ERDC/EL TN-20-4
September 2020

Optimization of LC-MS/MS Parameters for Analysis of Per- and Polyfluoroalkyl Substances (PFAS)

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PURPOSE: Integrate US Environmental Protection Agency (USEPA) Method 537 on current instrumentation to provide per- and polyfluoroalkyl substances (PFAS) analytical capabilities for the US Army Engineer Research and Development Center (ERDC), US Army Corps of Engineers (USACE) and the Department of Defense (DoD).

BACKGROUND: Per- and polyfluoroalkyl substances, collectively known as PFAS, are synthetic molecules used for myriad purposes in industrial, military, and consumer products. The compounds are persistent in the environment due to the very strong C—F[1] bond and may exhibit carcinogenic, teratogenic, and bioaccumulative properties (Ding and Peijnenburg 2013; Gorrochategui et al. 2014). Due to concerns based on these properties, advisory limits have been set at a total concentration of 70 ng/L[2] as a sum of each analyte's individual concentration. Of particular interest for the DoD is the use of PFAS in firefighting applications as a major component of aqueous film–forming foam (AFFF), used extensively in training exercises and emergency fire response.

Materials and Methods. The 14 analytes in USEPA 537 were purchased as an analytical standard with a stock concentration of 2 µg/mL, and the isotopically labeled internal standards (IS) and surrogates (SUR) were purchased from Wellington Laboratories (Guelph, Ontario, Canada), as listed in table 1. Ammonium acetate (99.99%) was purchased from Sigma Aldrich (St. Louis, Missouri). Optima (mass spectrometry, or MS) grade water and methanol for use as diluents and high-performance liquid chromatography (HPLC) mobile phase were purchased from Fisher Scientific (Hampton, New Hampshire). All dilutions and samples were made and stored in polypropylene containers. HyperSep Retention PEP solid-phase extraction (SPE) cartridges were purchased from Thermo Scientific (Waltham, Masssachusetts).

1. For a full list of the spelled-out forms of the chemical elements used in this document, please refer to *US Government Publishing Office Style Manual*, 31st ed. (Washington, DC: US Government Publishing Office, 2016), 265, https://www.govinfo.gov/content/pkg/GPO-STYLEMANUAL-2016/pdf/GPO-STYLEMANUAL-2016.pdf.

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Table 1. Perfluoroalkyl substances (PFASs) analyzed by EPA 537.

Chemical	Acronym	Precursor ion (m/z)	Product ion (m/z)	Collision energy (V)
perfluorobutanesulfonic acid	PFBS	298.94299	79.9569	40
perfluorohexanenoic acid	PFHxA	312.97281	268.9829	12
$^{13}C_2$-perfluorohexanoic acid	$^{13}C_2$-PFHxA	314.97856	269.9866	10
perfluorohepatnoic acid	PFHpA	362.96962	318.9798	10
perfluorohexanesulfonic acid	PFHxS	398.9366	79.9569	60
Perfluorooctanoic acid	PFOA	412.96643	368.9767	10
$^{13}C_2$-perfluoroocatnoic acid	$^{13}C_2$-PFOA	502.94495	79.9569	10
perfluorononanoic acid	PFNA	462.96323	418.9736	18
perfluorooctanesulfonic acid	PFOS	498.93022	79.9569	40
$^{13}C_2$-perfluorooctanesulfonic acid	$^{13}C_2$-PFOS	502.94495	79.9570	40
perfluorodecanoic acid	PFDA	512.96004	468.9702	11
$^{13}C_2$-perfluorodecanoic acid	$^{13}C_2$-PFDA	514.96790	469.9739	10
perfluoroundecanoic acid	PFUnA	562.95684	518.9674	10
N-methyl perfluorooctanesulfonamidoacetic acid	NMeFOSAA	569.96733	418.9735	18
d_3-N-methyl perfluorooctanesulfonamidoacetic acid	d_3-NMeFOSAA	572.98730	418.9740	10
N-ethyl perfluorooctanesulfonamidoacetic acid	NEtFOSAA	583.98298	418.9735	18
d_5-N-ethyl perfluorooctanesulfonamidoacetic acid	d_5-NEtFOSAA	589.01575	418.9737	20
perfluorododecanoic acid	PFDoA	612.95365	568.9644	10
perfluorotridecanoic acid	PFTrDA	662.95046	618.96141	10
pefluorotetradecanoic acid	PFTA	712.94726	668.9584	10

Samples of PFAS were analyzed on a ThermoFisher HPLC-MS/MS (Vanquish LC, Orbitrap High Resolution Accurate Mass-Mass Spectrometer, or HRAM-MS) equipped with a Hypersil C8 delay column (3 x 50 mm, particle size 5 µm, Thermo Scientific) to ensure PFAS leached from parts intrinsic to the system from eluting with sample or standard PFAS. A 50 µL aliquot of standards or unknown samples was separated across a Hypersil GOLD aQ C-18 analytical HPLC column (2.1 x 150 mm, 3 µm, Thermo Scientific) maintained at 40°C during chromatographic separations. A gradient mobile phase pumped at 0.300 mL min^{-1} (A: 20mM ammonium acetate; B: LC-MS methanol) through PEEK tubing with the gradient described in table 2. The samples were analyzed in parallel reaction monitoring mode (PRM) with parameters described in table 2. Optimized collision energies for each compound and their precursor and product ions are listed in table 1.

Calibration curves were generated by dilution of analytical standards with 96:4 MeOH:H_2O (v/v) from 156 ng/L to 20 µg/L and injected using the above described instrument parameters. Each calibration standard was spiked with isotopically labeled PFAS IS to final concentrations of 1, 3, and 4 µg/L for $^{13}C_2$-PFOA, $^{13}C_2$PFOS, and d_3-NMeFOSAA, respectively. Individual calibration points are reported as a ratio of the peak areas of PFAS/IS to eliminate any bias due to fluctuations of the ionization efficiency. The average percent relative standard deviation for the standards and surrogates was found to be 6.8% for 13 samples.

Table 2. Instrumentation parameters.

LC Gradient Parameters			MS Parameters			
time	%A	%B	probe position	C	auxilary gas temperature	325 °C
0	70	30	voltage	2.5 kV	m/z range	70-740
1	70	30	sheath gas	25 AU	resolution	35,000
25	10	90	auxilary gas	8 AU	microscans	5
27.5	10	90	capillary temperature	300 °C	automatic gain control target	100,000
30	70	30	S-lens RF level	50 AU	max injection time	100 ms

Solid phase extraction cartridges were conditioned with 15 mL of MeOH and equilibrated with 18 mL of MS grade water. To each conditioned cartridge was added 2–3 mL of MS grade water to prevent drying before loading 250 mL of sample fortified with 10 µL of 1, 1, and 4 µg/L of $^{13}C_2$-PFHxA, $_{31}C^2$-PFDA, and d_5-NEtFOSAA, respectively, directly from sample bottles using prerinsed polyethylene transfer tubes. All samples were loaded at a flow rate of approximately 10–15 mL/min. Once full samples were loaded onto SPE cartridges, sample bottles were rinsed with two 7.5 mL aliquots of MS grade water and transferred through the transfer tubes to load any residual sample onto cartridges. Cartridges were then dried under vacuum for five minutes at room temperature on the manifold. After drying, analyte elution was achieved by rinsing the sample bottles with two 4 mL aliquots of MeOH through the transfer tubes and collected in a 15 mL HDPE centrifuge tube. After collection of the eluent, methanol extracts were evaporated to dryness under nitrogen in a 60°C water bath. Once dried, extracts were reconstituted to 1 mL using 96:4 (v/v) MeOH:water containing the same concentration of IS as the calibration curve.

Results and Discussion. Separation of the analytes via HPLC was achieved with the above described method (figure 1). There are some coeluting compounds, PFNA and PFOS, and NetFOSAA and PFUnA; however, the molecules have different precursor and product ions (table 1, above) allowing for concomitant detection and quantification by the mass spectrometer.

Injection of calibration standards resulted in linear calibration responses and are summarized in table 3, along with representative calibration curves for PFOA and PFOS in figure 2. The 156 ng/L standard for NMeFOSAA and the 312 ng/L standard for NEtFOSAA were not observed, and the 156 ng/L standard for PFBS returned a calculated concentration outside of the ±50% tolerance for standards below the proposed minimum reporting limit (MRL, detailed below).

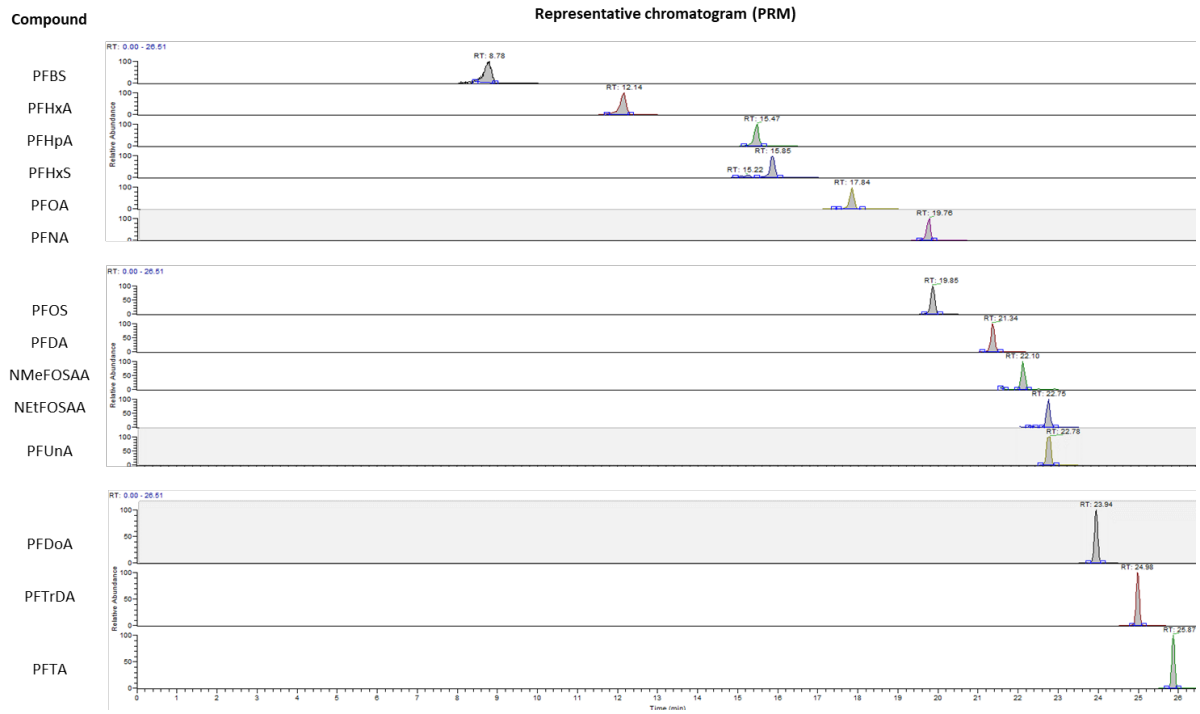

Figure 1. Representative chromatogram of 2.5 µg/L PFAS standard showing adequate separation for the analysis of PFAS compounds.

Table 3. PFAS Calibration Curves.

PFAS	equation	R^2	low concentration (ng/L)	error in calculated concentration
PFBS	0.2151x	0.9986	312	44
PFHxA	0.3898x	0.9991	156	28
PFHpA	0.7515x	0.9996	156	16
PFHxS	0.9808x	0.9997	156	7
PFOA	1.0282x	0.9994	156	2
PFNA	0.2323x	0.9966	156	26
PFOS	0.2676x	0.9992	156	5
PFDA	0.7272x	0.9982	156	1
NMeFOSAA	0.8686x	0.9995	312	24
NEtFOSAA	0.8999x	0.9994	625	14
PFUnA	5.7424x	0.9974	156	6
PFDoA	7.0131x	0.9971	156	7
PFTrA	6.0424x	0.9987	156	22
PFTA	6.3090x	0.9993	156	27

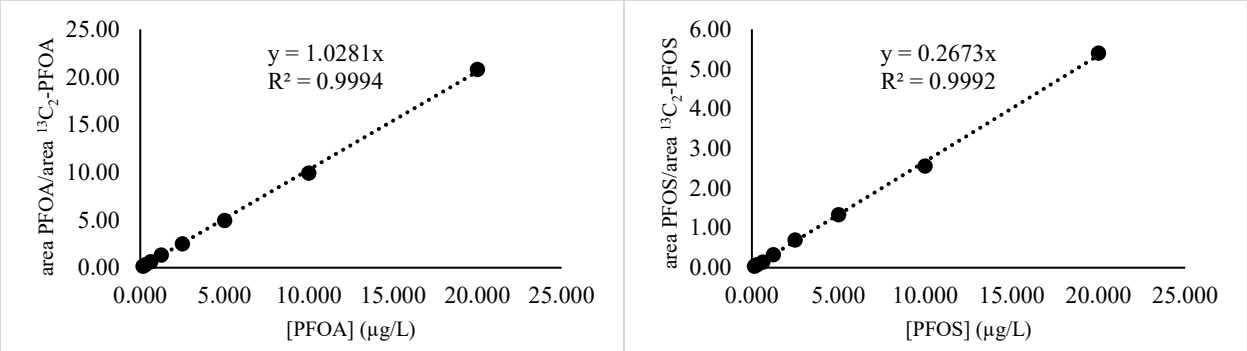

Figure 2. Calibration curves for PFOA and PFOS, respectively, from left to right.

Fortification of laboratory blank water samples with PFAS standards and subsequent analysis following SPE concentration allowed for the determination of MRLs for 12 of the 14 compounds. These results are summarized in table 4. The extraction procedure was precise, meeting the goal of ±20% RSD for each of the analytes; however, in each case the percent recovery was less than 100% (64.8–95.7%). For two of the compounds, NEtFOSAA and PFTA, the recoveries fell outside of the allowable window of ±30% for the method validation; yet their respective calibration curves were still linear to concentrations of 625 and 156 ng/L, respectively. The analytical method was further explored by calculating the half range for the prediction interval of results (HR_{PIR}) and the upper and lower limits of the range based on the standard deviation for each analyte sample set and the number of samples tested (n).

Table 4. Method reporting limits of PFAS compounds.

PFAS	n	Fortified [PFAS] (ng/L)	Extracted [PFAS] (µg/L)	Avg. Measured [PFAS] (µg/L)	Std. dev.	%RSD	avg. % recovery	HR_{PIR}	Upper PIR Limit (%)	Lower PIR Limit (%)
PFBS	7	750	10.5	9.42	0.99	10.54	89.7	3.93	127.2	52.3
PFHxA	7	750	10.5	7.81	0.47	5.98	74.4	1.85	92.0	56.7
PFHpA	7	750	10.5	8.60	0.41	4.76	82.0	1.62	97.4	66.5
PFHxS	7	750	10.5	9.49	0.44	4.67	90.4	1.76	107.2	73.7
PFOA	7	750	10.5	8.52	0.37	4.31	81.1	1.46	95.0	67.2
PFOS	7	750	10.5	10.05	0.55	5.5	95.7	2.19	116.5	74.8
PFNA	7	750	10.5	8.64	0.36	4.21	82.3	1.44	96.0	68.6
PFDA	7	750	10.5	8.73	0.35	4.01	83.2	1.39	96.4	70.0
NMeFOSAA	7	750	10.5	7.78	0.61	7.79	74.1	2.40	96.9	51.2
PFUnA	7	750	10.5	9.08	0.62	6.84	86.5	2.46	110	63.1
NEtFOSAA	7	750	10.5	6.80	0.60	8.85	64.8	2.39	87.5	42.1
PFDoA	7	750	10.5	8.20	0.47	5.69	78.1	1.85	95.7	60.5
PFTrDA	7	750	10.5	8.58	0.45	5.25	81.7	1.78	98.7	64.7
PFTA	7	750	10.5	6.84	0.56	8.22	65.1	2.23	86.3	43.9

SUMMARY: The USEPA Method 537 was implemented on current analytical instrumentation in place at the ERDC Environmental Laboratory (ERDC-EL) Environmental Chemistry Branch (EPC) to analyze aqueous samples containing PFAS. Calibrations were linear from 156 ng/L–20 µg/L for 11 of the 14 compounds, with the exception of PFBS (312 ng/L), NMeFOSAA (312 ng/L), and

NEtFOSAA (625 ng/L). MRL's of 750 ng/L were determined for 12 of 14 compounds. NEtFOSAA and PFTA were the only analytes with slightly higher calculated MRLs. For NEtFOSAA, this slighter higher MRL is likely due to instrumentation limitations, as this particular compound was not observed in the calibration curve below 625 ng/L; however, the PFTA gave satisfactory results to 312 ng/L in the calibration curves, and therefore the poor performance observed is likely due to poor recovery from SPE cartridges. Further optimization of the method is currently underway to achieve MRL values below the USEPA advisory limit of 70 ng/L for each of the compounds. These optimizations include both instrumental parameters as well as sample extraction and preconcentration (SPE) procedures.

Similar method development and validation studies are currently underway to evaluate complementary analytical capabilities on a newly acquired HPLC triple quadrupole mass spectrometer (QqQ, Agilent, G6495B), anticipated to yield detection limits in the range of 1 ng/L and MRL values in the 10 ng/L ranges. Additionally, method modification and refinement is needed for application of these techniques to more complex natural matrices, including complex aqueous, soil or sediment, and tissue samples. Use of the HRAM MS mode in environmental fate, remediation, and degradation studies should allow detection and identification of degradation products.

Future work on PFAS degradation and remediation efforts may also employ other advanced analytical capabilities available within ERDC-EL. For example, volatile PFAS degradation products may also be detected and identified using thermal desorption gas chromatography-mass spectrometry (TD-GC-MS). Alternatively, ion chromatography (IC) could be employed for determination of defluorination efficiency. Additionally, chemical structure determination of parent and daughter PFAS compounds can potentially be elucidated using advanced ^{1}H, ^{13}C, and ^{19}F nuclear magnetic resonance (NMR) spectroscopy.

ADDITIONAL INFORMATION: This technical note was prepared by Dr. Lee C. Moores, research chemist, Environmental Laboratory (EL), US Army Engineer Research and Development Center (ERDC) (Lee.C.Moores@usace.army.mil), Dr. Ashely Kimble, research chemist, EL, ERDC, Dr. Rebecca Crouch, research chemist, EL, ERDC, Mr. Garrett George, research chemist, EL, ERDC, Mr. David Henderson, research biologist, EL, ERDC, Dr. Bobbi Stromer, research chemist, EL, ERDC, Dr. Lauren Soblosky, senior scientist I, HX5, Mr. Jared Smith, research chemist, EL, ERDC, and Dr. Anthony Bednar, research chemist, EL, ERDC (Anthony.J.Bednar@usace.army.mil).

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> Moores, Lee, Ashley Kimble, Garrett George, David Henderson, Bobbi Stromer, Rebecca Crouch, Lauren Soblosky, Jared Smith, and Anthony Bednar. 2020. *Optimization of LC-MS/MS Parameters for Analysis of Per- and Polyfluoroalkyl Substances (PFAS)*. ERDC EL Technical Notes (ERDC/EL TN-20-4). Vicksburg, MS: US Army Engineer Research and Development Center.

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