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Applikationsbericht

Repeatability and Reproducibility of the Oasis™ WAX/GCB for PFAS Analysis Cartridges in Aqueous Samples for EPA Method 1633

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Abstract

In January 2024, EPA Method 1633 was finalized. EPA Method 1633 is the first method to incorporate the determination of 40 PFAS compounds across many challenging environmental sample matrices outside of drinking water, including non-potable waters (ground water, surface water, and wastewater) as well as soil, biosolids, and tissue by LC-MS/MS analysis. EPA Method 1633 requires use of both weak anion exchange (WAX) solid phase extraction with graphitized carbon black (GCB) cleanup and is a performance-based method allowing for modifications as long as acceptance criteria for recoveries and %RSDs are met.

This application note highlights the sample extraction for non-potable aqueous samples in EPA Method 1633 using a dual-phase, or bilayer cartridge: Oasis WAX/GCB for PFAS Analysis meeting the method acceptance criteria and displays reproducibility of the cartridges.

Benefits

- Oasis WAX/GCB for PFAS Analysis, a dual-phase cartridge is reproducible and repeatable for EPA Method

1633 aqueous samples in both inter- and intra-batch assays

- Reduction of manual steps, overall sample preparation time by use of the dual-phase SPE cartridge
- Acceptance criteria for recoveries and %RSDs are met for EPA Method 1633 for aqueous samples

Introduction

With EPA Method 1633 finalized, this introduces the first comprehensive US EPA method focused outside of drinking water for PFAS determination.[1] As the method incorporates a wide variety of matrices including ground water, surface water, wastewater, soil, biosolids, and tissue, their sample preparation to ensure reproducibility, sensitivity, and robustness is critical. In EPA Method 1633, the sample preparation incorporates two sorbents, WAX and GCB. For aqueous samples, extraction through a WAX cartridge is described first, followed by GCB clean up. However, the method is performance-based and gives requirements for establishing equivalency.[1] Oasis WAX/GCB for PFAS Analysis cartridges can alternatively be used and have been tested and shown to meet the acceptance criteria for multiple non-potable water sources as described previously.[2] Oasis WAX/GCB is a dual-phase, or bilayer cartridge which combines both sorbents into a single device benefitting the user by removing total manual steps in the sample preparation workflow and reducing time by up to 20% compared to use of loose GCB and a WAX cartridge. Additionally, Oasis WAX/GCB for PFAS Analysis undergoes a QC-batch release test for common PFAS to ensure cleanliness during the SPE process reducing the risk of false positives.

This application note uses Oasis WAX/GCB for PFAS Analysis on non-potable water samples showing that the acceptance criteria for EPA Method 1633 is met across three separate product lots of WAX/GCB with six replicates within each lot. This demonstrates the robustness of the SPE product in EPA 1633 workflows and the repeatability and reproducibility both inter- and intra-batch of WAX/GCB cartridges to ensure confidence in out-of-the-box performance of the cartridges lot after lot for use with complex matrices, like those in EPA Method 1633.

Figure 1. Oasis WAX/GCB for PFAS Analysis Cartridges.

Experimental

Sample Preparation and Extraction

Sample pretreatment:

Fill each sample bottle with 250 mL water. Spike 5 µL EIS Wellington stock to all sample bottles, **Check** pH with strips, and adjust to ≤6.

Add lab control sample to lab control bottle

Cartridge pretreatment:

Pack glass wool into half the height of the SPE barrel, loosely

Condition with 15 mL of 1% NH_4OH in methanol at 5 mL/min

Condition with 5 mL 0.3 M formic acid at 5 mL/min at 5 mL/min

Load:

Load 250 mL of sample to each cartridge reservoir at 5 mL/min

Air **Dry** for ~15s

Wash:

Let **dry**

Rinse the reservoir with 10 mL reagent water

Rinse the reservoir with 5 mL of 1:1 0.1M formic acid:methanol

Elute and collect:

Let **dry**

Place collection tubes in manifold.

Rinse with 5 mL 1% NH_4OH in methanol slowly

Post treat and transfer

Add 25 µL acetic acid to each sample extract

Add 5 µL NIS to samples

Vortex.

Transfer ~500 µL to injection vial for LCMS analysis

Note: rinse all sample bottles, manifold, needles, reservoirs, and clean hood with Methanol prior to start.

LC-MS Analysis

UPLC:	ACQUITY™ UPLC™ I-Class PLUS FTN, 50 µL Extension Loop
MPA:	2 mM ammonium acetate in water
MPB:	2 mM ammonium acetate in acetonitrile
Columns:	Analytical column: ACQUITY Premier BEH™ C_{18} 2.1 x 50 mm, 1.7 µm (p/n: 186009452) Isolator column: Atlantis™ Premier BEH C_{18} AX 2.1 x 50 mm, 5.0 µm (p/n: 186009407)
Column temperature:	35 ˚C
Sample temperature:	8 ˚C
Injection. volume:	2 µL
Wash solvent:	50:50 MeOH: H_2O
Purge solvent:	10:90 MeOH: H_2O
MS:	Xevo™ TQ-XS
Capillary voltage:	0.5 kV
Desolvation temperature:	350 °C
Desolvation flow:	900 L/hr

Vials:	700 µL Polypropylene Screw Cap Vials (p/n: 186005219)

UPLC Gradient Table

Time (min)	Flow (mL/min)	% MPA	% MPB	Curve
0	0.3	95	5	initial
0.5	0.3	75	25	6
3	0.3	50	50	6
6.5	0.3	15	85	6
7	0.3	5	95	6
8.5	0.3	5	95	6
9	0.3	95	5	6
11	0.3	95	5	6

Results and Discussion

High repeatability both inter- and intra-batch from non-potable water samples using dual-phase Oasis WAX/GCB for PFAS Analysis cartridges. Recoveries and RSD% meet acceptance criteria of EPA Method 1633.

		Lot 1		Lot 2		Lot 3	
Name	1633 Recovery acceptance criteria	Avg %Rec	# Reps	Avg %Rec	# Reps used	Avg %Rec	# Reps
PFBA	70–135	111	6	129	6	98	6
PFPeA	70–135	97	6	115	6	89	6
PFHxA	70–135	107	6	125	6	96	6
PFHpA	70–135	97	6	121	6	98	6
PFOA	65–155	97	6	117	6	96	6
PFNA	70–140	101	6	118	6	95	6
PFUnDA	65–140	110	6	116	6	98	6
PFDoDA	70–135	106	6	123	6	98	6
PFDA	70–130	103	6	113	6	100	6
PFTriDA	60–145	100	6	117	6	100	6
PFTreDA	70–145	105	6	116	6	101	6
PFBS	70–140	101	6	121	6	103	6
PFPeS	70–135	100	6	123	6	102	6
PFHxS	70–135	101	6	113	6	97	6
PFHpS	70–140	102	6	120	6	98	6
PFOS	70–140	102	6	121	6	97	6
PFNS	70–135	96	6	113	6	97	6
PFDS	70–135	93	6	114	6	103	6
PFDoDS	45–135	80	6	104	6	88	6
GenX (HFPO–DA)	70–135	103	6	117	6	96	6
ADONA	70–135	103	6	118	6	88	6
9ClPF3ONS	70–145	103	6	114	6	88	6
11ClPF3OUdS	50–150	96	6	108	6	85	6
4_2 FTS	70–135	96	6	108	6	90	6
6_2 FTS	70–135	122	6	137	4	119	6
8_2 FTS	70–140	100	6	119	6	102	6
FOSA	70–135	107	6	117	6	108	6
NMeFOSA	70–135	115	6	126	5	104	6
NEtFOSA	70–130	111	6	130	6	99	6
NMeFOSAA	65–140	112	6	115	6	95	6
NEtFOSAA	70–130	109	6	116	6	102	6
NMeFOSE	70–135	106	6	120	6	96	6
NEtFOSE	70–130	105	6	111	5	94	6
3:3 FTCA	70–130	107	6	102	5	103	6
5:3 FTCA	70–130	101	6	107	5	108	6
7:3 FTCA	55–130	106	6	109	6	107	6
PFMPA	60–140	102	6	118	6	104	6
PFMBA	65–145	104	6	118	6	100	6
Cholic acid	Not listed	120	6	105	6	123	6
PFEESA	70–135	103	6	117	6	101	6
NFDHA	65–140	105	6	120	6	107	6
M4 PFBA	52–130	75	6	82	6	86	6
M5_PFPeA	40–130	74	6	79	6	83	6
M5_PFHxA	40–130	74	6	80	6	84	6
M4_PFHpA	40–130	75	6	75	6	81	6
M8_PFOA	40–130	78	6	80	6	86	6
M9_PFNA	40–130	76	6	81	6	88	6
M6_PFDA	40–130	74	6	82	6	83	6
M7_PFUnDA	30–130	70	6	81	6	85	6
M_PFDoDA	10–130	68	6	76	6	80	6
M2_PFTreDA	10–130	55	6	69	6	75	6
M3_PFBS	40–135	75	6	76	6	84	6
M3_PFHxS	40–130	76	6	76	6	87	6
M8_PFOS	40–130	76	6	77	6	86	6
M2_42FTS	40–200	84	6	92	6	99	6
M2_62FTS	40–200	73	6	72	6	84	6
M2_82FTS	40–300	77	6	78	6	85	6
M8_FOSA	40–130	67	6	71	6	81	6
M3_GenX (M3–HFPO–DA)	40–130	73	6	79	6	84	6
D3_NMeFOSAA	40–170	66	6	75	6	82	6
D5_NEtFOSAA	25–135	65	6	74	6	83	6
dNMeFOSA	10–130	50	6	44	6	58	6
dNEtFOSA	10–130	52	6	45	6	61	6
d7 NMeFOSE	10–130	67	6	69	6	78	6
d9 NEtFOSE	10–130	66	6	69	6	78	6
M3 PFBA_NIS	50–200	158	6	125	6	125	6
M2 PFHxA_NIS	50–200	157	6	127	6	126	6
M4 PFOA_NIS	50–200	152	6	121	6	121	6
M5 PFNA_NIS	50–200	155	6	122	6	120	6
M2 PFDA_NIS	50–200	156	6	122	6	124	6
18O2 PFHxS_NIS	50–200	153	6	126	6	121	6
M4 PFOS_NIS	50–200	157	6	126	6	121	6

Figure 2. Demonstration of Oasis WAX/GCB for PFAS Analysis

performance repeatability requiring no protocol optimization, with recovery within 1633 acceptance criteria. Green displays values within 1633 acceptance criteria. For those labeled as "Not listed": EPA 1633 does not provide acceptance criteria for this compound. Note: 2/6 replicates excluded for 6_2 FTS in one lot.

		Lot 1		Lot 2		Lot 3	
Name	1633 %RSD acceptance criteria (≤)	Avg %Rec	# Reps	Avg %Rec	# Reps	Avg %Rec	# Reps
PFBA	21	6	6	21	5	5	6
PFPeA	23	7	6	22	6	6	6
PFHxA	24	7	6	22	6	8	6
PFHpA	28	7	6	23	6	5	6
PFOA	27	8	5	22	6	8	6
PFNA	28	10	6	18	6	7	6
PFUnDA	26	10	6	15	6	12	6
PFDoDA	29	3	6	22	6	8	6
PFDA	21	16	5	20	6	7	6
PFTriDA	29	4	6	13	6	9	6
PFTreDA	27	6	4	18	6	7	6
PFBS	23	5	6	20	6	5	6
PFPeS	25	8	6	17	6	7	6
PFHxS	27	9	6	21	6	4	6
PFHpS	30	9	6	21	6	12	6
PFOS	29	11	6	18	6	8	6
PFNS	29	10	6	16	6	8	6
PFDS	30	10	6	17	6	5	6
PFDoDS	35	9	6	19	6	8	6
GenX (HFPO-DA)	23	9	6	17	6	9	6
ADONA	23	10	6	17	6	8	6
9ClPF3ONS	30	18	6	18	6	9	6
11ClPF3OUdS	35	22	6	17	6	8	6
4_2 FTS	27	15	6	19	6	7	6
6_2 FTS	32	10	6	3	6	9	6
8_2 FTS	33	12	6	24	6	4	6
FOSA	22	6	5	22	6	13	6
NMeFOSA	30	5	4	23	5	7	4
NEtFOSA	26	7	5	25	5	6	4
NMeFOSAA	32	8	6	28	6	8	6
NEtFOSAA	28	13	6	22	6	4	6
NMeFOSE	29	7	6	22	6	6	6
NEtFOSE	21	6	6	7	6	5	6
3:3 FTCA	23	14	6	5	6	10	6
5:3 FTCA	24	9	6	3	6	4	6
7:3 FTCA	34	14	6	20	6	7	6
PFMPA	23	7	6	19	6	6	6
PFMBA	27	6	6	22	6	6	6
Cholic acid	Not listed	17	6	4	6	22	6
PFEESA	25	7	6	23	6	7	6
NFDHA	37	6	6	23	6	8	6
M4 PFBA	Not listed	17	6	19	6	3	6
M5_PFPeA	Not listed	17	6	19	6	4	6
M5_PFHxA	Not listed	15	6	20	6	5	6
M4_PFHpA	Not listed	14	6	19	6	4	6
M8_PFOA	Not listed	13	6	19	6	3	6
M9_PFNA	Not listed	11	6	18	6	4	6
M6_PFDA	Not listed	14	6	16	6	2	6
M7_PFUnDA	Not listed	16	6	19	6	3	6
M_PFDoDA	Not listed	16	6	21	6	3	6
M2_PFTreDA	Not listed	21	6	20	6	4	6
M3_PFBS	Not listed	19	6	21	6	6	6
M3_PFHxS	Not listed	15	6	20	6	3	6
M8_PFOS	Not listed	16	6	19	6	5	6
M2_42FTS	Not listed	16	6	18	6	4	6
M2_62FTS	Not listed	9	6	19	6	6	6
M2_82FTS	Not listed	14	6	22	6	10	6
M8_FOSA	Not listed	18	6	21	6	3	6
M3_GenX	Not listed	18	6	19	6	13	6
D3_NMeFOSAA	Not listed	17	6	21	6	2	6
D5_NEtFOSAA	Not listed	21	6	23	6	6	6
dNMeFOSA	Not listed	19	6	25	6	8	6
dNEtFOSA	Not listed	18	6	24	6	9	6
d7 NMeFOSE	Not listed	18	6	20	6	5	6
d9 NEtFOSE	Not listed	19	6	20	6	6	6
M3 PFBA_NIS	Not listed	9	6	11	6	6	6
M2 PFHxA_NIS	Not listed	8	6	11	6	6	6
M4 PFOA_NIS	Not listed	10	6	11	6	8	6
M5 PFNA_NIS	Not listed	9	6	11	6	7	6
M2 PFDA_NIS	Not listed	8	6	10	6	5	6
18O2 PFHxS_NIS	Not listed	11	6	10	6	6	6
M4 PFOS_NIS	Not listed	9	6	13	6	5	6

Figure 3. Demonstration of Oasis WAX/GCB for PFAS Analysis

performance repeatability requiring no protocol optimization, with %RSD within 1633 acceptance criteria. Green displays values within 1633 acceptance criteria. For those labeled "Not Listed": EPA 1633 does not provide %RSD criteria for this compound.

Conclusion

This study demonstrates the reproducibility of the dual-phase Oasis WAX/GCB for PFAS Analysis cartridges for determination of 40 PFAS and standards using the ACQUITY UPLC I-Class System and Xevo TQ-XS Mass Spectrometer. The cartridges are suitable for PFAS analysis in accordance with EPA 1633 guidelines for recovery and %RSD. The WAX/GCB cartridges show excellent repeatability across multiple product lots and within multiple replicates of each lot across non-potable water samples. The data demonstrates Oasis WAX/GCB for PFAS Analysis cartridges are ideally suited for PFAS analysis from complex matrices, such as non-potable waters like those described in EPA Method 1633. Out of the box performance is expected lot to lot and within a lot for SPE when using Oasis WAX/GCB for PFAS Analysis.

Ordering Information

Description	P/N
Oasis WAX/GCB for PFAS Analysis 6 cc Vac Cartridge, 200 mg WAX, 50 mg GCB, 60 µm WAX Particle Size, 30/pk	186011110
Oasis WAX/GCB for PFAS Analysis 6 cc Vac Cartridge, 200 mg WAX, 50 mg GCB, 60 µm WAX Particle Size, 300/pk	186011111
ACQUITY Premier BEH C_{18} Column, 1.7 µm, 2.1 × 50 mm, 1/pk	186009452
Atlantis Premier BEH C_{18} AX Column, 5 µm, 2.1 × 50 mm, 1/pk	186010926
Polypropylene 12 × 32 mm Screw Neck Vial, 700 µL Volume, 100/pk	186005219
Blue, 12 × 32 mm Screw Neck Cap and Preslit PTFE/Silicone Septum, 100/pk	186000305
Long Needle Valve for Sep-Pak Device Vacuum Manifold, 20/pk	WAT200685
Sep-Pak™ Reservoir adaptor 12/box	WAT054260
Waters Extraction Manifold, 20-position	186008998

References

1. US Environmental Protection Agency. Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS, Final version. 31 January 2024.
2. Organtini, K.; Rosnack, K.; Plummer, C.; Hancock, P.; Burt, O. Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Accordance With EPA 1633 Part 2: Analysis of Aqueous Matrices. Waters Application Note. 720008143. 2023.

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