UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON D.C., 20460

Analytical Chemistry Branch
701 Mapes Road
Ft. Meade, Maryland 20755-5350

OFFICE OF
CHEMICAL SAFETY AND
POLLUTION PREVENTION

September 28, 2021

MEMORANDUM

SUBJECT: EPA's Analytical Chemistry Branch Method for the Analysis of PFAS in Oily Matrix.
ACB Project B21-02

FROM: Thuy Nguyen, Chief
Analytical Chemistry Branch
Biological and Economic Analysis Division

To: Kimberly Nesci, Director
Biological and Economic Analysis Division
EPA Office of Pesticide Programs

The EPA's Analytical Chemistry Branch (ABC) Laboratory at Fort Meade has developed and validated a method for the analysis of twenty-eight (28) per-and polyfluoroalkyl substance (PFAS) compounds (listed in **Appendix I**) extracted from oily matrices. This memorandum describes the processing and analysis method for these PFAS compounds from samples of oily matrix, such as pesticide products formulated in oil, petroleum distillates, or mineral oils. The method limit of quantitation is 0.025 ppb for most of the analytes, based on the lowest level validated at the ACB laboratory.

METHOD SUMMARY

Briefly, oily samples are passed through a Florisil solid phase extraction (SPE) cartridge and the oily matrix is washed off the SPE by a mixed solvent of hexane and ethyl acetate (9/1, v/v). The PFAS compounds are eluted from the SPE with methanol/acetone mixture (9/1, v/v). The collected eluate samples are concentrated and analyzed with a liquid chromatography tandem mass spectrometer (LC-MS/MS). The instrumental analysis of samples with LC-MS/MS follows the

analysis described in EPA Method 537.1[1]. Isotopically labeled internal standards are used for quantitation. Isotopically labeled extraction standards are added prior to sample processing and the recoveries from each sample are monitored. Detailed information of the method, including sample preparation and analysis can be found in **Appendix II.**

METHOD VALIDATION SUMMARY

A single laboratory validation of the method was conducted at the ACB using a control oil matrix (middle distillate of crude oil). Aliquots of the control oil sample were fortified with PFAS analytes at four different concentration levels between 0.025 and 0.630 ppb in replicates (triplicate at two levels and quintuplicate at two other levels). Recoveries of the PFAS analytes and the isotopically labeled extraction standards were evaluated to determine the performance of the method.

Matrix enhancement was present for some analytes. The recoveries of these analytes, including their respective isotopically labeled extraction standards, are consistently high (greater than 120%) at every level of fortification when calibration standards in solvent (no matrix present) are used for quantitation. **Table 1** is the summary of the analyte recoveries obtained from the single lab method validations. Only the analytes listed in **Table 1** have been validated by the ACB.

Because the method is validated thus far only at the ACB, it is highly recommended that a method validation be conducted the first time a laboratory uses this method and/or if additional analytes are added to this method. Minor modifications may be necessary if acceptable results cannot be achieved during validation.

NOTE: As mentioned above, this method was validated at ACB using a clean oil matrix. When analyzing a product formulated in oil, petroleum distillates, or mineral oils, matrix interference is expected. Therefore, it is recommended that any detection of PFAS at low levels (around or below 10 times the reported limits of quantitation) be confirmed using different analytical techniques. Otherwise, the compound(s) should be reported as "tentatively identified." The ACB relies on a high resolution accurate mass (HRAM) Mass Spectrometer coupled with liquid chromatography (LC-MS) for confirmation. The instrument parameters for this confirmation technique are listed in **Appendix II**.

[1] https://cfpub.epa.gov/si/si_public_record_Report.cfm?dirEntryId=343042&Lab=NERL

Table 1. ACB Method Validation Study Results -
Summary of the recoveries of selected PFAS compounds and their labeled extraction standards from a fortified oil matrix at different concentration levels

Targeted PFAS compounds

	Average recoveries (%) from each fortification level				
Analyte	***0.025 ppb***	***0.050 ppb***	***0.126 ppb***	***0.630 ppb***	***Average***
PFBA	24%	51%	31%	71%	44%
PFBS	211%	201%	120%	139%	168%
PFPeA	80%	59%	30%	58%	57%
PFPeS	25%	33%	80%	107%	61%
PFHxA	0%	62%	61%	60%	45%
PFHxS	119%	118%	147%	175%	140%
PFHpA	101%	94%	65%	65%	81%
PFHpS	69%	74%	95%	120%	90%
PFOA	61%	50%	162%	157%	107%
PFOS	82%	90%	127%	110%	102%
PFNA	73%	80%	68%	61%	71%
PFNS	96%	97%	178%	136%	127%
PFDA	87%	94%	91%	101%	93%
PFDS	116%	121%	215%	224%	169%
PFUdA	80%	84%	136%	162%	115%
PFDoA	85%	97%	154%	216%	138%
PFDoS	109%	117%	219%	210%	164%
PFTrDA	69%	79%	149%	148%	111%
PFTeDA	80%	95%	140%	152%	117%
PFHxDA	122%	146%	133%	178%	145%
PFODA	158%	169%	398%	382%	277%
FOSAA	41%	65%	153%	104%	91%
N-MeFOSAA	76%	89%	98%	99%	91%
N-EtFOSAA	20%	31%	93%	107%	63%
HFPO-DA	69%	79%	57%	60%	67%
NaDONA	36%	44%	34%	34%	37%
9Cl-PF3ONS	102%	112%	159%	179%	138%
11Cl-PF3OUdS	69%	75%	124%	125%	98%

Labeled Extraction Standards

Average recoveries (%) from each fortification level

Analyte	*0.025 ppb*	*0.050 ppb*	*0.126 ppb*	*0.630 ppb*	*Average*
M2PFTeDA	84%	84%	139%	154%	115%
M3PFBS	166%	160%	157%	166%	162%
M3PFHxS	121%	115%	122%	149%	127%
M4PFHpA	103%	89%	77%	64%	83%
M5PFHxA	140%	124%	71%	69%	101%
M5PFPeA	84%	72%	49%	46%	63%
M6PFDA	92%	97%	121%	110%	105%
M7PFUdA	75%	68%	122%	127%	98%
M8PFOA	44%	42%	115%	109%	78%
M8PFOS	91%	96%	113%	111%	102%
M9PFNA	84%	83%	87%	70%	81%
MPFBA	85%	79%	92%	92%	87%
MPFDoA	102%	104%	224%	210%	160%
M NMeFOSAA	94%	98%	126%	102%	105%
M NEtFOSAA	35%	36%	106%	101%	69%
M3HFPO-DA	63%	60%	52%	43%	54%

APPENDICES

I. Full names and CAS numbers of analytes.

II. Sample preparation and analysis of selected PFAS from oily matrices

APPENDIX I -

CHEMICAL ABSTRACTS SERVICE (CAS) REGISTRY NUMBERS and CHEMICAL NAMES

Analyte	CAS #	Name
PFBA	375-22-4	Perfluorobutanoic Acid
PFBS	375-73-5	Perfluorobutanesulfonic Acid
PFPeA	2706-90-3	Perfluoropentanoic Acid
PFPeS	2706-91-4	Perfluoropentanesulfonic Acid
PFHxA	307-24-4	Perfluorohexanoic Acid
PFHxS	355-46-4	Perfluorohexanesulfonic Acid
PFHpA	375-85-9	Perfluoroheptanoic Acid
PFHpS	375-92-8	Perfluoroheptanesulfonic Acid
PFOA	335-67-1	Perfluorooctanoic Acid
PFOS	1763-23-1	Perfluorooctanesulfonic Acid
PFNA	375-95-1	Perflurononanoic Acid
PFNS	68259-12-1	Perfluorononanesulfonic Acid
PFDA	335-76-2	Perfluorodecanoic Acid
PFDS	335-77-3	Perfluorodecanesulfonic Acid
PFUdA	2058-94-8	Perfluoroundecanoic Acid
PFDoA	307-55-1	Perfluorododecanoic Acid
PFDoS	79780-39-5	Perfluorododecanesulfonic Acid
PFTrDA	72629-94-8	Perfluorotridecanoic Acid
PFTeDA	376-06-7	Perfluorotetradecanoic Acid
PFHxDA	67905-19-5	Perflurohexadecanoic Acid
PFODA	16517-11-6	Perfluorooctadecanoic Acid
FOSAA	2806-24-8	Perfluorooctane sulfonamidoacetic Acid
N-MeFOSAA	2355-31-9	N-Methyl Perfluorooctane sulfonoamidoacetic Acid
N-EtFOSAA	2991-50-6	N-Ethyl Perfluorooctane sulfonoamidoacetic Acid
HFPO-DA	13252-13-6	Hexafluoropropylene oxide dimer acid
NaDONA	958445-44-8	Sodium dodecafluoro-3H-4,8-dioxanonanoate
9Cl-PF3ONS	756426-58-1	9-chlorohexadecafluoro-3-oxanonane-1-sulfonic acid
11Cl-PF3OUdS	763051-92-9	11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid

Appendix II -

Sample Preparation and Analysis of Selected PFAS from Oily Matrices

Section 1. SAMPLE PREPARATION

1. Measure 4 g (about 5 ml) of samples into a 15 ml polypropylene tube.
2. Prepare a procedural blank and a laboratory blank spike (LBS) samples (5 ml of hexane each).
3. Prepare a matrix spike (MS) sample with one of the samples.
4. Fortify each sample with 50 µl of extraction standards (PFAC-ES-W, 10 ng/ml, isotopically labeled PFAS standards).
5. Fortify the appropriate samples (LBS, MS) with PFAS standard (PFAC-MXC-W, 10 ng/ml, native PFAS).
6. Mix by vortex.
7. Prepare Florisil SPE (1 g) on a SPE manifold. The Florisil SPE cartridges should have non-PFTE frits (e.g., BondElut). Ensure the SPE manifold does not have PTFE valves and dripping tubes.
8. Condition the Florisil SPE by passing through 15 ml of 1% acetic acid in methanol/acetone (9/1, v/v), followed by 5 ml of hexane/ethyl acetate (9/1, v/v).
9. Load the samples on to the SPE. Apply a vacuum to the manifold to ensure the flow through the SPE is dropwise (1-2 ml/min).
10. After all the samples pass through the SPE (do not let the SPE goes to dry), rinse the sample tubes with three aliquots of 4 ml of hexane/ethyl acetate (9/1, v/v). Each time transfer the rinse solution to the SPE and let the hexane/ethyl acetate pass through the SPE. Continue to pull the vacuum to ensure all solvent has passed through (no more dripping).
11. Place 15 ml polypropylene collection tubes under the SPE. Add 10 ml of 1% of acetic acid in methanol/acetone (9/1, v/v) to each SPE and collect the eluant.
12. Remove the collected samples from the manifold and concentrate to dryness/near dryness under a stream of N_2 in a water bath (40-50^{o}C).
13. Add 50 µl of internal standard (PFAC-IS-W, 10 ng/ml) to each sample.
14. Add about 400 µl of 0.1% acetic acid in methanol/water (9/1, v/v) to each sample and vortex.
15. Transfer the samples to polypropylene autosampler vials with polyethylene caps for instrumental analysis, either by liquid chromatography tandem mass spectrometer (LC-MS/MS) (**Section 2.**) or by high resolution accurate mass (HRAM) mass spectrometer coupled with liquid chromatography (HRAM LC-MS, **Section 3.**)
16. A 0.2 µm nylon syringe filter may be used if samples look cloudy and need filtration.

SECTION 2. SAMPLE ANALYSIS by LC-MS/MS

Calibration Standards

Standard materials were purchased from commercial sources. Calibration standards are made in methanol/water (95/5, v/v, with 0.1% acetic acid) in the range of 0.02 ng/ml to 20 ng/ml. Prepared calibration standards contain all native, extraction standard and internal standard analytes.

Instrument Analysis

Sample analysis is performed with a liquid chromatography tandem mass spectrometer (LC-MS/MS), following EPA method 537.1 "*Determination of Selected Per- and Polyfluorinated Alkyl Substances in Drinking Water by Solid Phase Extraction and Liquid Chromatography / Tandem Mass Spectrometry (LC-MS/MS)*".

A Waters Acquity BEH C_{18} column (2.1 mm x 100 mm, 1.7 µm) and a gradient of 5 mM ammonium acetate aqueous solution / 5 mM ammonium acetate in acetonitrile mobile phases were used for compound separation. The plumbing of the LC has been replaced with PEEK tubing. A short trap column is placed between the pump head and autosampler valve. Mass spectrometer is operated under ESI- multiple reaction monitoring mode. Instrument parameters for LC and MS/MS are listed in **Table 1** and **Table 2,** respectively. Monitored MS transitions are listed in **Table 3**.

Table 1. LC Instrument Parameters.

LC			
Column	Waters Acquity BEH C18 column (2.1 mm x 100 mm, 1.7 µm)		
Injection Volume	20 µL		
Column Temp.	35ºC		
Flow rate	300 µL/min.		
Mobile Phases & Gradient	**Time (min.)**	**5 mM ammonium acetate in water**	**5 mM ammonium acetate in acetonitrile**
	0	90%	10%
	4	75%	25%
	6	50%	50%
	11	5%	95%
	15	5%	95%
	17	90%	10%
	22	90%	10%

Table 2. MS/MS Instrument Parameters

MS/MS	
Polarity	Negative
Ionization	ESI
Scan type	MRM
Curtain gas	30 psi
Ionspray voltage	-4,500 V
Nebulizer current	2.85 µA
Source temperature	450ºC
Ion source gas 1	45 psi
Ion source gas 2	40 psi

Table 3. Analyte Monitored MS Transitions.

Compound	Parent mass	Product mass	DP	CE	CXP
Natives					
PFBA	213	169	-40	-14	-17
PFBS	299	80	-70	-66	-9
	299	99	-70	-36	-11
PFPeA	263	219	-25	-12	-11
PFPeS	349	80	-80	-72	-9
	349	99	-80	-38	-11
PFHxA	313	269	-15	-14	-11
	313	119	-15	-26	-15
PFHxS	399	80	-75	-90	-9
	399	99	-75	-78	-11
PFHpA	363	319	-60	-14	-55
	363	169	-60	-24	-19
PFHpS	449	80	-115	-102	-19
	449	99	-115	-86	-11
PFOA	413	369	-5	-14	-9
	413	169	-5	-22	-17
PFOS	499	80	-80	-108	-9
	499	99	-80	-60	-11
PFNA	463	419	-35	-14	-21
	463	219	-35	-24	-13

Compound	Parent mass	Product mass	DP	CE	CXP
PFNS	549	80	-85	-120	-9
	549	99	-85	-112	-13
PFDA	513	469	-45	-16	-27
	513	269	-45	-26	-33
PFDS	599	80	-65	-132	-9
	599	99	-65	-116	-11
PFUdA (PFUnA)	563	519	-40	-16	-27
	563	169	-40	-30	-15
PFDoA	613	569	-30	-32	-27
	613	169	-30	-18	-15
PFDoS	699	80	-40	-168	-21
PFTrDA	663	619	-50	-18	-31
	663	169	-50	-36	-19
PFTeDA	713	669	-50	-18	-33
	713	169	-50	-34	-17
PFHxDA	813	769	-60	-22	-27
PFODA	913	869	-20	-15	-15
FOSAA	556	498	-65	-44	-29
N-MeFOSAA	570	419	-20	-26	-23
	570	483	-20	-24	-35
N-EtFOSAA	584	419	-30	-30	-25
	584	483	-30	-24	-39
HFPO-DA	329	169	-15	-18	-15
	329	285	-15	-8	-17
NaDONA	377	251	-30	-16	-13
	377	85	-30	-34	-9
9Cl-PF3ONS	531	351	-50	-36	-19
	531	99	-50	-76	-11
11Cl-PF3OUdS	631	451	-50	-40	-25
	631	83	-50	-86	-9

Extraction Standards					
Compound	Parent mass	Product mass	DP	CE	CXP
M2PFTeDA	715	670	-35	-18	-55
M3PFBS	302	99	-60	-36	-15
M3PFHxS	402	99	-65	-74	-11
M5PFPeA	268	223	-50	-22	-13
M6PFDA	519	474	-25	-14	-27
M7PFUdA	570	525	-25	-16	-13
M8PFOA	421	376	-20	-12	-45
M8PFOS	507	80	-50	-110	-9
M9PFNA	472	427	-25	-14	-27
MPFBA	217	172	-60	-14	-15
MPFDoA	615	570	-35	-18	-39
M NMeFOSAA	573	419	-45	-28	-11
M NEtFOSAA	589	419	-5	-28	-23
M3HFPO-DA	332	287	-5	-8	-17
Internal Standards					
M3PFBA	216	172	-35	-6	-11
MPFDA	515	470	-40	-16	-27
M2PFOA	415	370	-20	-14	-21
MPFOS	503	99	-10	-106	-41
	503	80	-10	-110	-9

Quantitation is based on internal standard method. The recoveries of the isotopically labelled extraction standards from each sample, in addition to the recoveries of spiked compounds from LBS and matrix spike, are monitored.

SECTION 3. SAMPLE ANALYSIS by HRAM LC-MS

This is an alternative instrumental method for analysis of the perfluoroalkyl substances (PFAS), as compared with the traditional technique using LC-MS/MS and can be used to confirm presence of PFAS at low levels (around or below 10 times the LC-MS/MS reported limits of quantitation). Equivalent analytical columns and materials may be used in place of those described in this method. The instrument parameters may be modified to enhance the performance of the method.

Calibration Standards (same as that in Section 2)

Standard materials were purchased from commercial sources. Calibration standards are made in methanol/water (95/5, v/v, with 0.1% acetic acid) in the range of 0.02 ng/ml to 20 ng/ml. Prepared calibration standards contain all native, extraction standard and internal standard analytes.

Instrument Analysis

Sample analysis is performed with a Q-Exactive™ HF high resolution accurate mass (HRAM) mass spectrometer coupled with liquid chromatography.

A Thermo Hypersil GOLD column (2.1 mm x 100 mm, 1.9 µm particle size) and a gradient of 5 mM ammonium acetate aqueous solution / 5 mM ammonium acetate in acetonitrile mobile phases were used for compound separation. A short trap column (Waters XBridge C_{18} 3.5 µm, 2.1mm x 50 mm) is placed between the pump head and autosampler. Mass spectrometer is operated in negative ESI mode and the data were acquired using Full MS and $ddMS^2$ methods. Instrument parameters for LC and MS/MS are listed in **Table 4** and **Table 5,** respectively. Quantitation and confirmation masses are listed in **Table 6**.

Table 4. LC Parameters.

Column	Thermo Hypersil GOLD column (2.1 mm x 100 mm, 1.9 µm particle size)		
Injection Volume	5 µL		
Column Temp.	40ºC		
Flow rate	300 µL/min.		
Mobile Phases & Gradient	**Time (min.)**	**5 mM ammonium acetate in water**	**5 mM ammonium acetate in acetonitrile**
	0	95%	5%
	9	5%	95%
	10	0%	100%
	13	0%	100%
	13.5	95%	5%
	17	95%	5%

Table 5. HRAM MS Instrument Parameters.

Ionization	ESI Negative
Scan type	Full MS and $ddMS^2$
Resolution	60,000
Inclusion	On
$ddMS^2$	Top 5
Sheath gas flow	48
Aux gas flow	11
Spray voltage	3
Capillary temperature	260
S-lens	60
Aux gas temperature	410

Table 6. Masses used in quantitation and confirmation.

Compound	m/z	m/z	m/z
Natives			
PFBA	212.9787	168.9885	
PFBS	298.9429	79.9561	
PFPeA	262.9760	218.9857	
PFPeS	348.9398	79.9550	
PFHxA	312.9730	268.9830	
PFHxS	398.9366	79.9546	
PFHpA	362.9699	318.9799	296.9780
PFHpS	448.9335	449.9360	79.9550
PFOA	412.9667	368.9767	
PFOS	498.9307		
PFNA	462.9636	418.9737	428.1716
PFNS	548.9274		
PFDA	512.9602	468.9703	
PFDS	598.9244		
PFUdA (PFUnA)	562.9572	518.9672	563.9606
PFDoA	568.9642	612.9542	
PFDoS	698.9182		
PFTrDA	662.9513	618.9610	663.9550
PFTeDA	712.9483	168.9886	668.9580
PFHxDA	812.9421	768.9515	
PFODA	912.9340	868.9441	

FOSAA	555.9520		
N-MeFOSAA	569.9678		
N-EtFOSAA	583.9836		
HFPO-DA	328.9680	284.9780	168.9885
NaDONA	376.9690	250.9760	84.9892
9Cl-PF3ONS	530.8975	350.1258	
11Cl-PF3OUdS	630.8899	632.8868	
Extraction Standards			
M2PFTeDA	714.9548		
M3PFBS	301.9531		
M3PFHxS	401.9467		
M4PFHpA	366.9831	321.9900	
M5PFHxA	317.9897	272.9966	
M5PFPeA	267.9928	222.9991	
M6PFDA	518.9803	473.9872	
M7PFUdA	569.9807	524.9872	
M8PFOA	420.9935	376.0002	
M8PFOS	506.9570		
M9PFNA	471.9936	427.0003	
M4PFBA	216.9923	171.9986	
MPFDoA	614.9609	569.9677	
M NMeFOSAA	572.9867		
M NEtFOSAA	589.0150		
M3HFPO-DA	286.9848	331.9780	
Internal Standards			
M3PFBA	215.9888	171.9987	
MPFDA	514.9670	469.9738	
M2PFOA	414.9733	369.9800	
MPFOS	502.9439		

Identifications are based on the retention times and accurate masses. Quantitation is based on internal standard method. The recoveries of the isotopically labelled extraction standards from each sample, in addition to the recoveries of spiked compounds from laboratory blank spike (LBS) and matrix spike (MS), are monitored.