PFAS

Semi-automated Solid Phase Extraction Cleanup of Soil Samples with LC-MS/MS Analysis of Per- and polyfluoroalkyl Substances (PFAS) in Accordance with EPA Method 1633

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Abstract

Purpose: In this study we demonstrate the sensitivity of the Thermo Scientific™ Altis Plus triple quadrupole MS applied to EPA Method 1633[1] through low method detection limits and limits of quantitation four times lower than those prescribed for the entire panel of analytes.

Methods: This work describes that workflow by leveraging the Thermo Scientific™ Dionex™ AutoTrace™ 280 PFAS Solid-Phase Extraction (SPE) instrument to automate the soil sample solid-phase extraction (SPE) cleanup stage that is described in EPA Method 1633. The workflow utilizes the Vanquish Flex Binary UHPLC, the TSQ Altis Plus triple quadrupole mass spectrometer, and the Thermo Scientific™ Chromeleon™ Chromatography Data System to achieve reproducible and precise sample quantitation to meet the regulatory requirements for both water and solid sample types.

Results: In this work an overview of the LC-MS/MS workflow for PFAS quantitation in solid samples will be discussed in detail.

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. The EPA's Method 1633 is one example of a regulatory method that mandates determining the quantitative results for 40 PFAS compounds. Laboratories face the challenge of meeting these requirements while improving sample throughput and maintaining data quality to meet their productivity needs. In this study, we show that the Auto Trace 280 PFAS and the TSQ Altis Plus together form a sensitive and consistent solution for EPA 1633.

Materials and methods

Consumables

Figure 1. List of chemicals and consumables used

Item	Product	Part number
PFAS delay column	Hypersil GOLD, 4.6 x 50 mm, 1.9 µm	25002-054630
Analytical column	Acclaim 120 C18, 2.1 x 50 mm, 2.2 µm	068981
Guard column	Acclaim 120 C18, 2.1 × 10 mm, 5 µm	069689
Guard column kit	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	W8-1
	Acetonitrile, UHPLC-MS grade, 1 L	A956-1
	Ammonium acetate, LC-MS grade, 50 g	A114-50
	Acetic acid, LC-MS grade, 1 mL ampoules	A113-10X1AMP
Other reagents	Methanol, UHPLC-MS grade, 1 L	A458-1
	Ammonium hydroxide, ACS Plus grade, 500 mL, glass bottle	A669-500
	Formic acid, LC-MS grade, 1 mL ampoules	A117-10X1AMP
Solids reference matrix	Ottawa sand	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	Phenomenex Strata™ PFAS (GCB/WAX), 50 mg/200mg/6mL, 30/pk	CS0-9214
AutoTrace 280 PFAS collection vials	Round bottom PP culture tubes	187261
Autosampler vials	Polypropylene, 1.5 mL, screw-top, Level 1	6ESV9-1PP
Autosampler caps	Polypropylene caps, 9 mm, screw-thread	C5000-50

Calibration Acceptance Criteria

A calibration curve of 9 points spanning over 3 orders of magnitude was used for evaluation. At LOQ, a signal to noise ratio of greater than or equal to 3:1 was required for quantitative and confirmation ions. If the compound has no confirmation ion, a ratio of 10:1 or greater was required. The relative standard error for all components was under 20% for each point of the calibration curve. The R^2 value must be above 0.99.

Method Detection Limits (MDL)

Five-gram portions of Ottawa sand were weighed out and spiked at a concentration twice that of the lowest calibration concentration. A summary of the full analytical protocol can be found in Figure 3. MDLs were calculated by multiplying the standard deviation of the calculated concentration of 7 samples by the Student's t-value for n-1.

Data Analysis

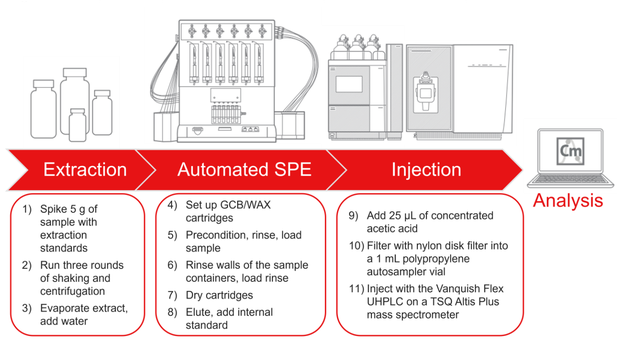
Data was collected and analyzed in Chromeleon CDS using reporting templates designed to meet the reporting requirements for EPA 1633. Analyte peaks must have a retention time match to the authentic standards and match the ion ratio +/- 50% of that of the middle of the calibration curve.

Figure 2. LC method[2]

Parameter	Value
Analytical column	Thermo Scientific™ Acclaim™ 120 C18, 2.1x50mm, 2.2 µm
Delay column	Thermo Scientific™ Hypersil GOLD™, 4.6 x 50 mm, 1.9 µm
Column temperature	40 C
Injection volume	5 µL
Autosampler temperature	20 C
Mobile phase	(A) H2O + 2% ACN + 2 mM ammonium acetate + 0.1% acetic acid (B) ACN + 2% H2O + 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

Figure 3. Simplified depiction of the EPA 1633 protocol for soil samples



Results

Figure 4. Calibration and method detection limit (MDL) results table (n=7). (*) Asterisk denotes quadratic calibration.

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

Figure 5. Extraction standard recoveries of the MDL samples. The blue dashed lines represent the MDLs listed in EPA 1633 from the validation study. Error bars represent +/- 1 standard deviation.

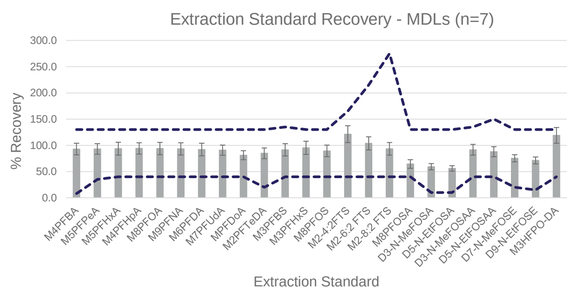
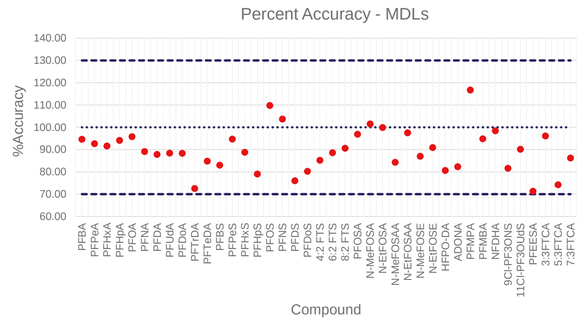


Figure 6. Percent accuracy for MDL samples (n=7) at concentration 2x of the LOQ.



Conclusions

- Method blanks and method detection limits for this workflow met the established method criteria.
- AutoTrace 280 PFAS scripts have been developed for extract cleanup on bimodal GCB/WAX cartridges.
- The TSQ Altis Plus MS demonstrates high sensitivity to go beyond currently established LOQs for EPA 1633.

References

1. U.S. EPA 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/2022-12/3rd%20Draft%20Method%201633%20December%202022%2012-20-22_508.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

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Trademarks/licensing

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