONGOING QUALITY CONTROL REQUIREMENTS (SUMMARY)

Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Sect. 8.1 - Sect. 8.5	Sample Holding Time	14 days with appropriate preservation and storage as described in Sections 8.1-8.5.	Sample results are valid only if samples are extracted within the sample holding time.
Sect. 8.5	Extract Holding Time	28 days when stored at room temperature in polypropylene centrifuge tubes.	Extract results are valid only if extracts are analyzed within the extract holding time.
Sect. 9.3.1	Laboratory Reagent Blank (LRB)	One LRB with each extraction batch of up to 20 samples.	Demonstrate that all method analytes are below 1/3 the MRL, and confirm that possible interferences do not prevent quantification of method analytes. If targets exceed 1/3 the MRL or if interferences are present, results for these subject analytes in the extraction batch are invalid.
Sect. 9.3.3	Laboratory Fortified Blank (LFB)	One LFB is required for each extraction batch of up to 20 Field Samples. Rotate the fortified concentrations between low, medium and high amounts.	Results of LFB analyses must be 70-130% of the true value for each method analyte for all fortified concentrations except the lowest CAL point. Results of the LFBs corresponding to the lowest CAL point for each method analyte must be 50-150% of the true value.
Sect. 9.3.4	Internal Standard (IS)	Internal standards, $^{13}C_2$-PFOA (IS#1), $^{13}C_4$-PFOS (IS#2), and d_3-NMeFOSAA (IS#3), are added to all standards and sample extracts, including QC samples. Compare IS areas to the average IS area in the initial calibration and to the most recent CCC.	Peak area counts for all ISs in all injections must be within ± 50% of the average peak area calculated during the initial calibration and 70-140% from the most recent CCC. If ISs do not meet this criterion, corresponding target results are invalid.
Sect. 9.3.5	Surrogate Standards (SUR)	Surrogate standards, $^{13}C_2$-PFHxA, $^{13}C_3$-HFPO-DA, $^{13}C_2$-PFDA, and d_5-NEtFOSAA, are added to all CAL standards and samples, including QC samples. Calculate SUR recoveries.	SUR recoveries must be 70-130% of the true value. If a SUR fails this criterion, report all results for sample as suspect/SUR recovery.

537.1 QA/QC Parameters

Method Reference	Requirement	Specification and Frequency	Acceptance Criteria
Sect. 9.3.6	Laboratory Fortified Sample Matrix (LFSM)	Analyze one LFSM per extraction batch (20 samples or less) fortified with method analytes at a concentration close to but greater than the native concentration, if known. Calculate LFSM recoveries.	Recoveries at mid and high levels must be within 70-130% and within 50-150% at the low-level fortified amount (near the MRL). If these criteria are not met, results are labeled suspect due to matrix effects.
Sect. 9.3.7	Laboratory Fortified Sample Matrix Duplicate (LFSMD) or Field Duplicates (FD)	Extract and analyze at least one FD or LFSMD with each extraction batch (20 samples or less). A LFSMD may be substituted for a FD when the frequency of detects are low. Calculate RPDs.	Method analyte RPDs for the LFMD or FD must be ≤30% at mid and high levels of fortification and ≤50% near the MRL. If these criteria are not met, results are labeled suspect due to matrix effects.
Sect. 9.3.8	Field Reagent Blank (FRB)	Analysis of the FRB is required only if a Field Sample contains a method analyte or analytes at or above the MRL. The FRB is processed, extracted and analyzed in exactly the same manner as a Field Sample.	If the method analyte(s) found in the Field Sample is present in the FRB at a concentration greater than 1/3 the MRL, then all samples collected with that FRB are invalid and must be recollected and reanalyzed.
Sect. 9.3.9	Peak Asymmetry Factor	Calculate the peak asymmetry factor for the first two eluting chromatographic peaks in a mid-level CAL standard during IDC and when chromatographic changes are made that affect peak shape.	Peak asymmetry factor of 0.8 - 1.5
Sect. 9.3.10	Quality Control Sample (QCS)	Analyze at least quarterly or when preparing new standards, as well as during the IDC.	Results must be within 70-130% of true value.
Sect. 10.2 and Sect. 9.3.2	Initial Calibration	Use IS calibration technique to generate a first or second order calibration curve forced through zero. Use at least five standard concentrations. Check the calibration curve as described in Sect. 10.2.6.	When each CAL standard is calculated as an unknown using the calibration curve, the analyte and SUR results must be 70-130% of the true value for all except the lowest standard, which must be 50-150% of the true value. Recalibration is recommended if these criteria are not met.
Sect. 9.3.2 and Sect. 10.3	Continuing Calibration Check (CCC)	Verify initial calibration by analyzing a low level (at the MRL or below) CCC prior to analyzing samples. CCCs are then injected after every 10 samples and after the last sample, rotating concentrations to cover the calibrated range of the instrument.	Recovery for each analyte and SUR must be within 70-130% of the true value for all but the lowest level of calibration. Recovery for each analyte in the lowest CAL level CCC must be within 50-150% of the true value and the SUR must be within 70-130% of the true value.

NOTE: Table 13 is intended as an abbreviated summary of QC requirements provided as a convenience to the method user. Because the information has been abbreviated to fit the table format, there may be issues that need additional clarification, or areas where important additional information from the method text is needed. In all cases, the full text of the QC in Sections 8-10 supersedes any missing or conflicting information in this table.

EPA 533

United States Environmental Protection Agency

METHOD 533: DETERMINATION OF PER- AND POLYFLUOROALKYL SUBSTANCES IN DRINKING WATER BY ISOTOPE DILUTION ANION EXCHANGE SOLID PHASE EXTRACTION AND LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY

1 Scope and Application

This is a solid phase extraction (SPE) liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the determination of select per- and polyfluoroalkyl substances (PFAS) in drinking water. Method 533 requires the use of MS/MS in Multiple Reaction Monitoring (MRM) mode to enhance selectivity. Accuracy and precision data have been generated in reagent water and drinking water for the compounds included in the Analyte List.

This method is intended for use by analysts skilled in the performance of solid phase extractions, the operation of LC-MS/MS instrumentation, and the interpretation of the associated data.

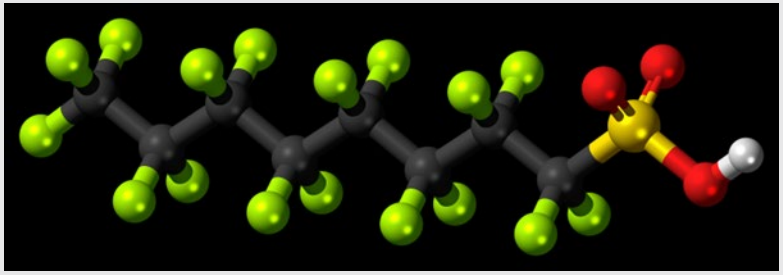
Analyte List

Analyte[a]	Abbreviation	CASRN
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF3OUdS	763051-92-9
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acd	9Cl-PF3ONS	756426-58-1
4,8-Dioxa-3*H*-perfluorononanoic acid	ADONA	919005-14-4
Hexafluoropropylene oxide dimer acid	HFPO-DA	13252-13-6
Nonafluoro-3,6-dioxaheptanoic acid	NFDHA	151772-58-6
Perfluorobutanoic acid	PFBA	375-22-4
Perfluorobutanesulfonic acid	PFBS	375-73-5
1*H*,1*H*, 2*H*, 2*H*-Perfluorodecane sulfonic acid	8:2FTS	39108-34-4
Perfluorodecanoic acid	PFDA	335-76-2
Perfluorododecanoic acid	PFDoA	307-55-1
Perfluoro(2-ethoxyethane)sulfonic acid	PFEESA	113507-82-7
Perfluoroheptanesulfonic acid	PFHpS	375-92-8
Perfluoroheptanoic acid	PFHpA	375-85-9
1*H*,1*H*, 2*H*, 2*H*-Perfluorohexane sulfonic acid	4:2FTS	757124-72-4
Perfluorohexanesulfonic acid	PFHxS	355-46-4
Perfluorohexanoic acid	PFHxA	307-24-4
Perfluoro-3-methoxypropanoic acid	PFMPA	377-73-1
Perfluoro-4-methoxybutanoic acid	PFMBA	863090-89-5
Perfluorononanoic acid	PFNA	375-95-1
1*H*,1*H*, 2*H*, 2*H*-Perfluorooctane sulfonic acid	6:2FTS	27619-97-2
Perfluorooctanesulfonic acid	PFOS	1763-23-1
Perfluorooctanoic acid	PFOA	335-67-1
Perfluoropentanoic acid	PFPeA	2706-90-3
Perfluoropentanesulfonic acid	PFPeS	2706-91-4
Perfluoroundecanoic acid	PFUnA	2058-94-8

[a] Some PFAS are commercially available as ammonium, sodium, and potassium salts. This method measures all forms of the analytes as anions while the identity of the counterion is inconsequential. Analytes may be purchased as acids or as any of the corresponding salts.

Isotope Dilution

- Isotopically labeled analytes are added to the sample prior to extraction
- Native analytes are extracted alongside their isotopic analogue
- The amount of analyte in solution is identified based on the extraction efficiency of the isotopically labeled analyte



Wikimedia Commons. https://commons.wikimedia.org/wiki/File:Perfluorooctanesulfonic-acid-3D-balls.png (Accessed 2023-04-18)

- PFOS: 500.119 amu
- M8PFOS: 508.055 amu

Weak Anion Exchange

- Stationary phase consists of positively-charged ligand that interacts with negatively charged analytes.
- The stationary phase is conditioned with a basic solution to ensure that ligands are positively charged.
- When negatively charged analytes pass through, they will bind to the stationary phase.
- After loading, the analytes are eluted from the stationary phase using a basic solution that will recharge the stationary phase, releasing the analytes.

ELI 537 MOD

Analyte Acronym	Formula		Analyte Acronym	Formula
PFBA	$C_3F_7CO_2H$		4:2 FTS	$CF_3(CF_2)_3(CH_2)_2SO_3Na$
PFPeA	$C_4F_9CO_2H$		6:2 FTS	$CF_3(CF_2)_5(CH_2)_2SO_3Na$
PFHxA	$C_5F_{11}CO_2H$		8:2 FTS	$CF_3(CF_2)_7(CH_2)_2SO_3Na$
PFHpA	$C_6F_{13}CO_2H$		FOSA	$CF_3(CF_2)_7SO_2NH_2$
PFOA	$C_7F_{15}CO_2H$		NMeFOSAA	$C_8F_{17}S(O_2)N(CH_3)CH_2CO_2H$
PFNA	$C_8F_{17}CO_2H$		NEtFOSAA	$C_8F_{17}S(O_2)N(C_2H_5)CH_2CO_2H$
PFDA	$C_9F_{19}CO_2H$		GenX (HFPO-DA)	$C_6H_4F_{11}NO_3$
PFUnA	$C_{10}F_{21}CO_2H$		ADONA	$C_7H_5F_{12}NO_4$
PFDoA	$C_{11}F_{23}CO_2H$		F53B Maj (9Cl)	$Cl(CF_2)_6O(CF_2)_2SO_3H$
PFTrDA	$C_{12}F_{25}CO_2H$		F53B Min (11Cl)	$Cl(CF_2)_8O(CF_2)_2SO_3H$
PFTA	$C_{13}F_{27}CO_2H$			
PFBS	$C_4F_9SO_3H$			
PFPeS	$C_5F_{11}SO_3H$			
PFHxS	$C_6F_{13}SO_3H$			
PFHpS	$C_7F_{15}SO_3H$			
PFOS	$C_8F_{17}SO_3H$			
PFNS	$C_9F_{19}SO_3H$			
PFDS	$C_{10}F_{21}SO_3H$			

ELI 537 MOD

- PFAS extraction of wastewater by WAX SPE (stacked cartridge with WAX bed and Graphetized Carbon Black (GCB) bed)
- PFAS extraction of soil with GCB pass-through SPE
- QA/QC parameters per DOD QSM Table B-15

537 MOD QA/QC Parameters

Sample Cleanup Procedure	Each sample and associated batch QC samples. Not applicable to AFFF and AFFF Mixture Samples.	ENVI-Carb™ or equivalent must be used on each sample and batch QC sample.	NA.	Flagging is not appropriate.	Cleanup should reduce bias from matrix interferences.

Table B-15. Per- and Polyfluoroalkyl Substances (PFAS) Using Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS) With Isotope Dilution or Internal Standard Quantification in Matrices Other Than Drinking Water

QC Check	Minimum Frequency	Acceptance Criteria	Corrective Action	Flagging Criteria	Comments
Instrument Sensitivity Check (ISC)	Prior to analysis and at least once every 12 hours.	Analyte concentrations must be at LOQ; concentrations must be within ±30% of their true values.	Correct problem, rerun ISC. If problem persists, repeat ICAL.	Flagging is not appropriate.	No samples shall be analyzed until ISC has met acceptance criteria. ISC can serve as the initial daily CCV.
Initial Calibration Verification (ICV)	Once after each ICAL, analysis of a second source standard prior to sample analysis.	Analyte concentrations must be within ±30% of their true value.	Correct problem, rerun ICV. If problem persists, repeat ICAL.	Flagging is not appropriate.	No samples shall be analyzed until calibration has been verified.
Continuing Calibration Verification (CCV)	Prior to sample analysis, after every 10 field samples, and at the end of the analytical sequence.	Concentration of analytes must range from the LOQ to the mid-level calibration concentration. Analyte concentrations must be within ±30% of their true value.	Immediately analyze two additional consecutive CCVs. If both pass, samples may be reported without reanalysis. If either fails, or if two consecutive CCVs cannot be run, perform corrective action(s) and repeat CCV and all associated samples since last successful CCV. Alternately, recalibrate if necessary; then reanalyze all associated samples since the last acceptable CCV.	If reanalysis cannot be performed, data must be qualified and explained in the Case Narrative. Apply Q-flag to all results for the specific analyte(s) in all samples since the last acceptable calibration verification.	Results may not be reported without valid CCVs. Instrument Sensitivity Check (ISC) can serve as a bracketing CCV.

537.1 vs 533 vs 537MOD

- Drinking water only
- Internal standard quantitation
- SDVB SPE
- LCMS analysis
- 14 analytes
- Evaporate to dryness

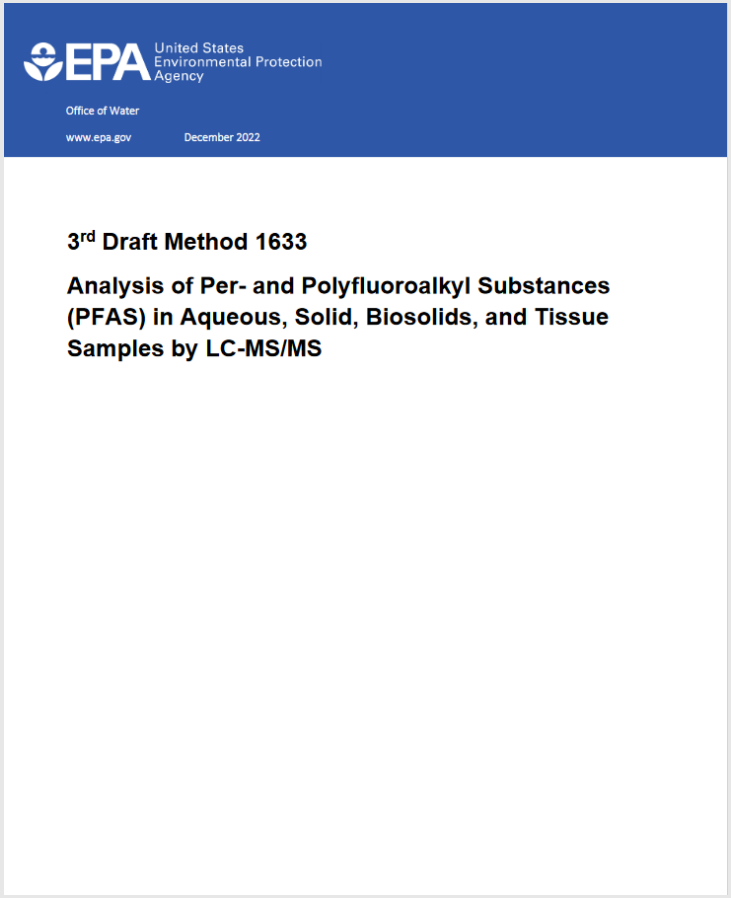
- Drinking water only
- Isotope Dilution
- WAX SPE
- LCMS analysis
- 25 analytes
- Evaporate to dryness

- Surface water, groundwater, and soil
- Isotope dilution
- WAX SPE
- LCMS analysis
- 28 analytes
- GCB cleanup step
- Do not evaporate to dryness.

537 MOD Limitations

- Dirty sample matrices can clog cartridges and transfer lines
- Sample extracts are not filtered or prescreened, so dilution and reanalysis are frequently required
- Minimal GCB cleanup for wastewater samples
- Sample extracts regularly contaminate instrumentation and cause premature wear on columns.

EPA 1633



EPA United States Environmental Protection Agency

Office of Water

www.epa.gov December 2022

3rd Draft Method 1633

Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS

EPA 1633

Table 1. Names, Abbreviations, and CAS Registry Numbers for Target PFAS, Extracted Internal Standards and Non-extracted Internal Standards[1]

Target Analyte Name	Abbreviation	CAS Number
Perfluoroalkyl carboxylic acids		
Perfluorobutanoic acid	PFBA	375-22-4
Perfluoropentanoic acid	PFPeA	2706-90-3
Perfluorohexanoic acid	PFHxA	307-24-4
Perfluoroheptanoic acid	PFHpA	375-85-9
Perfluorooctanoic acid	PFOA	335-67-1
Perfluorononanoic acid	PFNA	375-95-1
Perfluorodecanoic acid	PFDA	335-76-2
Perfluoroundecanoic acid	PFUnA	2058-94-8
Perfluorododecanoic acid	PFDoA	307-55-1
Perfluorotridecanoic acid	PFTrDA	72629-94-8
Perfluorotetradecanoic acid	PFTeDA	376-06-7
Perfluoroalkyl sulfonic acids		
Acid Form		
Perfluorobutanesulfonic acid	PFBS	375-73-5
Perfluoropentansulfonic acid	PFPeS	2706-91-4
Perfluorohexanesulfonic acid	PFHxS	355-46-4
Perfluoroheptanesulfonic acid	PFHpS	375-92-8
Perfluorooctanesulfonic acid	PFOS	1763-23-1
Perfluorononanesulfonic acid	PFNS	68259-12-1
Perfluorodecanesulfonic acid	PFDS	335-77-3
Perfluorododecanesulfonic acid	PFDoS	79780-39-5
Fluorotelomer sulfonic acids		
1*H*,1*H*, 2*H*, 2*H*-Perfluorohexane sulfonic acid	4:2FTS	757124-72-4
1*H*,1*H*, 2*H*, 2*H*-Perfluorooctane sulfonic acid	6:2FTS	27619-97-2
1*H*,1*H*, 2*H*, 2*H*-Perfluorodecane sulfonic acid	8:2FTS	39108-34-4
Perfluorooctane sulfonamides *		
Perfluorooctanesulfonamide	PFOSA	754-91-6
N-methyl perfluorooctanesulfonamide	NMeFOSA	31506-32-8
N-ethyl perfluorooctanesulfonamide	NEtFOSA	4151-50-2
Perfluorooctane sulfonamidoacetic acids *		
N-methyl perfluorooctanesulfonamidoacetic acid	NMeFOSAA	2355-31-9
N-ethyl perfluorooctanesulfonamidoacetic acid	NEtFOSAA	2991-50-6
Perfluorooctane sulfonamide ethanols *		
N-methyl perfluorooctanesulfonamidoethanol	NMeFOSE	24448-09-7
N-ethyl perfluorooctanesulfonamidoethanol	NEtFOSE	1691-99-2
Per- and Polyfluoroether carboxylic acids		
Hexafluoropropylene oxide dimer acid	HFPO-DA	13252-13-6
4,8-Dioxa-3*H*-perfluorononanoic acid	ADONA	919005-14-4
Perfluoro-3-methoxypropanoic acid	PFMPA	377-73-1
Perfluoro-4-methoxybutanoic acid	PFMBA	863090-89-5
Nonafluoro-3,6-dioxaheptanoic acid	NFDHA	151772-58-6

Table 1. Names, Abbreviations, and CAS Registry Numbers for Target PFAS, Extracted Internal Standards and Non-extracted Internal Standards[1]

Target Analyte Name	Abbreviation	CAS Number
Ether sulfonic acids		
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	9Cl-PF3ONS	756426-58-1
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF3OUdS	763051-92-9
Perfluoro(2-ethoxyethane)sulfonic acid	PFEESA	113507-82-7
Fluorotelomer carboxylic acids		
3-Perfluoropropyl propanoic acid	3:3FTCA	356-02-5
2*H*,2*H*,3*H*,3*H*-Perfluorooctanoic acid	5:3FTCA	914637-49-3
3-Perfluoroheptyl propanoic acid	7:3FTCA	812-70-4
EIS Compounds		
Perfluoro-n-[$^{13}C_4$]butanoic acid	$^{13}C_4$-PFBA	NA
Perfluoro-n-[$^{13}C_5$]pentanoic acid	$^{13}C_5$-PFPeA	
Perfluoro-n-[1,2,3,4,6-$^{13}C_5$]hexanoic acid	$^{13}C_5$-PFHxA	
Perfluoro-n-[1,2,3,4-$^{13}C_4$]heptanoic acid	$^{13}C_4$-PFHpA	
Perfluoro-n-[$^{13}C_8$]octanoic acid	$^{13}C_8$-PFOA	
Perfluoro-n-[$^{13}C_9$]nonanoic acid	$^{13}C_9$-PFNA	
Perfluoro-n-[1,2,3,4,5,6-$^{13}C_6$]decanoic acid	$^{13}C_6$-PFDA	
Perfluoro-n-[1,2,3,4,5,6,7-$^{13}C_7$]undecanoic acid	$^{13}C_7$-PFUnA	
Perfluoro-n-[1,2-$^{13}C_2$]dodecanoic acid	$^{13}C_2$-PFDoA	
Perfluoro-n-[1,2-$^{13}C_2$]tetradecanoic acid	$^{13}C_2$-PFTeDA	
Perfluoro-1-[2,3,4-$^{13}C_3$]butanesulfonic acid	$^{13}C_3$-PFBS	
Perfluoro-1-[1,2,3-$^{13}C_3$]hexanesulfonic acid	$^{13}C_3$-PFHxS	
Perfluoro-1-[$^{13}C_8$]octanesulfonic acid	$^{13}C_8$-PFOS	
Perfluoro-1-[$^{13}C_8$]octanesulfonamide	$^{13}C_8$-PFOSA	
N-methyl-d$_3$-perfluoro-1-octanesulfonamidoacetic acid	D$_3$-NMeFOSAA	
N-ethyl-d$_5$-perfluoro-1-octanesulfonamidoacetic acid	D$_5$-NEtFOSAA	
1*H*,1*H*,2*H*,2*H*-Perfluoro-1-[1,2-$^{13}C_2$]hexane sulfonic acid	$^{13}C_2$-4:2FTS	
1*H*,1*H*,2*H*,2*H*-Perfluoro-1-[1,2-$^{13}C_2$]octane sulfonic acid	$^{13}C_2$-6:2FTS	
1*H*,1*H*,2*H*,2*H*-Perfluoro-1-[1,2-$^{13}C_2$]decane sulfonic acid	$^{13}C_2$-8:2FTS	
Tetrafluoro-2-heptafluoropropoxy-$^{13}C_3$-propanoic acid	$^{13}C_3$-HFPO-DA	
N-methyl-d$_7$-perfluorooctanesulfonamidoethanol	D$_7$-NMeFOSE	
N-ethyl-d$_9$-perfluorooctanesulfonamidoethanol	D$_9$-NEtFOSE	
N-ethyl-d$_5$-perfluoro-1-octanesulfonamide	D$_5$-NEtFOSA	
N-methyl-d$_3$-perfluoro-1-octanesulfonamide	D$_3$-NMeFOSA	
NIS Compounds		
Perfluoro-n-[2,3,4-$^{13}C_3$]butanoic acid	$^{13}C_3$-PFBA	NA
Perfluoro-n-[1,2,3,4-$^{13}C_4$]octanoic acid	$^{13}C_4$-PFOA	
Perfluoro-n-[1,2-$^{13}C_2$]decanoic acid	$^{13}C_2$-PFDA	
Perfluoro-n-[1,2,3,4-$^{13}C_4$]octanesulfonic acid	$^{13}C_4$-PFOS	
Perfluoro-n-[1,2,3,4,5-$^{13}C_5$] nonanoic acid	$^{13}C_5$-PFNA	
Perfluoro-n-[1,2-$^{13}C_2$]hexanoic acid	$^{13}C_2$-PFHxA	
Perfluoro-1-hexane[$^{18}O_2$]sulfonic acid	$^{18}O_2$-PFHxS	

[1] The target analyte names are for the acid and neutral forms of the analytes. See Table 2 for the names and CASRN of the corresponding anion forms, where applicable.

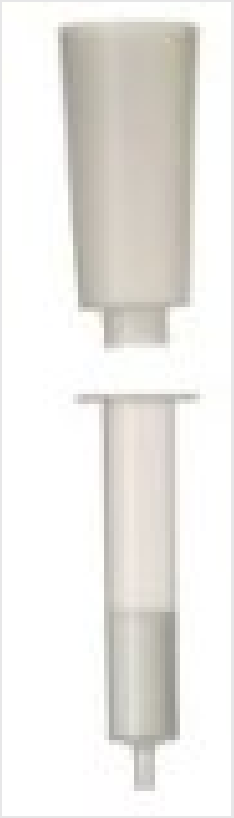
NA Not assigned a CASRN

* Analytes in this class may not perform as well as others (see Section 1.6)

1633 advantages

- Transfer lines are not used and silanized glass wool is added to cartridges, so there is reduced risk of clogging
- A specific procedure is written for when cartridges do clog
- GCB cleanup on extract
- Prescreening of all samples
- Samples with >50mg TSS are rejected
- Subsampling is allowed
- Extracts are filtered prior to instrumental analysis
- Extract concentration step is eliminated (no “blow-down”)
- 12-minute run time
- Bile acid calibration

Sample Loading



QA/QC parameters

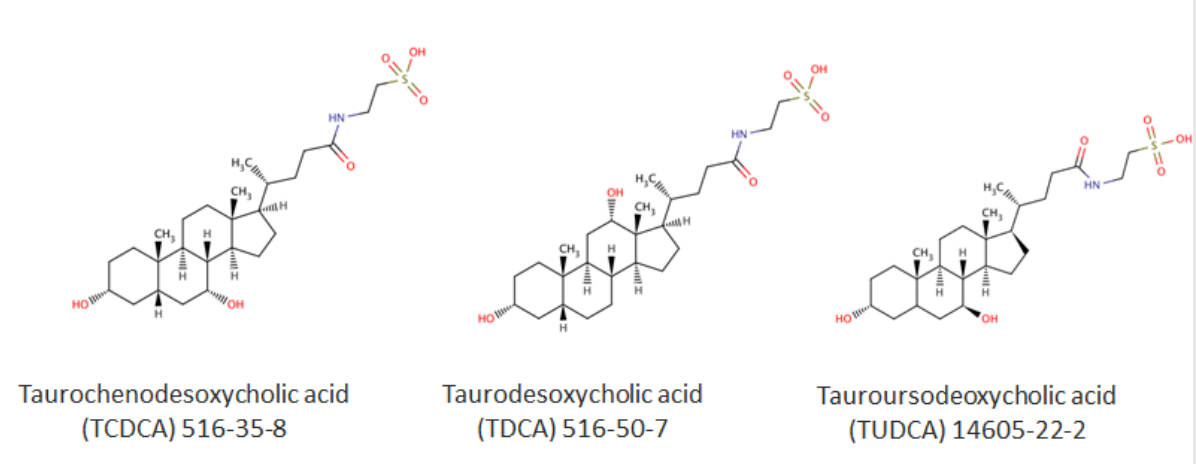
Table 5. IPR/OPR/LLOPR Acceptance Limits for Wastewater Samples

	Aqueous Matrix [1, 2]			
	IPR			
Compounds	Recovery (%)	RSD (%)	OPR Recovery (%)	LLOPR Recovery (%)
PFBA	60 – 147	20	58 – 148	44 - 157
PFPeA	56 – 150	20	54 – 152	57 - 148
PFHxA	59 – 148	25	55 – 152	62 - 149
PFHpA	60 – 149	25	54 – 154	56 - 150
PFOA	55 – 158	25	52 – 161	57 - 161
PFNA	64 – 144	25	59 – 149	53 - 157
PFDA	57 – 142	25	52 – 147	43 - 158
PFUnA	54 – 153	30	48 – 159	50 - 155
PFDoA	73 – 133	25	64 – 142	60 - 141
PFTrDA	52 – 145	25	49 – 148	52 - 140
PFTeDA	49 - 158	25	47 – 161	52 - 156
PFBS	66 – 141	20	62 – 144	63 - 145
PFPeS	66 – 144	25	59 – 151	58 - 144
PFHxS	62 – 141	25	57 – 146	44 - 158
PFHpS	59 – 148	25	55 – 152	51 - 150
PFOS	61 – 145	20	58 – 149	43 - 162
PFNS	57 – 143	25	52 – 148	46 - 151
PFDS	56 – 142	25	51 – 147	50 - 144
PFDoS	41 – 140	30	36 – 145	30 - 138
4:2FTS	77 – 135	25	67 – 146	52 - 158
6:2FTS	75 – 137	30	61 – 151	48 - 158
8:2FTS	79 – 136	30	63 – 152	46 - 165
PFOSA	65 – 144	20	61 – 148	47 - 163
NMeFOSA	76 – 132	25	63 – 145	54 - 155
NEtFOSA	75 – 129	25	65 – 139	49 - 156
NMeFOSAA	69 – 134	25	58 – 144	32 - 160
NEtFOSAA	65 – 140	25	59 – 146	51 - 154
NMeFOSE	79 – 129	20	71 – 136	56 - 151
NEtFOSE	79 – 126	25	69 – 137	60 - 147
HFPO-DA	72 – 135	25	63 – 144	58 - 154
ADONA	75 – 138	20	68 – 146	61 - 148
PFMPA	55 – 141	25	51 – 145	48 - 150
PFMBA	59 – 145	20	55 – 148	49 - 154
NFDHA	63 – 146	35	48 – 161	47 - 160
9Cl-PF3ONS	72 – 140	30	56 – 156	44 - 167
11Cl-PF3OUdS	61 – 140	35	46 – 156	36 - 158
PFEESA	57 – 149	20	56 – 151	56 - 144
3:3FTCA	66 – 126	20	62 – 129	32 - 161
5:3FTCA	68 – 130	20	63 – 134	39 - 156
7:3FTCA	55 – 133	25	50 – 138	36 - 149
$^{13}C_4$-PFBA	10 – 130	30	10-130	10-130
$^{13}C_5$-PFPeA	35 – 150	30	40 -150	40 -150
$^{13}C_5$-PFHxA	55 – 150	30	40 -150	40 -150
$^{13}C_4$-PFHpA	55 – 150	30	40 -150	40 -150
$^{13}C_8$-PFOA	60 – 140	30	30-140	30-140
$^{13}C_9$-PFNA	55 – 140	30	30-140	30-140
$^{13}C_6$-PFDA	50 – 140	30	20-140	20-140

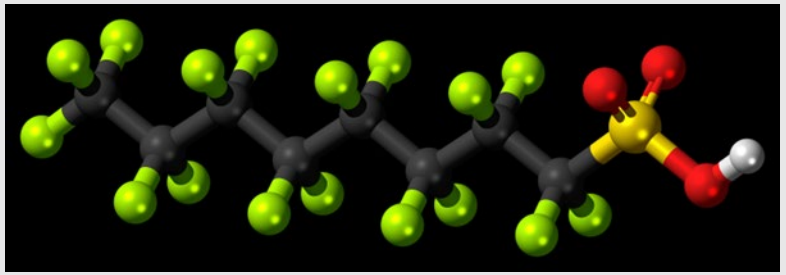
Bile Acid Calibration

QC Check	Minimum Frequency	Acceptance Criteria	
Ion Transitions (Precursor-> Product)	Every field sample, standard, blank, and QC sample.	In order to avoid biasing results high due to known interferences for some transitions, the following transitions must be used for the quantification of the following analytes: PFOA: 413 → 369 PFOS: 499 → 80 PFHxS: 399 → 80 PFBS: 299 → 80 4:2 FTS: 327 → 307 6:2 FTS: 427 → 407 8:2 FTS: 527 → 507 NEtFOSAA: 584 → 419 NMeFOSAA: 570 → 419 If these transitions are not used, the reason must be technically justified and documented (e.g., alternate transition was used due to observed interferences).	N

Bile Acid Calibration



SGS Axys. Bile Acids Interferences in PFAS Analysis - SGS AXYS(Accessed 2023-04-18)



Wikimedia Commons. https://commons.wikimedia.org/wiki/File:Perfluorooctanesulfonic-acid-3D-balls.png (Accessed 2023-04-18)

- PFOS: 500.119 amu
- Bile salts: 499.2 amu

EPA 1633 Implementation Challenges

- Sub 2um column is used, so UHPLC instrumentation is required (~16,000 psi)
- Standards are sold as multiple individual mixes
- Additional consumables and solutions are required and must be tested for PFAS interference/enhancement/suppression
- Additional analytes means tedious data analysis
- EPA has not finalized the method

UHPLC

- HPLC that operates in excess of 6,000 psi
- >2um column with high flow rate
- Greater separation and resolution are achieved with a shorter run time

1633 Development at ELI

- Setup of the new Shimadzu UHPLC was completed April 25th
- The UHPLC will be integrated with the mass spec the week of May 8th
- Compound optimization on Mass Spec will be completed week of May 15th
- Initial MDL study to follow
- We plan on accepting 1633 samples by July 8th, 2023, barring accreditation/approvals

Thank you

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