Application note | 002771

Environmental

LC-MS/MS analysis of per- and polyfluoroalkyl substances (PFAS) in soil samples in accordance with EPA Method 1633

Featuring semi-automated solid phase extraction cleanup

Authors

Cynthia Grim[1], Kevin McHale[2], Toby Astill[1]

[1]Thermo Fisher Scientific, San Jose, CA

[2]Thermo Fisher Scientific, Somerset, NJ

Keywords

EPA Method 1633, per- and polyfluoroalkyl substances, PFAS, environmental contaminants, solid phase extraction, soil, AutoTrace 280 PFAS, Vanquish Flex Binary UHPLC, TSQ Altis Plus triple quadrupole mass spectrometer, Chromeleon CDS

Application benefits

- The Thermo Scientific™ TSQ Altis™ Plus mass spectrometer represents the most sensitive triple quadrupole for targeted PFAS testing across multiple matrix types.
- The Thermo Scientific™ Dionex™ AutoTrace™ 280 PFAS Solid-Phase Extraction (SPE) instrument is compatible with the SPE elution method specified in EPA Method 1633 and devoid of contamination for the 40 specified PFAS analytes.
- The Thermo Scientific™ Chromeleon™ Chromatography Data System templates for EPA Method 1633 provide a flexible and comprehensive solution to method reporting requirements.

Goal

To demonstrate the measurement of 40 per- and polyfluoroalkyl substances (PFAS) in soil samples with precision and accuracy following EPA Method 1633 on the TSQ Altis Plus triple quadrupole mass spectrometer

Introduction

PFAS workflows are continuously being developed to meet evolving testing requirements for per- and polyfluoroalkyl substances (PFAS) worldwide, driven by increasing health concerns related to these persistent chemicals in our environment. Global regulatory

organizations are developing and publishing testing requirements to standardize the application of PFAS testing, considering extended compound lists, various sample matrices, and lower detection limits. EPA Method 1633 is one example of a regulatory method that mandates determining the quantitative results for 40 PFAS compounds.[1] Laboratories face the challenge of meeting these requirements while improving sample throughput and maintaining data quality to meet their productivity needs. Previously, this was difficult due to a fragmented approach across sample preparation, analytical detection, and data processing. However, a holistic method from a single vendor offers a comprehensive and seamless workflow solution.

This work describes that workflow by leveraging the Dionex AutoTrace 280 PFAS SPE instrument to automate the soil sample solid-phase extraction (SPE) cleanup stage that is described in EPA Method 1633. The workflow utilizes the Thermo Scientific™ Vanquish Flex™ Binary UHPLC, the TSQ Altis Plus triple quadrupole mass spectrometer, and the Chromeleon Chromatography Data System to achieve reproducible and precise sample quantitation to meet the regulatory requirements for both water and solid sample types. In this work, an overview of the LC-MS/MS workflow for PFAS quantitation in solid samples will be discussed in detail.

Experimental

Chemicals and consumables

A list of suggested products can be found in Table 1. All products are from Thermo Fisher Scientific unless specifically noted. The solvents used were all UHPLC-MS grade from Thermo Fisher Scientific (as they contain the lowest amounts of PFAS).

Sample preparation

Ottawa sand for the initial method detection limit (MDL) study was baked at 400 °C overnight to ensure a blank matrix. Stainless steel utensils for weighing samples were similarly baked before use. PFAS standards were obtained from Wellington Laboratories (Guelph, ON) and stored at 4 °C until use. Ampoules were transferred to 1.5 mL polypropylene autosampler vials after opening for subsequent uses.

Table 1. Suggested materials for EPA Method 1633

Item	Product	Part number
PFAS delay column	Thermo Scientific™ Hypersil GOLD™, 4.6 × 50 mm, 1.9 µm	25002-054630
Analytical column	Thermo Scientific™ Acclaim™ 120 C18, 2.1 × 50 mm, 2.2 µm	068981
Guard column*	Thermo Scientific™ Acclaim™ 120 C18, 2.1 × 10 mm, 5 µm	069689
Guard column kit*	Thermo Scientific™ Acclaim™ guard kit (holder and coupler) V-2	069707
Strong solvent loop	Strong solvent loop	6036.22
Mobile phase chemicals	Water, UHPLC-MS grade, 1 L, Thermo Scientific™	W8-1
	Acetonitrile, UHPLC-MS grade, 1 L, Thermo Scientific™	A956-1
	Ammonium acetate, LC-MS grade, 50 g, Fisher Chemical™	A114-50
	Acetic acid, LC-MS grade, 1 mL ampoules, Fisher Chemical™	A113-10X1AMP
Other reagents	Methanol, UHPLC-MS grade, 1 L, Thermo Scientific™	A458-1
	Ammonium hydroxide, ACS Plus grade, 500 mL, glass bottle, Fisher Chemical™	A669-500
	Formic acid, LC-MS grade, 1 mL ampoules, Fisher Chemical™	A117-10X1AMP
Solids reference matrix	Ottawa sand, Fisher Chemical™	S23-3
Centrifuge tubes	15 mL conical PP centrifuge tubes	05-539-12
	50 mL conical PP centrifuge tubes	05-539-13
Syringes	Luer-slip syringes, PE barrels, PP plungers, 5 mL	S7510-5
Filters	Disposable syringe filters, 22 mm, 0.2 µm, nylon membrane	CH4513-NN
SPE cartridges	GCB/WAX, 50 mg/200 mg/6 mL	CS0-9214
Dionex AutoTrace 280 PFAS collection vials	Round bottom PP culture tubes	187261
Autosampler vials	Thermo Scientific™ SureSTART!™ polypropylene, 1.5 mL, screw-top, Level 1	6ESV9-1PP
Autosampler caps	Polypropylene caps, 9 mm, screw-thread	C5000-50

* For laboratories running high-volume and/or highly matrixed samples; not used for this data set

Five gram portions of Ottawa sand were weighed out into 50 mL centrifuge tubes. Samples were fortified with native PFAS to a concentration matching the second lowest calibration level. 2.5 mL of ultrapure water was added to each sample to better mimic real-world samples as per section 11.3.2 of EPA Method 1633. 25 µL of MPFAC-HIF-EIS (extraction standard solution) was added to each sample and mixed by vortex for 10 seconds to distribute. The samples were then left to equilibrate for 30 minutes.

Once the equilibration period was over, 10 mL of 0.3% ammonium hydroxide was added by 10 mL pipette to each sample. Samples were shaken by hand to disperse, sonicated for 30 minutes to extract, then centrifuged to settle the sediment. Supernatant for each sample was poured into a new 50 mL centrifuge tube. Another similar round of extraction was done with 15 mL of 0.3% ammonium hydroxide. The third round of extraction was done with 5 mL of 0.3% ammonium hydroxide and was only briefly shaken by hand before centrifugation and supernatant transfer.

The resulting supernatant from the three rounds of extraction was evaporated at 1.2 L/min with nitrogen gas at 55 °C until 10.5 mL of total solution was left (8 mL of methanol, 2.5 mL of added water). During this process, samples were briefly mixed after the first 25 minutes as well as every ten minutes thereafter. Ultrapure water was added to each sample to a final volume of 40 mL, targeting a final solvent composition of 20% methanol. The pH was checked by pH paper to be within 6.0–7.0 and adjusted as necessary.

Dionex AutoTrace 280 PFAS instrument method

To prepare the Dionex AutoTrace 280 PFAS instrument, sample lines were placed in a bottle of methanol and the “rinse sample path” script was run. The system was loaded with freshly prepared solutions of 1% methanolic ammonium hydroxide and 0.3 M formic acid. Then, fresh GCB/WAX cartridges were inserted, and the conditioning process was started. Round bottom polypropylene culture tubes were racked to collect eluent.

The settings and script for the AutoTrace 280 PFAS instrument can be found in Tables A1 and A2, respectively.

To perform the semi-automated solid phase extraction, sample lines were placed in the 50 mL centrifuge tubes containing the sample extracts and the script was started. The first pause in the script (step 5) indicated a point to check that the sample lines were in the corresponding sample tubes. During the second and third pause, the centrifuge tubes were rinsed with 5 mL of reagent water to perform bottle rinses. The fourth pause was used to add 5 mL of 1:1 0.1% formic acid/methanol solution to each sample centrifuge tube. While the cartridges were briefly dried, 25 µL of MPFAC-HIF-IS (internal standard solution) was added to each polypropylene culture tube. 5 mL of 1% methanolic ammonium hydroxide was added to each tube to prepare for elution. Once samples eluted into the round bottom culture tubes, 25 µL of concentrated acetic acid was added to each sample extract. Extracts were filtered with a 5 mL polypropylene syringe and nylon syringe filter before analysis by LC-MS/MS. Filters and syringes were pre-rinsed with 5 mL of methanol to remove any potential contaminant PFAS. Figure 1 shows a brief representation of the full procedure from sample weighing to analysis.

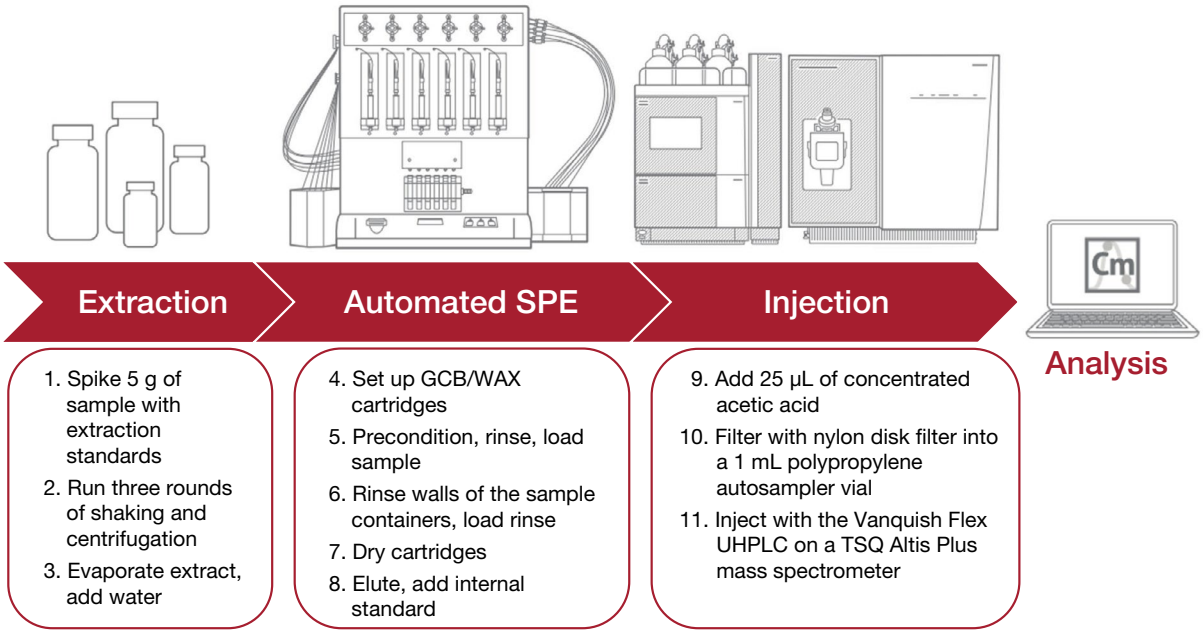

Figure 1. Visualization of the procedure

Liquid chromatography

HPLC configuration and method parameters matched those described in a previous application note,[2] aside from the use of a Thermo Scientific™ Hypersil GOLD™, 4.6 × 50 mm, 1.9 µm delay column (P/N 25002-054630, included in the PFAS upgrade kit), which slightly modified the gradient and retention times. The updated gradient along with the rest of the LC method can be found in Table 2.

Table 2. Solvents and additives

Parameter	Value	
Analytical column	Acclaim 120 C18, 2.1 × 50 mm, 2.2 µm (P/N 068981)	
Delay column	Hypersil GOLD, 4.6 × 50 mm, 1.9 µm (P/N 25002-054630)	
Column temperature	40 °C	
Injection volume	5 µL	
Autosampler temperature	20 °C	
Mobile phase	A: H_2O + 2% ACN + 2 mM ammonium acetate + 0.1% acetic acid B: ACN + 2% H_2O + 2 mM ammonium acetate + 0.1% acetic acid	
Flow rate	0.4 mL/min	
Gradient	Time (min)	%B
	0.0	10
	1.0	30
	5.0	46
	10.0	76
	10.5	86
	11.3	86
	11.4	10
	14.0	10

Mass spectrometry

Mass spectrometer parameters used were the same as described in a previous application note,[2] aside from the RF Lens values which are different for the TSQ Altis Plus mass spectrometer. Table A3 lists the full SRM table along with the new RF Lens values for the TSQ Altis Plus mass spectrometer.

Data analysis

All LC-MS/MS data were acquired and processed using the Chromeleon Chromatography Data System (CDS), version 7.2. Templates have been developed to allow laboratories to export data to meet EPA Method 1633 reporting requirements, such as %RSE, surrogate recoveries, and MDLs, and are included in this workflow. Figure 2 depicts an example of the surrogate recovery template.

Results and discussion

Calibration data

The same nine calibration concentrations used for the water experiments from the previous application note[2] were prepared for this soil data on the TSQ Altis Plus mass spectrometer. Calibration curves for all target PFAS were fit using 1/x weighting and not forced through zero. Based on reviewing all acceptance criteria data, ADONA, 4:2 FTS, 8:2 FTS, 5:3 FTCA, and 7:3 FTCA were determined to be best described by a quadratic calibration function. A quadratic calibration is permitted by the method as long as there are at least seven calibration levels.[1] All other compounds were fit by a linear calibration curve. Most curve fits can be described by a %RSE (relative standard error) of less than 10%, with all PFAS targets, including those with quadratic fits, having %RSE values below 20% (Table 3).

Surrogate Recovery Report: M8PFOA

Sequence Details							
Sequence Name:					*Created On:*		
Directory:	**PFAS_Soil**				*Created By:*	**Altis Plus**	
Data Vault:	**ChromeleonLocal**				*Updated On:*		
No. of Injections:					*Updated By:*	**cynthia.grim**	
Calibration Source:							
Injection Name	**Analysis_Type**	**Spike Amount ng/mL** MS Quantitation	**Rec. Amt. ng/mL** MS Quantitation	**Recovered %** MS Quantitation	**Valid Range** MS Quantitation	**Outside Limit?** MS Quantitation	**Manually Integrated?** MS Quantitation
MDL 2	Minimum Detection Limit (MDL)	2.5	3.00	119.3	70 - 130		
MDL 4	Minimum Detection Limit (MDL)	2.5	2.25	89.3	70 - 130		
MDL 1	Minimum Detection Limit (MDL)	2.4	2.35	96.2	70 - 130		
MDL 5	Minimum Detection Limit (MDL)	2.5	2.14	86.2	70 - 130		
MDL 7	Minimum Detection Limit (MDL)	2.5	2.25	91.0	70 - 130		
MDL 6	Minimum Detection Limit (MDL)	2.5	2.13	85.3	70 - 130		
MDL 3	Minimum Detection Limit (MDL)	2.4	2.20	89.9	70 - 130		
					Number of failed tests:	**0**	
This page is automatically repeated for all surrogates							

Figure 2. An example of a surrogate recovery page in the report template editor. These templates can be further modified to individual lab requirements.

Table 3. Initial method detection limit results

Analyte	Cal 1 (ng/g)	%RSE	MDL (n=7) (ng/g)	%Accuracy
PFBA	0.20	3%	0.062	95%
PFPeA	0.10	3%	0.016	93%
PFHxA	0.05	3%	0.008	92%
PFHpA	0.05	2%	0.012	94%
PFOA	0.05	5%	0.008	96%
PFNA	0.05	2%	0.007	89%
PFDA	0.05	3%	0.007	88%
PFUdA	0.05	3%	0.007	88%
PFDoA	0.05	10%	0.013	88%
PFTrDA	0.05	6%	0.011	73%
PFTeDA	0.05	11%	0.009	85%
PFBS	0.05	3%	0.012	83%
PFPeS	0.05	6%	0.011	95%
PFHxS	0.05	4%	0.013	89%
PFHpS	0.05	5%	0.011	79%
PFOS	0.05	7%	0.034	110%
PFNS	0.05	7%	0.020	104%
PFDS	0.05	3%	0.014	76%
PFDoS	0.05	5%	0.013	80%
4:2 FTS*	0.20	2%	0.046	85%
6:2 FTS	0.20	18%	0.038	89%
8:2 FTS*	0.20	3%	0.036	91%
PFOSA	0.05	2%	0.015	97%
N-MeFOSA	0.05	6%	0.024	102%
N-EtFOSA	0.05	6%	0.019	100%
N-MeFOSAA	0.05	9%	0.023	84%
N-EtFOSAA	0.05	11%	0.019	98%
N-MeFOSE	0.50	6%	0.116	87%
N-EtFOSE	0.50	4%	0.098	91%
HFPO-DA	0.10	3%	0.024	81%
ADONA*	0.10	3%	0.037	82%
PFMPA	0.10	19.7%	0.022	117%
PFMBA	0.10	4%	0.014	95%
NFDHA	0.10	4%	0.024	98%
9Cl-PF3ONS	0.10	15%	0.044	82%
11Cl-PF3OUdS	0.10	17%	0.050	90%
PFEESA	0.10	4%	0.013	71%
3:3FTCA	0.25	10%	0.086	96%
5:3FTCA*	1.25	5%	0.227	74%
7:3FTCA*	1.25	6%	0.236	86%

(*) Denotes quadratic calibration

Method detection limits data

The reported method detection limits exhibit excellent quantitative accuracy, with all but five native PFAS compounds falling within 20% of the theoretical spiked concentration. Furthermore, eighteen compounds exhibited accuracy within 10% of the theoretical spiked concentration. All MDL results for native PFAS analytes are listed in Table 3.

The average extracted internal standard recoveries for the method detection limit samples for every compound were within the method acceptance criteria found in Table 8 of the EPA Method.[1] This information is visualized in Figures 3 and 4.

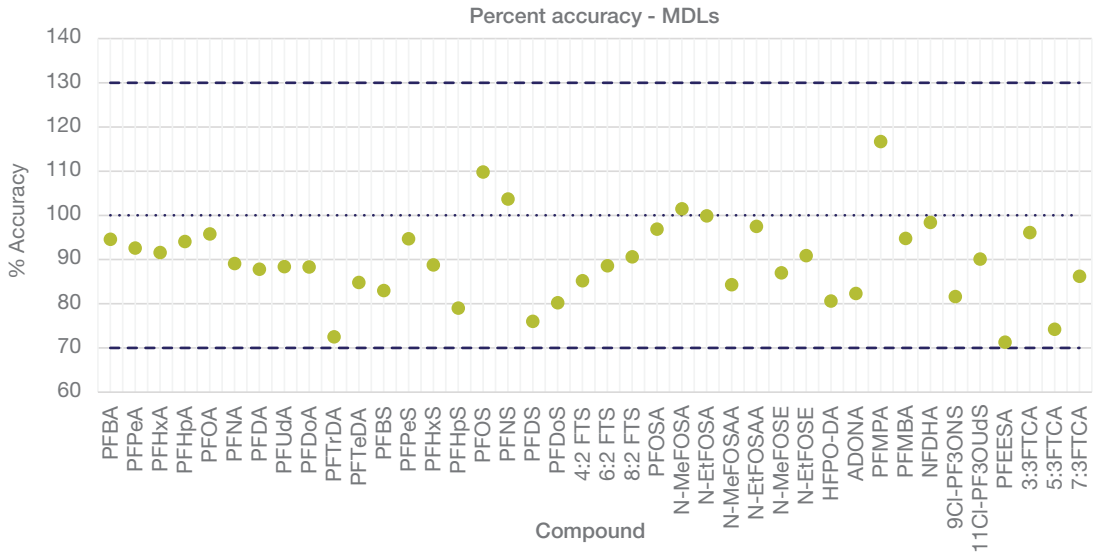

Figure 3. Percent accuracy of the native PFAS analytes calculated from the MDL sample set (n=7). The bounding dashed lines mark ±30% accuracy, while the dots represent the mean percent accuracy of each native analyte.

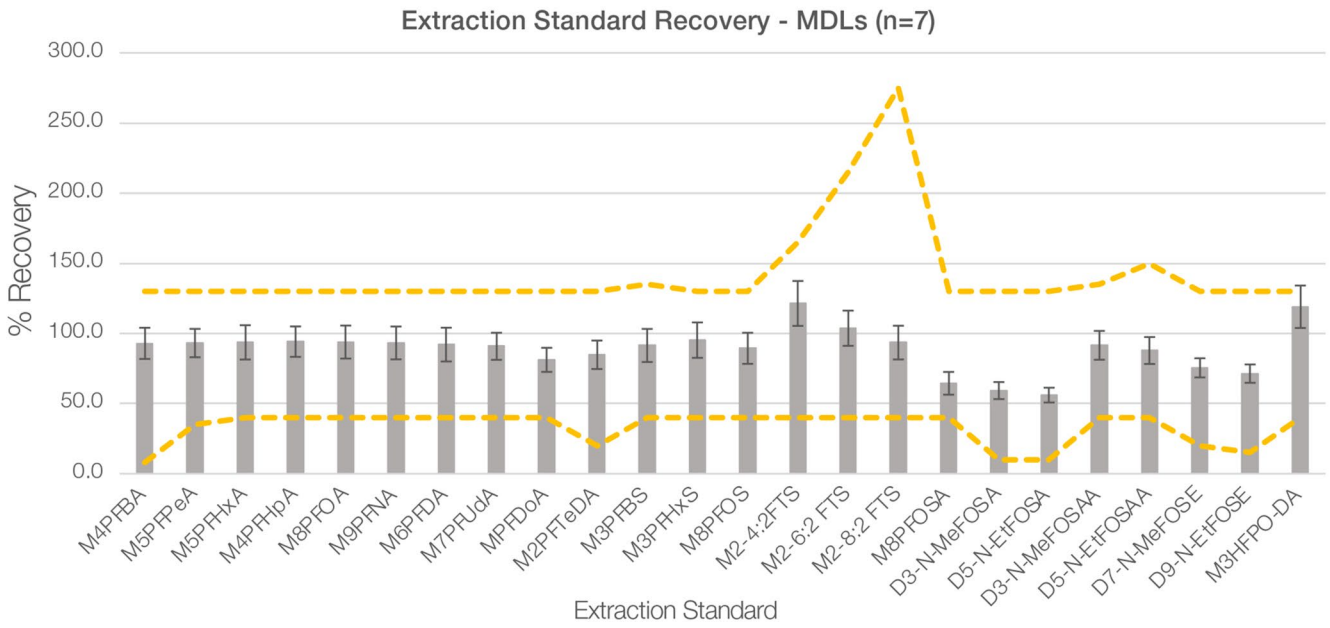

Figure 4. Average percent PFAS surrogate recoveries taken from the MDL sample set (n=7). The bounding dashed lines mark the surrogate recovery acceptance limits found in EPA Method 1633 Table 8. Gray bars represent the extracted internal standard recovery for the MDL samples; error bars signify ±1 standard deviation.

Conclusions

The workflow presented demonstrates excellent accuracy and precision for the analysis of PFAS in soil samples when the Dionex AutoTrace 280 PFAS instrument is applied to the cleanup of soil extracts in accordance with EPA Method 1633.

- The complete workflow in this application note with listed reagents, consumables, and procedures ensure that PFAS contamination in laboratory method blanks meet the method criteria.
- Specific scripts on the Dionex AutoTrace 280 PFAS instrument for cleanup of extracts are ready to be implemented in any analytical lab.
- The Dionex AutoTrace 280 PFAS instrument, when combined with the Vanquish Flex UHPLC and TSQ Altis Plus mass spectrometer, will boost productivity in the lab by potentially avoiding costly errors when using standard vacuum manifolds that require constant operator attention and the risk of poor precision and accuracy, often resulting in re-extraction of samples.

Acknowledgements

We acknowledge our Thermo Fisher Scientific colleagues Rahmat Ullah and Gopal Bera for assistance in developing and programming the Dionex AutoTrace 280 PFAS instrument script.

References

1. U.S. EPA 4th Method 1633, Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS, January 2024. https://www.epa.gov/system/files/documents/2024-01/method-1633-final-for-web-posting.pdf
2. Thermo Fisher Scientific Application Note 002348: Quantitation of per- and polyfluoroalkyl substances (PFAS) in aqueous samples by LC-MS/MS following EPA Draft Method 1633. https://assets.thermofisher.com/TFS-Assets/CMD/Application-Notes/an-002348-lsms-pfas-epa-method-1633-an002348-na-en.pdf

Appendix

Table A1. Dionex AutoTrace 280 PFAS instrument settings

Flow rates	
Cond flow	15.0 mL/min
Load flow	5.0 mL/min
Rinse flow	20.0 mL/min
Elute flow	5.0 mL/min
Cond air push	15.0 mL/min
Rinse air push	20.0 mL/min
Elute air push	5.0 mL/min
SPE parameters	
Push delay	5 s
Air factor	1.0
Autowash volume	1.0 mL
Instrument parameters	
Max elution volume	12.0 mL
Exhaust fan on	Yes
Beeper on	Yes
Solvent	**Nomenclature**
Solvent 1	1% Methanolic NH_4OH
Solvent 2	0.3 M Formic acid

Table A2. Dionex AutoTrace 280 PFAS instrument method for EPA Method 1633 soil samples

#	Step
1	Process four samples using the following method steps:
2	Condition cartridge with 10.0 mL of 1% methanolic NH_4OH into solvent waste
3	Condition cartridge with 5.0 mL of 1% methanolic NH_4OH into solvent waste
4	Condition cartridge with 5.0 mL of 0.3 M formic acid into solvent waste
5	Pause and alert operator, resume when continue is pressed
6	Load 50.0 mL of sample onto cartridge
7	Pause and alert operator, resume when continue is pressed
8	Load 15.0 mL of sample onto cartridge
9	Pause and alert operator, resume when continue is pressed
10	Load 15.0 mL of sample onto cartridge
11	Pause and alert operator, resume when continue is pressed
12	Load 15.0 mL of sample onto cartridge
13	Dry cartridge with gas for 0.4 min
14	Pause and alert operator, resume when continue is pressed
15	Manually rinse sample container with 20.0 mL to collect
16	End

Note 10 mL of dead volume along sample path (steps 6-15)

Table A3 (part 1). Timed SRM on the TSQ Altis Plus mass spectrometer

Compound	Retention time (min)	RT window (min)	Precursor (*m/z*)	Product (*m/z*)	Collision energy (V)	RF lens (V)	Min dwell time (ms)
PFBA	2.1	1.5	213	169	9	30	77.9
M3PFBA	2.1	1.5	216	172	9	30	77.9
MPFBA	2.1	1.5	217	172	9	30	77.9
PFMPA	3.1	0.8	229	85	10.5	31	64.8
PFMPA	3.1	0.8	229	185	7	31	64.8
PFPeA	3.6	0.8	263	219	8.5	33	18.3
M5PFPeA	3.6	0.8	268	223	8.5	33	18.3
PFMBA	3.7	0.8	279	85	10.5	38	18.3
PFMBA	3.7	0.8	279	235	7.5	38	18.3
4:2FTS	4	0.8	327	81	28	100	16.5
4:2FTS	4	0.8	327	307	20	100	16.5
M2-4:2FTS	4	0.8	329	81	28	100	16.5
M2-4:2FTS	4	0.8	329	309	20	100	16.5
NFDHA	4.1	0.8	295	85	22	33	16.5
NFDHA	4.1	0.8	295	201	8	33	16.5
PFHxA	4.2	0.8	313	119	19	36	16.5
PFHxA	4.2	0.8	313	269	9	36	16.5
MPFHxA	4.2	0.8	315	119	19	36	16.5
MPFHxA	4.2	0.8	315	270	9	36	16.5
M5PFHxA	4.2	0.8	318	120	19	36	16.5
M5PFHxA	4.2	0.8	318	273	9	36	16.5
PFBS	4.3	0.8	298.9	80	32	111	16.5
PFBS	4.3	0.8	298.9	99	29	111	16.5
M3PFBS	4.3	0.8	302	80	32	111	16.5
M3PFBS	4.3	0.8	302	99	29	111	16.5
HFPO-DA_CO2	4.5	0.8	285	169	7	25	16.5
HFPO-DA_CO2	4.5	0.8	285	185	17	25	16.5
13C3-HFPO-DA	4.5	0.8	287	169	7	35	16.5
13C3-HFPO-DA	4.5	0.8	287	185	17	35	16.5
PFEESA	4.7	0.8	314.9	83	19	60	16.5
PFEESA	4.7	0.8	314.9	135	22	60	16.5
PFHpA	5	0.8	363	169	17	41	19.3
PFHpA	5	0.8	363	319	9.5	41	19.3
M4PFHpA	5	0.8	367	322	9.5	41	19.3
3:3FTCA	5.2	0.8	241	117	32	35	19.3
3:3FTCA	5.2	0.8	241	177	7	35	19.3
PFPeS	5.2	0.8	348.9	80	35	120	19.3
PFPeS	5.2	0.8	348.9	99	32	120	19.3
ADONA	5.3	0.8	377	85	22	46	19.3
ADONA	5.3	0.8	377	251	10	46	19.3
PFHxS	6.1	2	398.9	80	38	124	19.3
PFHxS	6.1	2	398.9	99	34	124	19.3
PFOA	5.85	1.5	413	169	17	46	19.3
PFOA	5.85	1.5	413	369	10	46	19.3

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Table A3 (part 2). Timed SRM on the TSQ Altis Plus mass spectrometer

Compound	Retention time (min)	RT window (min)	Precursor (*m/z*)	Product (*m/z*)	Collision energy (V)	RF lens (V)	Min dwell time (ms)
M4PFOA	5.85	1.5	417	172	17	46	19.3
M8PFOA	5.85	1.5	421	376	10	46	19.3
6:2FTS	5.5	0.8	427	81	30	123	19.3
6:2FTS	5.5	0.8	427	407	22.5	123	19.3
M2-6:2FTS	5.5	0.8	429	81	30	123	19.3
M2-6:2FTS	5.5	0.8	429	409	22.5	123	19.3
M3PFHxS	6.1	0.8	402	80	38	124	28.8
M3PFHxS	6.1	0.8	402	99	34	124	28.8
MPFHxS	6.1	0.8	403	84	38	124	28.8
PFNA	6.7	0.8	463	219	17	50	26.8
PFNA	6.7	0.8	463	419	10.5	50	26.8
MPFNA	6.7	0.8	468	423	10.5	50	26.8
M9PFNA	6.7	0.8	472	427	10.5	50	26.8
PFHpS	7.2	0.8	448.9	80	40	129	20.4
PFHpS	7.2	0.8	448.9	99	37	129	20.4
PFOS	7.9	2	498.9	80	46	132	18.2
PFOS	7.9	2	498.9	99	40	132	18.2
8:2FTS	7.3	0.8	527	81	33	137	20.4
8:2FTS	7.3	0.8	527	507	26	137	20.4
M2-8:2FTS	7.3	0.8	529	81	33	137	20.4
M2-8:2FTS	7.3	0.8	529	509	26	137	20.4
5:3FTCA	7.7	0.8	341	217	25	42	20.4
5:3FTCA	7.7	0.8	341	237	13	42	20.4
PFDA	7.7	0.8	513	269	17	54	20.4
PFDA	7.7	0.8	513	469	11	54	20.4
MPFDA	7.7	0.8	515	470	11	54	20.4
M6PFDA	7.7	0.8	519	474	11	54	20.4
MPFOS	7.9	0.8	503	80	46	132	20.4
MPFOS	7.9	0.8	503	99	40	132	20.4
M8PFOS	7.9	0.8	507	80	46	132	20.4
M8PFOS	7.9	0.8	507	99	40	132	20.4
N-MeFOSAA	9	2	570	419	18	93	18.2
N-MeFOSAA	9	2	570	483	16	93	18.2
N-MeFOSAA	9	2	570	512	19	93	18.2
d3-N-MeFOSAA	9	2	573	419	18	93	18.2
PFUdA	8.5	0.8	563	269	18	58	18.2
PFUdA	8.5	0.8	563	519	11	58	18.2
M7PFUdA	8.5	0.8	570	525	11	58	18.2
9Cl-PF3ONS	8.6	0.8	530.9	350.9	25	130	18.2
9Cl-PF3ONS_37Cl	8.6	0.8	532.9	352.9	25	130	18.2
PFNS	8.9	0.8	548.9	80	49	144	18.2
PFNS	8.9	0.8	548.9	99	43	144	18.2
N-EtFOSAA	9.7	2	584	419	20	94	18.2
N-EtFOSAA	9.7	2	584	483	18	94	18.2

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Table A3 (part 3). Timed SRM on the TSQ Altis Plus mass spectrometer

Compound	Retention time (min)	RT window (min)	Precursor (*m/z*)	Product (*m/z*)	Collision energy (V)	RF lens (V)	Min dwell time (ms)
N-EtFOSAA	9.7	2	584	526	20	94	18.2
d5-N-EtFOSAA	9.7	2	589	419	20	94	18.2
PFDoA	9.2	0.8	612.9	169	25	62	18.2
PFDoA	9.2	0.8	612.9	569	11.5	62	18.2
MPFDoA	9.2	0.8	615	570	10.5	62	18.2
7:3FTCA	9.5	0.8	441	317	20	46	18.2
7:3FTCA	9.5	0.8	441	337	11	46	18.2
PFDS	9.5	0.8	598.9	80	50	160	18.2
PFDS	9.5	0.8	598.9	99	46	160	18.2
PFTrDA	9.8	0.8	662.9	169	26	66	18.2
PFTrDA	9.8	0.8	662.9	618.96	12	66	18.2
11Cl-PF3OUdS	10	0.8	630.9	450.94	27	140	18.2
11Cl-PF3OUdS_37Cl	10	0.8	632.9	452.94	27	140	18.2
FOSA	10.1	0.8	497.9	78	30	132	18.2
FOSA	10.1	0.8	497.9	169	27	132	18.2
FOSA	10.1	0.8	497.9	478	23	132	18.2
M8FOSA	10.1	0.8	506	78	30	132	18.2
NMeFOSE	11	2	616	59	16	65	20.3
d7-NMeFOSE	11	2	623	59	16	65	20.3
PFTeDA	10.5	0.8	712.9	169	28	71	20.3
PFTeDA	10.5	0.8	712.9	668.96	12.5	71	20.3
M2PFTeDA	10.5	0.8	715	670	12.5	71	20.3
NMeFOSA	11.2	2	512	169	26	130	20.3
NMeFOSA	11.2	2	512	219	24	130	20.3
d3-NMeFOSA	11.2	2	515	219	24	130	20.3
NEtFOSE	11.5	2	630	59	16	68	23.1
d9-NEtFOSE	11.5	2	639	59	16	68	23.1
PFDoS	10.9	0.8	698.9	80	53	190	23.1
PFDoS	10.9	0.8	698.9	99	48	190	23.1
NEtFOSA	11.7	2	526	169	26	138	24.7
NEtFOSA	11.7	2	526	219	23	138	24.7
d5-NEtFOSA	11.7	2	531	219	23	138	24.7

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