



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

UCT Products ECDVB156P (6 mL cartridge with PE frits containing 500mg SDVB)

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Method Summary

A 250-mL water sample is spiked with surrogates then passed through a solid phase extraction (SPE) cartridge containing polystyrenedivinylbenzene (SDVB). The compounds are eluted from the solid phase with methanol. The extract is concentrated to dryness with N₂ then adjusted to a 1-mL volume with 96:4 methanol: water (vol/vol %) after adding the internal standards (IS). A 10-μL injection is made into an LC with a C18 column interfaced to an MS/MS.

Method 537 Analytes

Analyte	Acronym	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	PFTTrDA	72629-94-8
Perfluoroundecanoic acid	PFUnA	2058-94-8

Interferences

- All glassware must be meticulously cleaned. Wash glassware with detergent and tap water, rinse with tap water, followed by a reagent water rinse
- Non-volumetric glassware can be heated in a muffle furnace at 400° C for 2 hours or solvent rinsed
- Volumetric glassware should be solvent rinsed. Do not heat in an oven above 120° C
- Store clean glassware inverted or capped
- **Do not cover glassware with aluminum foil because PFAAs can be potentially transferred from the aluminum foil**

Note: PFAA standards, extracts and samples should not come in contact with any glass containers or pipettes as these analytes can potentially adsorb to glass

- PFAA analyte, IS and SUR standards commercially purchased in glass ampoules are acceptable however all subsequent dilutions must be prepared and stored in polypropylene containers
- Method interferences may be caused by contaminants in solvents, reagents (including reagent water), sample bottles and caps, and other sample processing hardware
- The method analytes in this method can also be found in many common laboratory supplies and equipment, such as PTFE (polytetrafluoroethylene) products, LC solvent lines, methanol, aluminum foil and SPE sample transfer lines. These items must be routinely demonstrated to be free from interferences (less than 1/3 the MRL for each method analyte) under the conditions of the analysis by analyzing laboratory reagent blanks as described in Section 9.3.1
- **Subtracting blank values from sample results is not permitted**
- Matrix interferences may be caused by contaminants that are co-extracted from the sample and will vary considerably from source to source
- Humic and/or fulvic material can be co-extracted during SPE and high levels can cause enhancement and/or suppression in the electrospray ionization source or low recoveries. Total organic carbon (TOC) is a good indicator of humic content of the sample

Sample Collection, Preservation, and Storage

- Samples must be collected in a 250-mL polypropylene bottle fitted with a polypropylene screw-cap
- The preservation reagent, listed below, is added to each sample bottle as a solid prior to shipment to the field
- The sample handler must wash their hands before sampling and wear nitrile gloves while filling and sealing the sample bottles.
- Open the tap and allow the system to flush until the water temperature has stabilized (approximately 3 to 5 min)
- Fill sample bottles, taking care not to flush out the sample preservation reagent
- After collecting the sample, cap the bottle and agitate until preservative is completely dissolved

Preservation Reagent

Compound	Amount	Purpose
Trizma®*	5.0 g/L	Buffer & free Cl removal

* Synonym: TRIS HCl (Tris(hydroxymethyl)aminomethane hydrochloride) CASRN 1185-53-1

SAFETY

- The toxicity or carcinogenicity of each reagent used in this method has not been defined. Each chemical should be treated as a potential health hazard
- PFOA has been described as “likely to be carcinogenic to humans.” Pure standard materials and stock standard solutions of these method analytes should be handled with suitable protection to skin and eyes, and care should be taken not to breathe the vapors or ingest the materials

Internal Standards and Surrogates

Internal Standards Table

Internal Standards	Acronym
Perfluoro-[1,2-¹³C₂]octanoic acid	¹³ C-PFOA
Sodium perfluoro-1-[1,2,3,4-¹³C₄]octanesulfonate	¹³ C-PFOS
N-deuteriomethylperfluoro-1-octanesulfonamidoacetic acid	d ₃ -NMeFOSAA

Internal Standard (IS) Primary Dilution Table

IS	Conc. of IS Stock (µg/mL)	Vol. Of IS Stock (µL)	Final Vol. of IS PDS (µL)	Final Conc. of IS PDS (ng/µL)
¹³C-PFOA	1000	5.0	5000	1.0
¹³C-PFOS	50	300.0	5000	3.0
d₃-NMeFOSAA	50	400.0	5000	4.0

Surrogates (SUR) Table

Surrogates	Acronym
Perfluoro-n-[1,2- ¹³ C ₂]hexanoic acid	¹³ C-PFHxA
Perfluoro-n-[1,2- ¹³ C ₂]decanoic acid	¹³ C-PFDA
N-deuterioethylperfluoro-1-octanesulfonamidoacetic acid	d ₅ -NEtFOSAA

Surrogate Primary Dilution Table

SUR	Conc. Of SUR Stock (µg/mL)	Vol. of SUR Stock (µL)	Final Vol. of SUR PDS (µL)	Final Conc. Of SUR PDS (ng/µL)
¹³ C-PFHxA	50	100.0	5000	1.0
¹³ C-PFDA	50	100.0	5000	1.0
d ₅ -NEtFOSAA	50	400.0	5000	4.0

IS Stock Standard Solutions

IS stocks solutions can be obtained as individual certified stock standards from Wellington Labs, Perkin Elmer or equivalent. Analysis of the IS is less complicated if the IS purchased contains only the linear isomer. IS stock standard solutions are stable for at least 6 months when stored at 4 °C.

Internal Standard Primary Dilution (IS PDS) Standard

Prepare (or purchase) IS PDS at a suggested concentration of 1-4 ng/µL in 96:4% (vol/vol) methanol:water. Use 10 µL of this 1-4 ng/µL solution to fortify the final 1-mL extracts (Sect. 11.5). This will yield a concentration of 10-40 pg/µL of each IS in the 1-mL extracts.

Analyte Solvent Dilution Table

Analyte	Analyte Stock Solvent
PFHxA	96:4% (vol/vol) methanol:water
PFHpA	96:4% (vol/vol) methanol:water
PFOA	96:4% (vol/vol) methanol:water
PFNA	96:4% (vol/vol) methanol:water
PFDA	96:4% (vol/vol) methanol:water
PFUnA	96:4% (vol/vol) methanol:water
PFDoA	96:4% (vol/vol) methanol:water
PFTTrDA	100% ethyl acetate
PFTA	100% ethyl acetate
PFBS	100% methanol
PFHxS	100% methanol
PFOS	100% methanol
NEtFOSAA	100% methanol
NMeFOSAA	100% methanol

Procedure

1) Cartridge Preparation

- a. Insert a **ECDVB156P** cartridge in a vacuum manifold or automated extraction system
- b. Add 15 mL of methanol to the cartridge and slowly draw through under vacuum

Note: Do not let the cartridge dry out after addition of methanol otherwise start over

- c. Add 18 mL of reagent water
- d. Draw through under vacuum but do not let water level drop below cartridge frit

2) Sample Extraction

- a. Adjust vacuum setting to achieve a flow rate of about 10-15 mL/min
- b. Draw water through sample cartridge
- c. After sample extraction, rinse sample bottles and reservoir with 2 x 7.5 mL aliquots of reagent water and add to cartridge
- d. Dry cartridge by drawing air through it for 5 minutes at high vacuum

3) Sample Elution

- a. Insert a clean collection tube in the vacuum manifold
- b. Rinse sample bottle and reservoir with 4 mL of methanol and add to cartridge
- c. Draw through cartridge in dropwise manner
- d. Rinse sample bottle and reservoir with another 4 mL of methanol and add to the cartridge
- e. Draw through cartridge in dropwise manner

4) Extract Concentration

- a. Concentrate the extract to dryness using a gentle stream of N₂ in a heated water bath 60-65° C
- b. Add the appropriate amount of 96:4% (vol/vol) methanol:water solution and the IS PDS to the collection vial
- c. Bring the volume to 1 mL and vortex
- a. Transfer a small aliquot with a plastic pipette to a polypropylene autosampler vial

NOTE: Do not transfer the entire 1-mL aliquot to the autosampler vial because the polypropylene autosampler caps do not reseal after injection. Do not store the extracts in the autosampler vials as evaporation losses can occur. Extracts can be stored in 15-mL centrifuge tubes

5) HPLC Analysis Conditions

LC Method Conditions

Time (min)	% 20 mM	% Methanol
Initial	60.0	40.0
1.0	60.0	40.0
25.0	10.0	90.0
32.0	10.0	90.0
32.1	60.0	40.0
37.0	60.0	40.0
Flow rate of 0.3 mL/min		10 µL injection
Waters Atlantis® dC ₁₈ 2.1 x 150 mm packed with 5.0 µm C ₁₈ stationary phase or equivalent		

ESI-MS Conditions

Polarity	Negative Ions
Capillary Needle Voltage	-3 kV
Cone Gas Flow	98 L/hr
Nitrogen Desolvation Gas	1100 L/hr
Desolvation Gas Temperature	350° C

MS/MS Method Conditions					
Segment	Analyte	Precursor Ion (m/z)	Product Ion (m/z)	Cone Voltage	Collision Energy (v)
1	PFBS	299	80	40	25
2	PFHxA	313	269	15	10
3	PFHpA	363	319	12	10
3	PFHxS	399	80	40	40
4	PFOA	413	369	15	10
4	PFNA	463	419	12	10
4	PFOS	499	80	40	40
5	PFDA	513	469	15	10
5	NMeFOSAA	570	419	25	20
5	NEtFOSAA	584	419	25	20
5	PFUnA	563	519	15	10
5	PFDoA	613	569	15	10
6	PFTA	663	619	15	10
6	PFTA	713	669	15	10
2	¹³ C-PFHxA	315	270	15	10
5	¹³ C-PFDA	515	470	12	12
5	d ₅ -NEtFOSAA	589	419	25	20
4	¹³ C-PFOA	415	370	15	10
4	¹³ C-PFOS	503	80	40	40
5	d ₃ -NMeFOSAA	573	419	25	20

Summarized from: Shoemaker, J.A., Grimmett P.E., Boutin, B.K., 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, National Exposure Research Laboratory, Office Of Research And Development, U. S. Environmental Protection Agency, Cincinnati, Ohio 45268

Listing of instrument manufacturers does not constitute endorsement by UCT

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