



TN-0155

Evaluation of Extraction Options for EPA Method 1633

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Introduction

PFAS compounds are present in all environmental matrices, including water, soil, air, and living organisms. Due to the persistent nature of these compounds, as well as their ability to be easily transported in the environment, there is a significant push to regulate them. The EPA has developed a PFAS Strategic Roadmap to outline the steps needed “to further the science and research, to restrict these dangerous chemicals from getting into the environment, and to immediately move to remediate the problem in communities across the country.” One of these steps was to develop and promulgate a standardized method of testing for PFAS in non-Drinking Water matrices. The EPA, in collaboration with the Department of Defense (DOD), has published Method 1633 to test for 40 PFAS compounds in wastewater, surface water, groundwater, soil, biosolids, sediment, landfill leachate, and fish tissue.

This method involves a two-step sample prep approach using a weak anion exchange (WAX) SPE cartridge and graphitized carbon black (GCB) clean-up in a powder format, known as dispersive Solid Phase Extraction (dSPE). For water samples, GCB is added after WAX extraction, but for soil samples the GCB is added before the WAX SPE step. The purpose of the additional GCB clean-up step is to eliminate matrix that can cause interference and reduce bias. GCB has been shown to remove organic acids (such as humic and cholic acids), which can suppress ionization and lead to low bias on the recoveries (especially for PFOS). However, limitations of using GCB are well known in that this media can bind to longer chain PFAS compounds and lead to lower recoveries. This is stated in the EPA Method 1633, “...It is important to minimize the time the sample extract is in contact with the carbon.” Besides these practical limitations, adding GCB in a dSPE step is very labor intensive and therefore not practical due to the extra time needed to add, mix, and centrifuge for each sample, especially in high throughput laboratories. This step can also lead to higher RSD values. To address these practical limitations, EPA Method 1633 Section 12.2.3 states, “The use of two stacked SPE cartridges, the first containing the WAX sorbent and the second containing the activated carbon, or other cartridge configurations may be employed in place of the use of the loose carbon described above.”

Strata™ PFAS cartridges were developed as a single cartridge stacked with Strata-X-AW and Strata GCB sorbents that function as a traditional SPE cartridge with a built-in polishing step to meet the method guidelines. We have previously demonstrated the utility of the Strata PFAS stacked SPE format for PFAS analysis following DOD QSM 5.2/ Table B15 for a variety of water matrices (TN-0145). We have shown that using a single, stacked WAX/GCB is cheaper, easier, and ultimately yields better recoveries for PFAS analytes from various water samples. This technical note presents a study to validate method performance for the broader compound list in EPA 1633 and demonstrate the same utility for both water and soil extracts.

Sample Preparation

As a guidance method, EPA Method 1633 makes provisions to demonstrate equivalency as described in section 9.1.2a “... laboratory is permitted certain options to improve separations or lower the costs of measurements. These options include alternative extraction, concentration, and clean-up procedures, and changes in sample volumes, columns, and detectors.” In addition, Section 7.1.7 indicates that “once the method is multi-laboratory validated, laboratories will have the flexibility to use carbon cartridges as long as all method QC criteria are met.” In order to demonstrate equivalency, the Initial Precision and Recovery (IPR) as described in EPA Method 1633 section 9.2.1 was used for the comparison studies for water and soil matrices. For the water

extract comparisons, 500 mL of reagent water per sample was used for 4 samples. For the soil comparison study, 5 g of Ottawa sand was used per sample for 4 samples, and all were spiked and extracted. Our extraction panel was expanded by adding three (3) additional analytes to reflect the California water board panel for PFAS compounds in wastewater discharges. Extracted Internal Standards (EIS) were added to all samples: 100 µL of Wellington MPFAC-HIF-ES 10x diluted + 13C2-PFHxDA at 25 ng/mL and 13C2D4-10:2-FTS at 100 ng/mL. The spike recoveries were compared with an injection internal standard fortified after extraction and results shown are in percent recovery. For details of the extraction procedure please refer to EPA Method 1633.

LC Conditions

Column: Fully Porous Sub-2 µm C18

Dimensions: 50 x 2.1 mm

Guard Column: SecurityGuard™ ULTRA for EVO-C18 (AJ0-9296)

Delay Column Dimensions: 30 x 4.6 mm

Mobile Phase: A: 2 mM Ammonium Acetate in (95:5, v/v) Water / Acetonitrile
B: Acetonitrile

Gradient:	Time (min)	%B	Flow Rate (mL/min)
	0	2	0.35
	0.2	2	0.35
	4	30	0.4
	7	55	0.4
	9	75	0.4
	10	95	0.4
	10.4	2	0.4
	11.8	2	0.4
	12	2	0.35

Injection Volume: 2 µL

Temperature: 40 °C

LC System: Agilent® 1290 Infinity

Detection: MRM

Detector: Agilent 6495

MS Conditions

Polarity: Negative

Capillary Voltage: 2000

Nebulizer Pressure (psi): 25

Gas Temperature: 120 °C

Gas Flow (L/min): 11

Sheath Gas Heater Temperature: 300 °C

Sheath Gas Flow: 11

Cell Accelerator Voltage: 4

Collision Energy (CE): Compound Dependent



Equivalency Studies from Water Samples

For water samples, the precision of the extractions was determined by comparing the %RSD of spiked native PFAS analytes between Waters® Oasis® WAX + dSPE with GCB, Strata™-X-AW + dSPE with GCB, and Strata PFAS WAX/GCB cartridges shown in **Figure 1**. Water samples were extracted using two different procedures based on the SPE cartridges. Samples were either extracted with WAX followed by GCB powder as described in EPA 1633 for the Oasis WAX and the Strata-X-AW cartridges. For the dual stacked Strata PFAS, the GCB cleanup step was not used. **Table 1** shows the PFAS panel, spike concentrations, % recoveries, and %RSD for the different SPE cartridges.

Figure 2 and **3** show the extraction precision of the PFAS analytes from the 4 water samples as %RSD for native and extracted internal standards (EIS). These results are compared with those obtained from the single lab, Draft Method (see labels "Table 5"). Overall, the precision was lower in our studies when compared to the published method. Though there were minor variations between the phases, the precision difference between the phases was not statistically significant within the sample size. Perfluorooctadecanoic Acid (PFODA) is not included in EPA Method 1633 but was added to this study as one of the standards tested per the CA water board recommendations.² This analyte had the poorest precision but still well below 15 %.

Figure 1. SPE Cartridges Used for the Comparison Study. Note the Order of the WAX/GCB vs. GCB/WAX for the Water and Soil Matrices, Respectively.

Waters Oasis WAX



150mg WAX

30 µm Particles
80 Å Pore Size
pKas 3.70 and 8.95
(Piperazine)

Strata-X-AW



150mg WAX
Part No.: [8B-S038-SCH](#)

30 µm Particles
80 Å Pore Size
pKa 6.09 and 9.27
(Ethylenediamine)

Strata PFAS
WAX/GCB



200mg WAX / 50mg GCB
Part No.: [CS0-9207](#)
For water extraction

WAX
50 µm Particles
80 Å Pore Size
GCB
120-400 mesh
85 m²/g surface area

Strata PFAS
GCB/WAX



50mg GCB / 200mg WAX
Part No.: [CS0-9214](#)
For soil/tissue extraction

GCB
120-400 mesh
85 m²/g surface area
WAX
50 µm particles
80 Å pore size

Table 1. Comparison of PFAS Percent Spike Recoveries in Water Extracts and %RSD Using Different SPE Phases.

Analyte	Spike (ng/L)	Strata-X-AW		Strata PFAS WAX/GCB		Oasis WAX	
		% Rec.	%RSD	% Rec.	%RSD	% Rec.	%RSD
PFBA	230.40	99	2.2	103	1	90	2
PFPeA	115.20	103	1.3	103	2	90	2
PFHxA	57.60	102	2.2	105	3	89	3
PFHpA	57.60	59	2.5	101	4	90	2
PFOA	57.60	58	1.3	102	4	91	3
PFNA	57.60	100	3.2	102	3	90	3
PFDA	57.60	99	3.4	103	1	89	3
PFUnA	57.60	105	1.5	104	2	90	2
PFDoA	57.60	102	2.9	103	4	91	3
PFTTrDA	57.60	104	4.5	110	4	100	1
PFTeDA	57.60	101	1.5	101	3	93	3
PFHxDA	57.60	98	1.5	103	2	96	6
PFoDA	57.60	116	4.6	76.2	14	87	13
PFBS	51.10	101	1.3	102	1	89	3
PFPeS	54.20	101	2.4	102	2	89	1
PFHxS	52.60	101	2.6	101	2	89	1
PFHpS	54.90	104	1.3	111	1	90	2
PFOS	53.50	104	1.5	105	3	90	2
PFNS	55.40	108	2.5	106	2	91	2
PFDS	55.60	105	0.77	107	2	89	3
PFDoS	55.90	98.3	2	90.5	5	81	5
4:2-FTS	216.00	102	0.32	102	2	90	4
6:2-FTS	218.90	98.9	2.5	97.1	2	88	2
8:2-FTS	221.20	102	2.4	103	4	89	6
10:2-FTS	222.60	97.9	4.3	105	3	83	5
PFOSA	57.60	102	1.6	105	2	91	2
MeFOSA	57.60	101	3.4	97.5	3	92	2
EtFOSA	57.60	101	4	102	4	92	4
MeFOSAA	57.60	106	2.9	102	1	91	4
EtFOSAA	57.60	101	2.8	104	4	90	1
MeFOSE	576.00	101	1.5	107	2	89	1
EtFOSE	576.00	103	0.4	102	1	92	1
HFPO-DA	230.40	107	0.79	108	2	87	1
ADONA	217.70	105	0.94	110	3	87	2
PFMPA	115.20	29	7	106	2	70	7
PFMBA	115.20	109	4.5	105	2	88	2
NFDHA	1152.00	103	2.3	125	10	82	5
9CI-PF3ONS	215.40	104	5.5	105	4	84	2
11CI-PF3OUDS	217.70	105	5.3	104	3	82	3
PFEESA	102.50	101	1.2	106	3	87	3
3:3-FTCA	288.00	80	14	101	2	66	6
5:3-FTCA	1440.00	101	1.5	101	3	90	4
7:3-FTCA	1440.00	101	0.9	100	4	91	4



Figure 2. %RSD of Spiked, Native PFAS Compounds from Water Samples. Precision Represents the Average of n=4 Extracts. The Green Dots are the %RSD from EPA Method 1633 "Table 5."

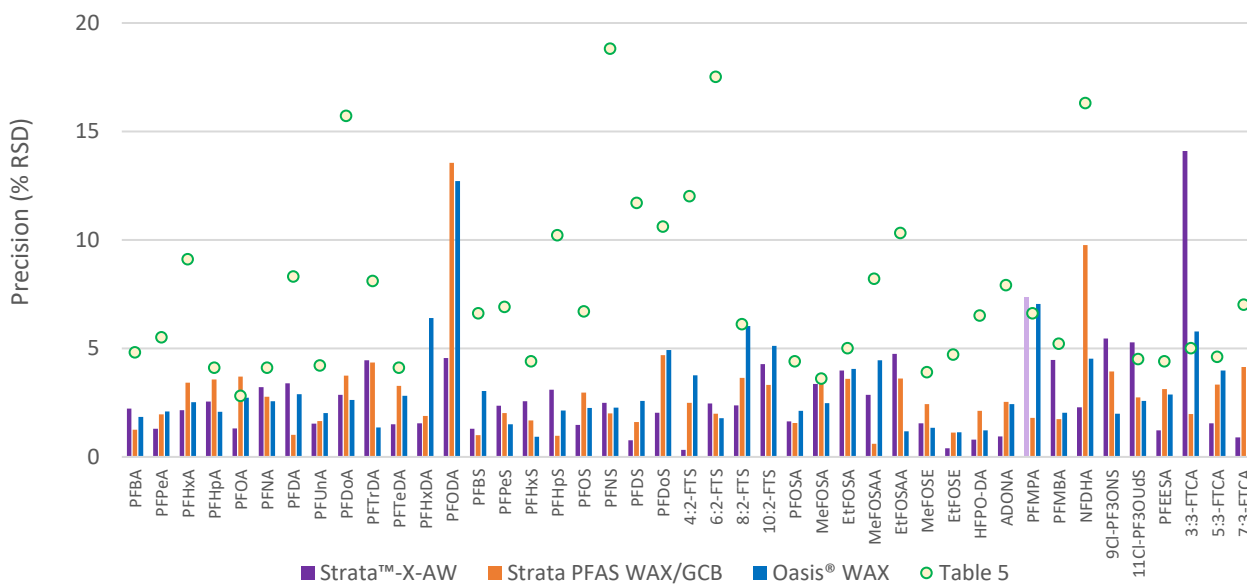
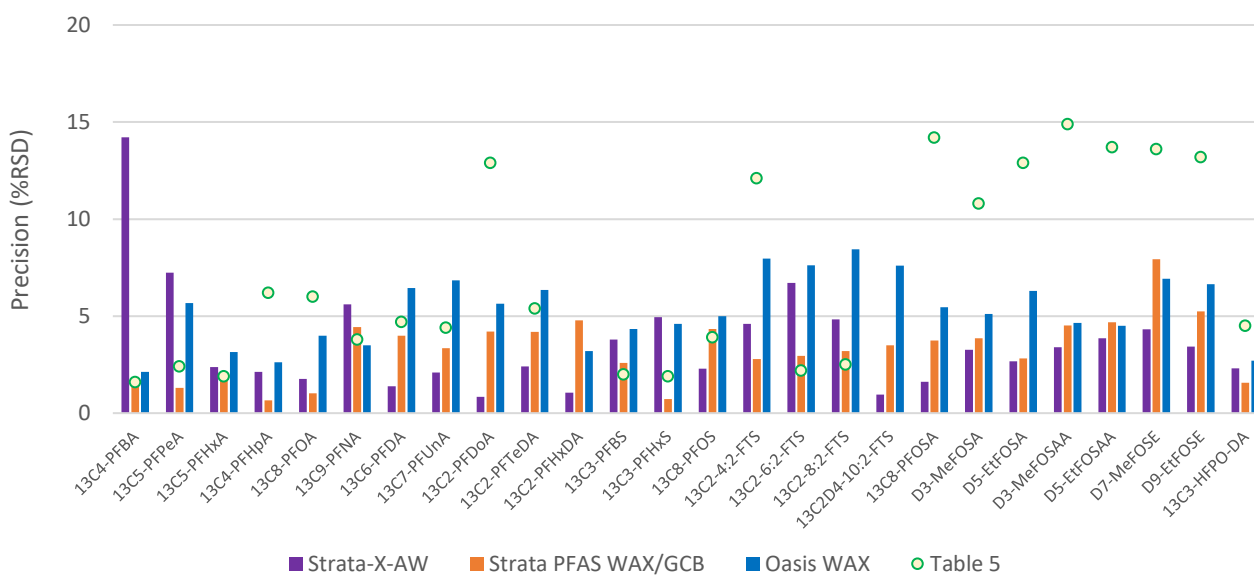


Figure 3. %RSD of Spiked, EIS from Water Samples. Precision Represents the Average of n=4 Extracts. The Green Dots are the %RSD from EPA Method 1633 "Table 5."



Equivalency Studies from Soil Extracts

Per EPA Method 1633, soil extraction requires using an initial 0.3 % Methanolic Ammonium Hydroxide (see section 11.3.4 - 11.3.7) extraction, GCB clean-up by dSPE, followed by SPE using a WAX cartridge. Thus, the dSPE clean-up and WAX SPE are in a reverse order compared to the water samples. To simplify clean-up and SPE into a single step, we used a stacked cartridge that had GCB on top of the WAX SPE (the reverse order) compared to the format used for the water samples. Table 2 shows the PFAS panel, spike concentrations, % recoveries, and %RSD comparison between Strata PFAS GCB/WAX and Oasis WAX + GCB dSPE. The precision for the soil extraction are shown as %RSD for both native and extracted internal standards in **Figures 4 and 5**, respectively. **These results are compared with those obtained from the single lab, Draft Method.** The %RSD for the native standards had excellent precision below 10 % for all PFAS standards except for PFODA. Both procedures produced equivalent results for the cartridge comparison tests.

Table 2. Comparison of PFAS Percent Spike Recoveries in Water Extracts and %RSD Using Different SPE Phases.

Analyte	Spike (ng/g)	Strata™ PFAS GCB/WAX		Oasis® WAX + GCB dSPE	
		% Rec.	%RSD	% Rec.	%RSD
PFBA	23.0	102	1.2	105	1.4
PFPeA	11.5	102	0.9	105	1.9
PFHxA	5.8	101	2.0	105	1.6
PFHpA	5.8	102	1.4	104	1.6
PFOA	5.8	100	2.8	104	2.4
PFNA	5.8	100	1.2	104	3.3
PFDA	5.8	97.3	1.6	102	2.7
PFUnA	5.8	100	3.6	107	3.1
PFDoA	5.8	102	1.3	108	3.3
PFTTrDA	5.8	103	1.8	104	3.9
PFTeDA	5.8	98.4	0.7	105	2.3
PFHxDA	5.8	95.3	4.8	106	5.2
PFoDA	5.8	73.8	21	138	23
PFBS	5.1	101	0.68	105	1.4
PFPeS	5.4	101	2.9	99.6	2.4
PFHxS	5.3	100.6	2.7	101	2.4
PFHpS	5.5	100	4.6	104	1.5
PFOS	5.3	99.7	4.4	104	2.4
PFNS	5.5	101	5.1	107	2.8
PFDS	5.6	98.5	4.7	102	2.8
PFDoS	5.6	91.0	5.9	106	6.0
4:2-FTS	21.6	103	3.3	101	3.2
6:2-FTS	21.9	99.8	2.3	107	5.0
8:2-FTS	22.1	96.6	3.3	105	2.6
10:2-FTS	22.3	101	3.6	105	4.3
PFOSA	5.8	99.4	1.4	112.6	5.6
MeFOSA	5.8	103	3.4	115	8.0
EtFOSA	5.8	105	7.4	116	8.2
MeFOSAA	5.8	101	3.7	104	5.4
EtFOSAA	5.8	104	3.2	107	3.2
MeFOSE	57.6	101	1.4	109	4.9
EtFOSE	57.6	99.5	1.5	104	6.4
HFPO-DA	23.0	106	1.3	102	1.5
ADONA	21.8	106	1.7	103	1.0
PFMPA	11.5	99.3	2.3	100	1.6
PFMBA	11.5	101	0.94	103	2.4
NFDHA	115.2	105	2.4	98.0	2.9
9CI-PF3ONS	21.5	107	4.2	108	2.9
11CI-PF3OUdS	21.8	103	2.9	108	3.9
PFEESA	10.3	101	2.2	102	1.1
3:3-FTCA	28.8	82.3	5.8	91.3	4.7
5:3-FTCA	144	98.4	1.8	101	0.93
7:3-FTCA	144	96.5	1.7	102	0.61



Figure 4. %RSD of Spiked, Native PFAS Compounds from Soil Extracts. Precision Represents the Average of n=4 Extracts. The Green Dots are the %RSD from EPA Method 1633 "Table 5."

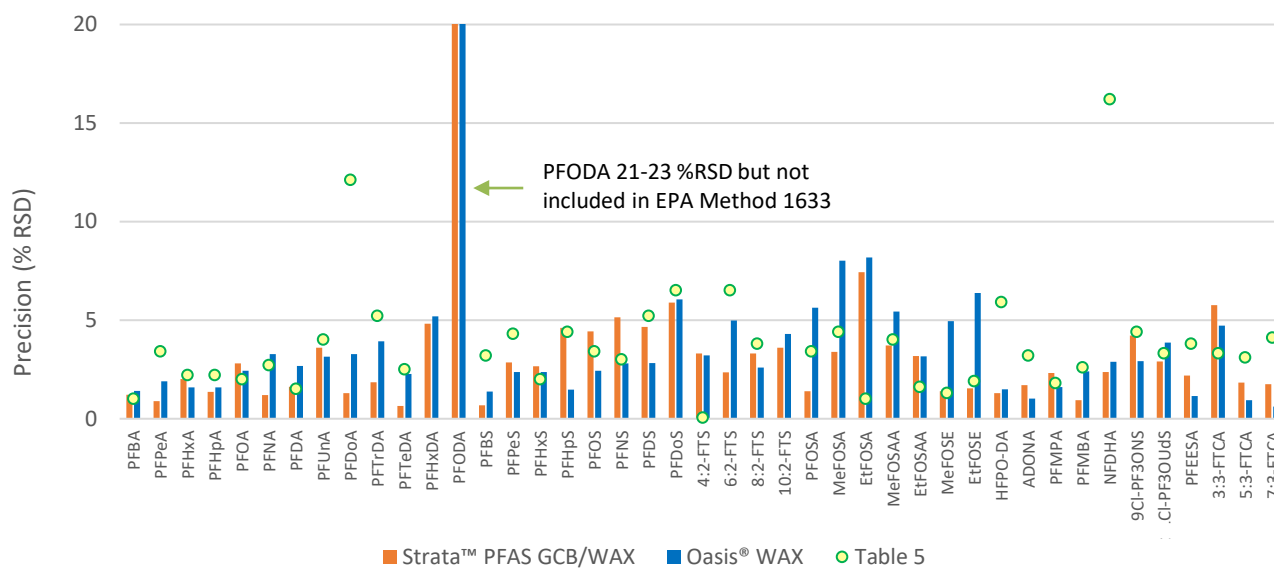
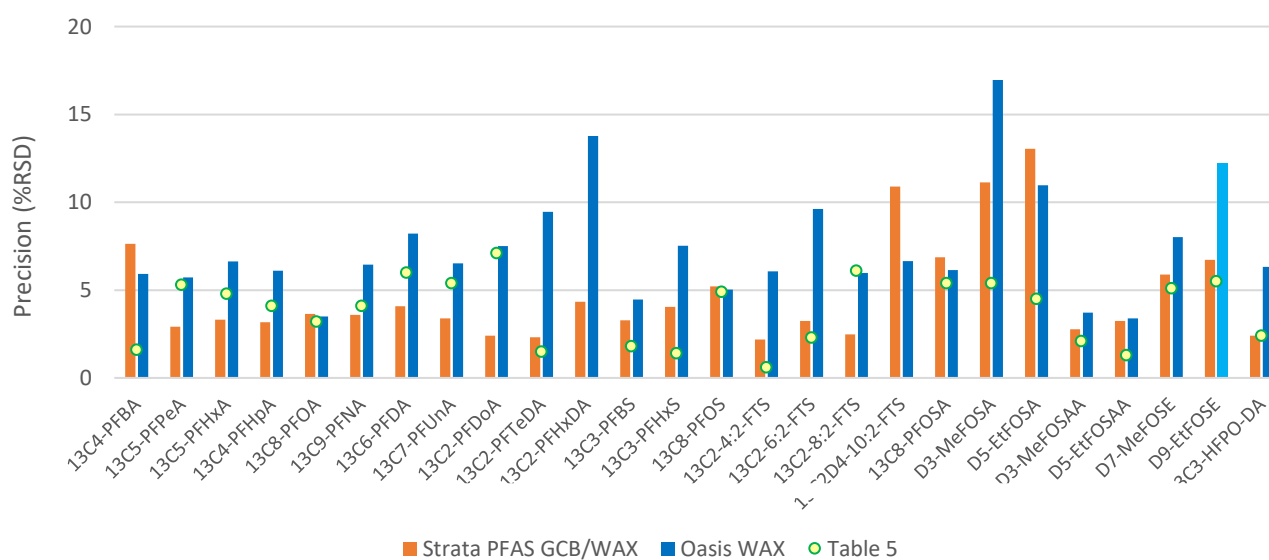


Figure 5. %RSD of Spiked, EIS from Soil Extracts. Precision Represents the Average of n=4 Extracts. The Green Dots are the %RSD from EPA Method 1633 "Table 5."



Conclusions

Our results demonstrate equivalence of Strata™ PFAS stacked SPE format (compared to WAX + GCB dSPE) for an EPA 1633 PFAS panel from water samples and soil extracts. Importantly, Strata PFAS dual layer cartridges (with elimination of dSPE) provides equivalent performance to WAX cartridges specified in EPA Method 1633 for all 40 EPA 1633 parameters plus PFHxDA, PFODA, and 10:2FTS for both soil and water, as per IDOC requirements (Section 9.1.2) and DOD QSM 5.4 Table B-24. For the soil extract, only PFODA (which is not included in EPA 1633 PFAS panel) failed the IDC for both procedures. In our laboratory, the elimination of adding GCB in a dSPE step reduces labor per analytical batch (20 samples) by approximately 30 minutes for manual cartridge SPE clean-up. Elimination of the filtration step would provide a further 30 min labor reduction. Incorporation of the dual layer cartridges into the workflow enables automation of the full clean-up procedure, with the potential for a significant reduction in labor and improvements in data reproducibility.

References

1. Office of Science and Technology. (2024, January). [Method 1633: Analysis of Per- and Polyfluoroalkyl Substances \(PFAS\) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS](#). (EPA 821-R-24-001). Environmental Protection Agency. Office of Water.
2. WATER CODE SECTIONS 13267 AND 13383 ORDER FOR THE DETERMINATION OF THE PRESENCE OF PER- AND POLYFLUOROALKYL SUBSTANCES AT PUBLICLY OWNED TREATMENT WORKSORDER WQ 2020-0015-DWQ. CA Water Board www.waterboards.ca.gov


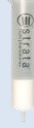


SecurityGuard™ ULTRA Cartridges Ordering Information

Material	Description	pH Stability	Column ID (mm)		
			2.1	3.0	4.6
			/3pk	/3pk	/3pk
EVO C18	(ODS, Octadecyl)	1.0 – 12.0	AJ0-9298	AJ0-9297	AJ0-9296
C18	(ODS, Octadecyl)	1.5 – 8.5*	AJ0-8782	AJ0-8775	AJ0-8768
C8	(MOS, Octyl)	1.5 – 8.5*	AJ0-8284	AJ0-8777	AJ0-8770
PFP	(Pentafluorophenyl)	1.5 – 8.5*	AJ0-8287	AJ0-8780	AJ0-8773
F5	(Pentafluorophenyl)	1.5 – 8.5*	AJ0-9322	AJ0-9321	AJ0-9320
Biphenyl	(Biphenyl)	1.5 – 8.5*	AJ0-9209	AJ0-9208	AJ0-9207
Phenyl	(Phenylhexyl)	1.5 – 8.5*	AJ0-8788	AJ0-8781	AJ0-8774
HILIC	(HILIC)	2.0 – 7.5	AJ0-8786	AJ0-8779	AJ0-8772
Polar C18	(Polar Functional C18)	1.5 – 8.5*	AJ0-9532	AJ0-9531	AJ0-9530

*pH stable 1.5 – 8.5 under gradient conditions. pH stable 1.5–10 under isocratic conditions.
[AJ0-9000](#) is the universal holder designed for use with 2.1mm, 3.0mm and 4.6mm ID cartridges.

Strata™-X-AW Ordering Information

Strata			
Format	Sorbent Mass	Part Number	Unit
Tube			
	100 mg	8B-S038-EBJ	3 mL (50/box)
	100 mg	8B-S038-ECH	6 mL (30/box)
	150 mg	8B-S038-SCH	6 mL (30/box)
	200 mg	8B-S038-FBJ	3 mL (50/box)
	200 mg	8B-S038-FCH	6 mL (30/box)
	500 mg	8B-S038-HBJ	3 mL (50/box)
	500 mg	8B-S038-HCH	6 mL (30/box)
Giga™ Tube			
	500 mg	8B-S038-HDG	12 mL (20/box)
	1 g	8B-S038-JDG	12 mL (20/box)
	1 g	8B-S038-JEG	20 mL (20/box)
	5 g	8B-S038-LFF	60 mL (16/box)

Strata PFAS Ordering Information

Strata		
Sorbent Mass	Part Number	Unit
200 mg / 50 mg (WAX/GCB)	CS0-9207	6 mL (30/box)
250 mg / 50 mg (WAX/GCB)	CS0-9215	6 mL (200/box)
250 mg / 100 mg (GCB/WAX)	CS0-9217	6 mL (30/box)
500 mg / 50 mg (WAX/GCB)	CS0-9208	6 mL (200/box)
50 mg / 200 mg (GCB/WAX)	CS0-9214	6 mL (30/box)

PFAS CRM Native Standards. All analytes at the same concentration in acid form for easy calculation and dilution.

Product	Part	Volume	Concentration
EPA 533 mix	AL0-101838	1 mL	2 µg/mL in Methanol
EPA 537.1 mix	AL0-101839	1mL	2 µg/mL in Methanol
EPA 533 + 537.1 mix	AL0-101840	1 mL	2 µg/mL in Methanol

Customized CRMs available. Contact Phenomenex for details.

Other Recommended Products for Your PFAS Methods

Description	Part No.
Luna™ Omega Column 3 µm PS C18 50 x 3 mm	00B-4758-Y0
Kinetex™ EVO Column 5 µm C18 100 x 2.1 mm	00D-4633-AN
Strata SDB-L 500 mg/6mL tubes, 30/pk	8B-S014-HCH
Verex™ Vial, 9 mm Screw, PP, 1.7 mL, 1000/pk	AR0-39P0-13
Verex Vial, 9 mm Screw, PP, 300 µL, 1000/pk	AR0-39P2-13
Verex Vial, 9 mm Screw, PP, 700 µL, 1000/pk	AR0-39P1-13
SecurityCAP™ mobile phase filter for PFAS testing, 6-month capacity, ¼ in-28 thread	AC2-0961-P
SecurityCAP mobile phase starter kit for PFAS testing, 3-port GL45 caps and 6-month safety filter	AC2-4345-P
SecurityCAP mobile phase starter kit for PFAS testing, 2-port GL45 caps and 6-month safety filter	AC2-4245-P

Columns and vials available in multiple sizes. Contact Phenomenex for details.



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Comparative separations may not be representative of all applications.
SecurityGuard is patented by Phenomenex. U.S. Patent No. 6,162,362.

CAUTION: this patent only applies to the analytical-sized guard cartridge holder, and does not apply to SemiPrep, PREP, or ULTRA holders, or to any cartridges.

Strata-X is patented by Phenomenex. U.S. Patent No. 7,119,145.

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