RESTEK Pure Chromatography

Simplifying EPA 1633 analyses with an improved dual bed solid-phase extraction method

Jason Hoisington[1], Alexis Shelow[1], Jason Thomas[1], Colton Myers[1], Diego A. Lopez[1]
[1]Restek Corporation, Bellefonte, PA

PFAS and EPA 1633

Per- and Polyfluoroalkyl Substances (PFAS) are synthetic organofluorine chemicals that have been linked to a range of health effects, including decrease immune response, vaccine effectiveness, cancer risk, among others.

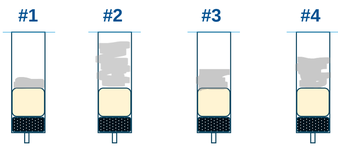
EPA Method 1633 was finalized early last year, and it represents a comprehensive analysis of 40 target PFAS compounds in different aqueous, solids, and tissue matrixes. This method uses a weak anion exchange (WAX) solid phase extraction (SPE), coupled with a dispersive graphitized carbon black (GCB) clean-up step prior to LC-MS/MS analysis on a C18 column.

Statement of Problem

1. Dispersive GCB can be a cumbersome and laborious step that requires further filtration before LC-M/MS analysis.

2. EPA Method 1633 requires analysis of large volume (up to 500 mL) samples of complex aqueous matrices with a varying degree of suspended solids that tend to clog the SPE cartridge, even with addition of glass wool or settling and centrifugation steps. A secondary SPE tube is necessary if clogging occurs.

Figure 1. Inter-cartridge variation from the amount of wool used to fill up to "half the height" of the SPE tube. Manual packing carried out in triplicates by four different lab technicians.



	#1	#2	#3	#4
Weight of wool used (n= 3)	39 mg	150 mg	51 mg	116 mg
Std. Dev.	2 mg	13 mg	7 mg	49 mg
%RSD	5%	8%	14%	42%

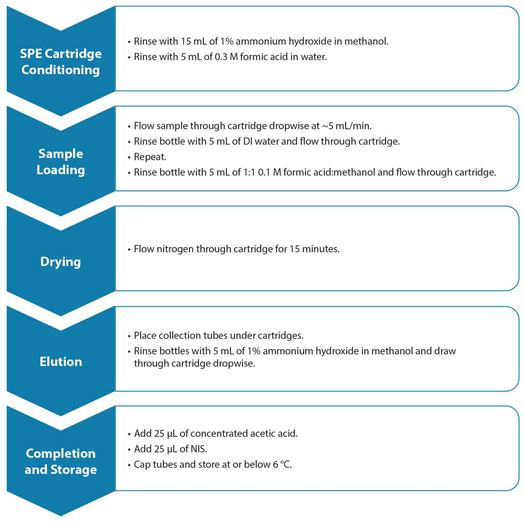
Solution Approach

A dual bed WAX/GCB cartridge that leverages an integrated Filter Aid; therefore, eliminating the need for dispersive carbon clean up, as well as the requirement to hand-pack deactivated glass wool in each tube.

Sample Preparation Workflow

Samples for precision, accuracy, and method detection limit (MDL) studies were prepared in polypropylene bottles using 500 mL deionized water spiked with 25 µL of extracted internal standards (EIS, Wellington Laboratories, Cat. # MPFAC-HIF-ES) as per EPA Method 1633, Section 11.2.4. Four samples for precision and recovery analysis were spiked with 200 µL of native PFAS standards (Wellington Laboratories, Cat. # EPA-1633STK), giving pre-extraction concentrations of 100-2500 ng/L. Seven MDL samples were spiked with 20 µL of a 20:1 dilution of the native standard, giving pre-extraction concentrations of 0.5-12.5 ng/L. Seven blank replicates were prepared for the MDL study as well. The MDL samples were prepared and analyzed over three days.

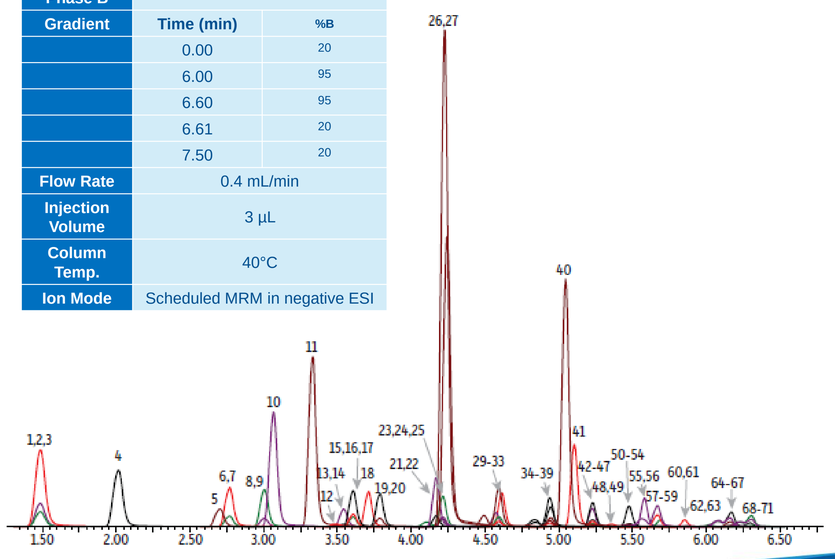
Figure 2. Sample preparation procedure per EPA 1633 using Thermo Scientific AutoTrace 280 PFAS System.



LC-MS/MS Parameters

Figure 3. Analytical conditions (Waters Xevo TQ-S with ACQUITY Premier UPLC) and chromatogram showing all target PFAS and isotopically labeled compounds.

Analytical Column	1.8 µm Force C18 50x2.1 mm id	
PFAS Delay Column	5 µm PFAS Delay 50x2.1 mm id	
Mobile Phase A	Water, 5mM Ammonium Acetate	
Mobile Phase B	Methanol	
Gradient	Time (min)	%B
	0.00	20
	6.00	95
	6.60	95
	6.61	20
	7.50	20
Flow Rate	0.4 mL/min	
Injection Volume	3 µL	
Column Temp.	40°C	
Ion Mode	Scheduled MRM in negative ESI	



Results and Discussion

Table 1. Results from MDL, Blank, Accuracy, and Precision experiments for native PFAS.

Compound	Abbreviation	MDL (ng/L)	Blank (ng/L)*	Accuracy (%)	%RSD
Perfluorobutanoic acid	PFBA	0.34	ND	112	5
Perfluoro-3-methoxypropanoic acid	PFMPA	0.20	ND	109	6
3-Perfluoropropyl propanoic acid	3:3FTCA	0.31	ND	95	6
Perfluoropentanoic acid	PFPeA	0.26	ND	111	6
Perfluorobutane sulfonate	PFBS	0.19	ND	101	5
Perfluoro-4-methoxybutanoic acid	PFMBA	0.18	ND	111	6
Perfluoro(2-ethoxyethane)sulfonic acid	PFEESA	0.15	ND	94	5
Nonafluoro-3,6-dioxaheptanoic acid	NFDHA	0.46	ND	121	5
1*H*,1*H*,2*H*,2*H*-perfluorohexane sulfonate	4:2 FTS	0.36	ND	104	5
2*H*,2*H*,3*H*,3*H*-Perfluorooctanoic acid	5:3FTCA	1.48	ND	102	6
Perfluorohexanoic acid	PFHxA	0.08	ND	113	5
Perfluoropentane sulfonate	PFPeS	0.07	ND	122	4
Hexafluoropropylene oxide dimer acid	HFPO-DA	0.40	ND	114	12
Perfluoroheptanoic acid	PFHpA	0.08	ND	109	5
Perfluorohexane sulfonate	PFHxS	0.07	ND	104	7
4,8-Dioxa-3*H*-perfluorononanoic acid	ADONA	0.39	ND	99	9
1*H*,1*H*,2*H*,2*H*-perfluorooctane sulfonate	6:2 FTS	0.37	ND	107	4
3-Perfluoroheptyl propanoic acid	7:3FTCA	2.32	ND	100	6
Perfluoroheptane sulfonate	PFHpS	0.76	ND	121	8
Perfluorooctanoic acid	PFOA	0.32	ND	104	2
Perfluorooctane sulfonate	PFOS	0.20	ND	112	7
Perfluorooctanesulfonamide	PFOSA	0.21	ND	81	11
Perfluorononanoic acid	PFNA	0.08	ND	114	5
N-methyl perfluorooctanesulfonamide	NMeFOSA	0.07	ND	104	13
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	9Cl-PF3ONS	0.61	ND	85	11
Perfluorononanesulfonic acid	PFNS	0.25	ND	79	7
Perfluorodecanoic acid	PFDA	0.18	ND	117	8
N-ethyl perfluorooctanesulfonamide	NEtFOSA	0.14	ND	112	15
1*H*,1*H*,2*H*,2*H*-perfluorodecane sulfonate	8:2 FTS	0.91	ND	113	11
Perfluoroundecanoic acid	PFUnA	0.26	ND	125	12
N-methyl perfluorooctanesulfonamidoacetic acid	NMeFOSAA	0.15	ND	95	12
N-ethyl perfluorooctanesulfonamidoacetic acid	NEtFOSAA	0.23	ND	93	12
Perfluorodecanesulfonic acid	PFDS	0.25	ND	94	23
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF3OUdS	0.56	ND	77	25
Perfluorododecanoic acid	PFDoA	0.27	ND	118	15
N-methyl perfluorooctanesulfonamidoethanol	NMeFOSE	1.11	ND	106	9
N-ethyl perfluorooctanesulfonamidoethanol	NEtFOSE	1.10	ND	111	9
Perfluorotridecanoic acid	PFTrDA	0.33	ND	108	15
Perfluorododecanesulfonic acid	PFDoS	0.13	ND	89	12
Perfluorotetradecanoic acid	PFTeDA	0.32	ND	102	23

Table 2. Results from Precision and Accuracy experiments for isotope dilution standards.

Compound	Abbreviation	Accuracy (%)	% RSD
Perfluoro-*n*-[1,2,3,4- 13C4]butanoic acid	13C4-PFBA	88	4
Perfluoro-*n*-[1,2,3,4,5-13C5]pentanoic acid	13C5-PFPeA	88	5
Sodium perfluoro-1-[2,3,4-13C3]butanesulfonate	13C3-PFBS	97	3
Sodium 1*H*,1*H*,2*H*,2*H*-perfluoro-1-[1,2- 13C2]hexane sulfonate	13C2-4:2FTS	95	8
Perfluoro-*n*-[1,2,3,4,6- 13C5]hexanoic acid	13C5-PFHxA	122	2
2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)13C3-propanoic acid	13C3-HFPO-DA	92	4
Perfluoro-*n*-[1,2,3,4- 13C4]heptanoic acid	13C4-PFHpA	107	4
Sodium perfluoro-1-[1,2,3-13C3]hexanesulfonate	13C3-PFHxS	102	4
Sodium 1*H*,1*H*,2*H*,2*H*-perfluoro-1-[1,2-13C2]-octane sulfonate	13C2-6:2FTS	83	5
Perfluoro-*n*-[13C8]octanoic acid	13C8-PFOA	106	5
Sodium perfluoro-[13C8]octanesulfonate	13C8-PFOS	77	7
Perfluoro-1-[13C8]octanesulfonamide	13C8-PFOSA	94	11
N-methyl-d 3-perfluoro-1-octanesulfonamidoacetic acid	D3-NMeFOSAA	85	9
N-ethyl-d 5-perfluoro-1-octanesulfonamidoacetic acid	D5-NEtFOSAA	87	11
Perfluoro-*n*-[13C9]nonanoic acid	13C9-PFNA	83	7
Perfluoro-*n*-[1,2,3,4,5,6-13C6]decanoic acid	13C6-PFDA	80	7
Sodium 1*H*,1*H*,2*H*,2*H*-perfluoro-1-[1,2- 13C2]-decane sulfonate	13C2-8:2FTS	84	5
N-methyl-D 7-perfluorooctanesulfonamidoethanol	D7-NMeFOSE	108	13
N-ethyl-D 9-perfluorooctanesulfonamidoethanol	D9-NEtFOSE	88	17
N-methyl-D 3-perfluoro-1-octanesulfonamide	D3-NMeFOSA	84	8
N-ethyl-D 5-perfluoro-1-octanesulfonamide	D5-NEtFOSA	111	10
Perfluoro-*n*-[1,2,3,4,5,6,7-13C7]undecanoic acid	13C7-PFUnA	78	13
Perfluoro-*n*-[1,2- 13C2]dodecanoic acid	13C2-PFDoA	45	24
Perfluoro-*n*-[1,2- 13C2]tetradecanoic acid	13C2-PFTeDA	36	21

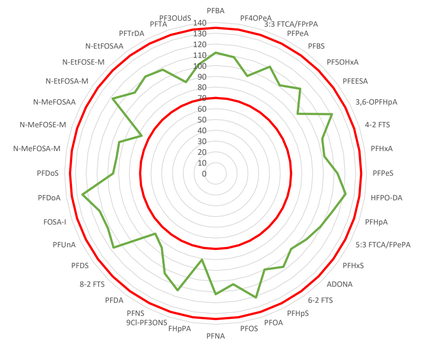


Figure 4. Accuracy (%) for all target PFAS compounds from four replicate spikes. Accuracy ranged from 77% to 125% of the spiked value, meeting the requirements in Table 5 of EPA Method 1633.

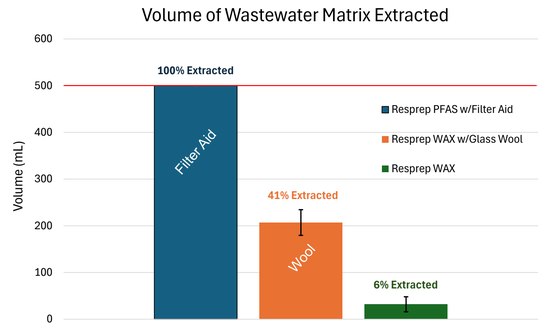


Figure 5. Average volume of 500 mL wastewater extracted before SPE clogging. Comparison between Filter Aid, glass wool, and control. ASTM substitute wastewater (ASTM International, D5905-98[2018]) diluted to ~100 mg/mL suspended solids was used.

Summary

Nondrinking water matrices present unique challenges to labs using SPE sample preparation. In this study, a dual bed WAX/GCB cartridge format exhibited consistency with the acceptance criteria in EPA method 1633. Furthermore, the *Filter Aid* greatly mitigates the clogging risk by this challenging matrix, allowing for 100% extraction of the recommended sample volume under method guidelines.

COI Disclosure: Authors are employed by Restek Corporation.