Analysis of PFAS in aqueous samples by SPE and LC-MS/MS according to EPA Method 1633

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Application benefits

- Successful determination of 40 per- and polyfluoroalkyl substances from non-potable water matrices according to EPA Method 1633
- High recovery rates were achieved with a bilayer dual phase SPE cartridge containing CHROMABOND® WAX and GCB
- Minimizing the debris and hazards of working with dispersive GCB as well as further reducing sample preparation time
- Fast and sensitive HPLC analysis on a NUCLEODUR® Pheny-Hexyl

MN products

REF 7300043
CHROMABOND® WAX / GCB

REF 760576.20
EC 100/2 NUCLEODUR® Phenyl-Hexyl, 3 µm

REF 760573.20
EC 50/2 NUCLEODUR® Phenyl-Hexyl, 3 µm

REF 702402
Screw closure, N 9, PP, blue, center hole, silicone white/polyimide orange, 1 mm, fluorine-free

REF 702009
Screw neck vial, N 9, 11.6 × 32.0 mm, 0.3 mL, inner cone, PP transparent

REF 730150N
CHROMABOND® SPE vacuum manifold for 12 positions

REF 730382
Reservoir columns, 70 mL, with adaptor for 1, 3, 6 mL CHROMABOND® SPE PP columns

REF 730564N
CHROMABOND® PP connectors for SPE vacuum manifolds

Keywords

EPA Method 1633, PFAS, WAX, weak anion exchanger, GCB, graphitized carbon black, non-potable water matrices, LC-MS/MS, delay column

Introduction

In January 2024, the United States Environmental Protection Agency (US EPA) has published the Method 1633 [1] for the analysis of per- and polyfluoroalkyl substances (PFAS) in aqueous, solid, biosolids, and tissue samples by LC-MS/MS. The method is for use in Clean Water Act (CWA) [2] for 40 PFAS compounds in aqueous, solid (soil, biosolids, sediment) and tissue samples by liquid chromatography/mass spectrometry (LC-MS/MS). Environmental samples are prepared and extracted using method-specific procedures. Sample extracts are subjected to cleanup procedures designed to remove interferences. Aqueous samples are spiked with isotopically labeled standards, extracted using solid-phase extraction (SPE) cartridges and undergo cleanup using carbon before analysis. The bilayer dual-phase SPE cartridge CHROMABOND® WAX / GCB allows to substitute a weak anion exchange (WAX) SPE cartridge and a dispersive Solid Phase extraction (dSPE) with a graphitized carbon black (GCB) in a powder format. This approach minimizes the time the sample extract is in contact with the GCB to reduce the risk of binding longer chain PFAS compounds. Another advantage is the time saving by eliminating weighing GCB, vortexing, centrifugation and filtration during dSPE procedure.

In this application note, a SPE method according to EPA Method 1633 using CHROMABOND® WAX / GCB is presented. High recovery rates with very good reproducibility are achieved for drinking water matrices. Finally, the extracts are analyzed using HPLC-MS/MS on a NUCLEODUR® Phenyl-Hexyl column.

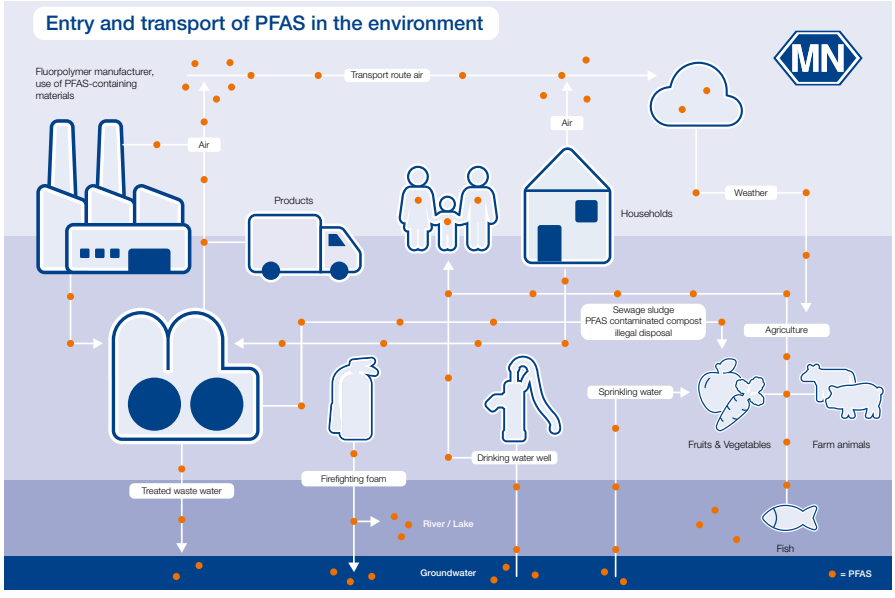

Figure 1: Entry and transport of PFAS in the environment.

Sample pretreatment

Solid phase extraction according to EPA Method 1633

Sample preparation:

This method is applicable to aqueous samples containing up to 50 mg of suspended solids per sample. The procedure requires the preparation of the entire sample. Subsampling should be avoided whenever possible. Typical sample size is 500 mL.

1. Homogenize the sample by inverting the sample 3 – 4 times and allowing the sample to settle. Do not filter the sample.

2. Check that the pH is 6.5 ± 0.5. If necessary, adjust pH with 50 % formic acid or ammonium hydroxide. The extract is now ready for solid-phase extraction (SPE).

3. Add the spiking solution containing the internal standard substances to the water sample (500 mL) in the sample bottle (adding 0,75 ng of each) and mix thoroughly by shaking.

SPE procedure:

Column:

CHROMABOND® WAX / GCB, 6 mL 200 mg / 50 mg (REF 7300043)

SPE accessories:

CHROMABOND® SPE vacuum manifold for 12 positions (REF 730150N); Reservoir columns, 70 mL, with adaptor for 1, 3, 6 mL CHROMABOND® SPE PP columns (REF 730382); CHROMABOND® PP connectors for SPE vacuum manifolds, (REF 730564N)

Put reservoir column on PP connector and connect it through adapter to CHROMABOND® WAX / GCB column.

Conditioning:

With 15 mL of 1 % methanolic ammonium hydroxide, followed by 5 mL of 0.3 M formic acid. Do not allow the SPE to run dry. Discard the wash solvents.

Sample application:

Add 500 mL water sample with a flow rate of 5 mL/min to the cartridge. (Do not let the sorbent material in the cartridge run dry and ensure it is always immersed in water.)

Bottle rinse:

Rinse the walls of the reservoir with 5 mL reagent water (twice) followed by 5 mL of 1:1 0.1 M formic acid/methanol.

Washing step:

Pass those rinses through the cartridge using vacuum. Discard the rinse solution.

Drying step:

Dry the cartridge by pulling air through for 15 seconds.

Elution:

Rinse the inside of the sample bottle and the SPE reservoir with 5 mL of 1 % methanolic ammonium hydroxide. Use vacuum to pull the elution solvent through the cartridge and into the collection tubes.

Neutralisation:

Add 25 µL of concentrated acetic acid to each sample eluted in the collection tubes and vortex to mix. Spike each sample with Non-extracted internal standard.

Analysis by HPLC-MS/MS

Chromatographic conditions

DELAY Column	EC 50/2 NUCLEODUR® Phenyl-Hexyl (REF 760573.20)
Column	EC 100/2 NUCLEODUR® Phenyl-Hexyl, 3 µm (REF 760576.20)
Eluent A	5 mM ammonium acetate in water
Eluent B	5 mM ammonium acetate in methanol
Gradient	Hold 40 % B for 1 min, in 8 min from 40 % B to 95 % B,hold 95 % B for 3 min, in 0.1 min to 40 % B, hold 40 % B for 2.9 min
Flow rate	0.3 mL/min
Temperature	40 °C
Injection volume	2 µL

MS conditions

Acquisition mode	SRM
Interface	ESI
Polarity	negative
Curtain Gas	30
Collision Gas	medium
Ionspray Voltage	-4500 V
Temperature	400 °C
Ion Source Gas 1	50
Ion Source Gas 2	60

Detection Window	60 sec

MRM transitions

Analyte	Abbreviation	CAS number	Q1 mass [Da]	Q3 mass [Da]	Retention time [min]
Perfluoro-*n*-butanoic acid	PFBA	375-22-4	212.90	168.80	1.76
Perfluoro-3-methoxypropanoic acid	PFMPA	377-73-1	229.00	85.00	2.15
3-Perfluoropropyl propanoic acid	3:3FTCA	356-02-5	241.00	177.00	3.00
Perfluoro-*n*-pentanoic	PFPeA	2706-90-3	262.88	219.00	3.15
Perfluoro-*n*-butanesulfonic acid	PFBS	375-73-5	298.93	98.90	3.50
Perfluoro-4-methoxybutanoic acid	PFMBA	863090-89-5	279.00	85.00	3.60
Perfluoro(2-ethoxyethane)sulfonic acid	PFEESA	113507-82-7	315.00	135.00	4.12
Nonafluoro-3,6-dioxaheptanoic acid	NFDHA	15772-58-6	295.00	201.00	4.35
1H, 1H, 2H, 2H-Perfluorohexane sulfonic acid	4:2FTS	757124-72-4	326.94	306.90	4.40
Perfluoropentansulfonic acid	PFPeS	2706-91-4	348.85	80.00	4.75
Perfluoro-*n*-hexanoic acid	PFHxA	307-24-4	312.91	268.80	4.76
Hexafluoropropylene oxide dimer acid	HFPO-DA	13252-13-6	284.99	168.70	5.00
Perfluoro-*n*-hexansulfonic acid	PFHxS	355-46-4	398.94	79.80	5.54
Perfluoro-*n*-heptanoic acid	PFHpA	375-85-9	362.93	318.80	5.57
2H, 2H, 3H, 3H-Perfluorooctanoic acid	5:3FTCA	914637-49-3	341.00	237.00	5.60
4,8-Dioxa-3H-perfluorononanoic acid	ADONA	919005-14-4	376.90	250.70	5.74
1H, 1H, 2H, 2H-Perfluorooctane sulfonic acid	6:2FTS	27619-97-2	426.93	406.90	6.31
Perfluoro-*n*-octanoic acid	PFOA	335-67-1	412.91	369.00	6.35
Perfluoro-*n*-heptanesulfonic acid	PFHpS	375-92-8	448.93	79.80	6.41
Perfluoro-*n*-nonanoic acid	PFNA	375-95-1	462.89	418.90	7.00
Perfluoro-*n*-octanesulfonic acid	PFOS	1763-23-10	498.84	79.90	7.00
3-Perfluoroheptyl propanoic acid	7:3FTCA	812-70-4	441.00	317.00	7.20
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	9Cl-PF3ONS	73606-19-6	530.75	350.70	7.43
1H,1H, 2H, 2H-Perfluorodecane sulfonic acid	8:2FTS	39108-34-4	526.00	506.80	7.51
Perfluoro-*n*-decanoic acid	PFDA	335-76-2	512.84	468.90	7.58
Perfluorononanesulfonic acid	PFNS	68259-12-1	548.81	79.90	7.59
N-methyl perfluorooctanesulfonamidoacetic acid	*N*-MeFOSAA	2355-31-9	569.80	418.90	7.90
Perfluorooctanesulfonamide	FOSA	754-91-6	497.87	77.90	7.94
Perfluoro-*n*-undecanoic acid	PFUnDA	2058-94-8	562.80	518.90	8.02
Perfluoro-*n*-decanesulfonic	PFDS	335-77-3	598.79	79.90	8.02
N-ethyl perfluorooctanesulfonamidoacetic acid	*N*-EtFOSAA	2991-50-6	583.81	418.80	8.14
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF3OUdS	763051-92-9	630.74	451.00	8.34
Perfluoro-*n*-dodecanoic acid	PFDoDA	307-55-1	612.79	568.90	8.40
Perfluorododecanesulfonic acid	PFDoS	79780-39-5	698.72	80.00	8.72
Perfluoro-*n*-tridecanoic acid	PFTrDA	72629-94-8	662.77	618.90	8.73
N-methyl perfluorooctanesulfonamide	*N*-MeFOSA	31506-32-8	512.00	169.00	8.84
N-ethyl perfluorooctanesulfonamidoethanol	NEtFOSE	1961-99-2	630.00	59.00	8.87
Perfluoro-*n*-tetradecanoic acid	PFTeDA	376-06-7	712.77	668.80	9.03
N-ethyl perfluorooctanesulfonamide	*N*-EtFOSA	4151-50-2	526.00	169.00	9.09
N-methyl perfluorooctanesulfonamidoethanol	NMeFOSE	24448-09-7	616.00	59.00	9.11
Surrogates					
Perfluoro-(2,3,4 – 13C3)butanoic acid	M3PFBA		216.00	172.00	1.74
Perfluoro-(13C4)butanoic acid	M4PFBA		216.94	171.90	1.75

Analyte	Abbreviation	CAS number	Q1 mass [Da]	Q3 mass [Da]	Retention time [min]
Perfluoro-(13C5)pentanoic acid	M5PFPeA		267.97	22.90	3.13
Sodium perfluoro-(2,3,4 – 13C3)butanesulfonate	M3PFBS		301.89	98.90	3.48
Sodium 1H,1H,2H,2H-perfluoro(1,2 – 13C2)hexanesulfonate	M2 – 4:2FTS		328.97	81.00	4.41
Perfluoro-(1,2 – 13C2)hexanoic acid	MPFHxA		315.00	270.00	4.53
Perfluoro-(1,2,3,4,6 – 13C5)hexanoic acid	M5PFHxA		318.00	272.80	4.53
Tetrafluoro-2-heptafluoropropoxy-13C3-propanoic acid	M3HPFO-DA		287.00	169.00	4.98
Perfluoro-(1,2,3,4 – 13C4)heptanoic acid	M4PFHpA		366.95	321.80	5.56
Perfluoro-1-hexane(18O2)sulfonic acid	MPFHxS		403.00	103.00	5.66
Sodium perfluoro-(1,2,3 – 13C3)hexanesulfonate	M3PFHxS		401.90	79.90	5.67
Sodium 1H,1H,2H,2H-perfluoro(1,2 – 13C2)octanesulfonate	M2 – 6:2FTS		428.94	81.00	6.29
Perfluoro-(13C8)octanoic acid	M8PFOA		420.95	376.00	6.35
Perfluoro-(1,2,3,4 – 13C4)octanoic acid	MPFOA		417.00	372.00	6.35
Perfluoro-(1,2,3,4 – 13C4)octanesulfonic acid	MPFOS		503.00	99.00	7.00
Perfluoro-(13C9)nonanoic acid	M9PFNA		471.94	427.00	7.05
Perfluoro-(1,2,3,4,5 – 13C5) nonanoic acid	MPFNA		468.00	423.00	7.06
Sodium perfluoro-(13C8)octanesulfonate	M8PFOS		506.91	98.90	7.07
Sodium 1H,1H,2H,2H-perfluoro(1,2 – 13C2)decanesulfonate	M2 – 8:2FTS		528.94	80.90	7.55
Perfluoro-(1,2,3,4,5,6 – 13C6)decanoic acid	M6PFDA		518.92	474.00	7.57
Perfluoro-(1,2 – 13C2)decanoic acid	MPFDA		515.00	470.00	7.57
N-methyl-d3 -perfluorooctanesulfonamidoacetic acid	d3-*N*-MeFOSAA		572.89	419.00	7.89
Perfluoro-(13C8)octanesulfonamide	M8FOSA		505.98	77.90	7.96
Perfluoro-(1,2,3,4,5,6,7 – 13C7)undecanoic acid	M7PFUdA		569.95	525.00	8.03
N-ethyl-d5 -perfluorooctanesulfonamidoacetic acid	d5-*N*-EtFOSAA		588.85	418.80	8.14
Perfluoro-(1,2 – 13C2)dodecanoic acid	MPFDoA		614.95	569.90	8.42
N-methyl-d3-perfluoro-1-octanesulfonamide	d3-*N*-MeFOSA		515.00	169.00	8.81
N-methyl-d7-perfluorooctanesulfonamidoethanol	d7-*N*-MeFOSE		623.00	59.00	8.85
Perfluoro-(1,2 – 13C2)tetradecanoic acid	M2PFTeDA		714.94	670.00	8.98
N-ethyl-d5-perfluoro-1-octanesulfonamide	d5-*N*-EtFOSA		531.00	169.00	9.03
N-ethyl-d9-perfluorooctanesulfonamidoethanol	d9-*N*-EtFOSE		639.00	59.00	9.08

Table 1: MRM transitions and retention times of native PFAS and isotopically labeled PFAS analytical standards.

Chromatogramms

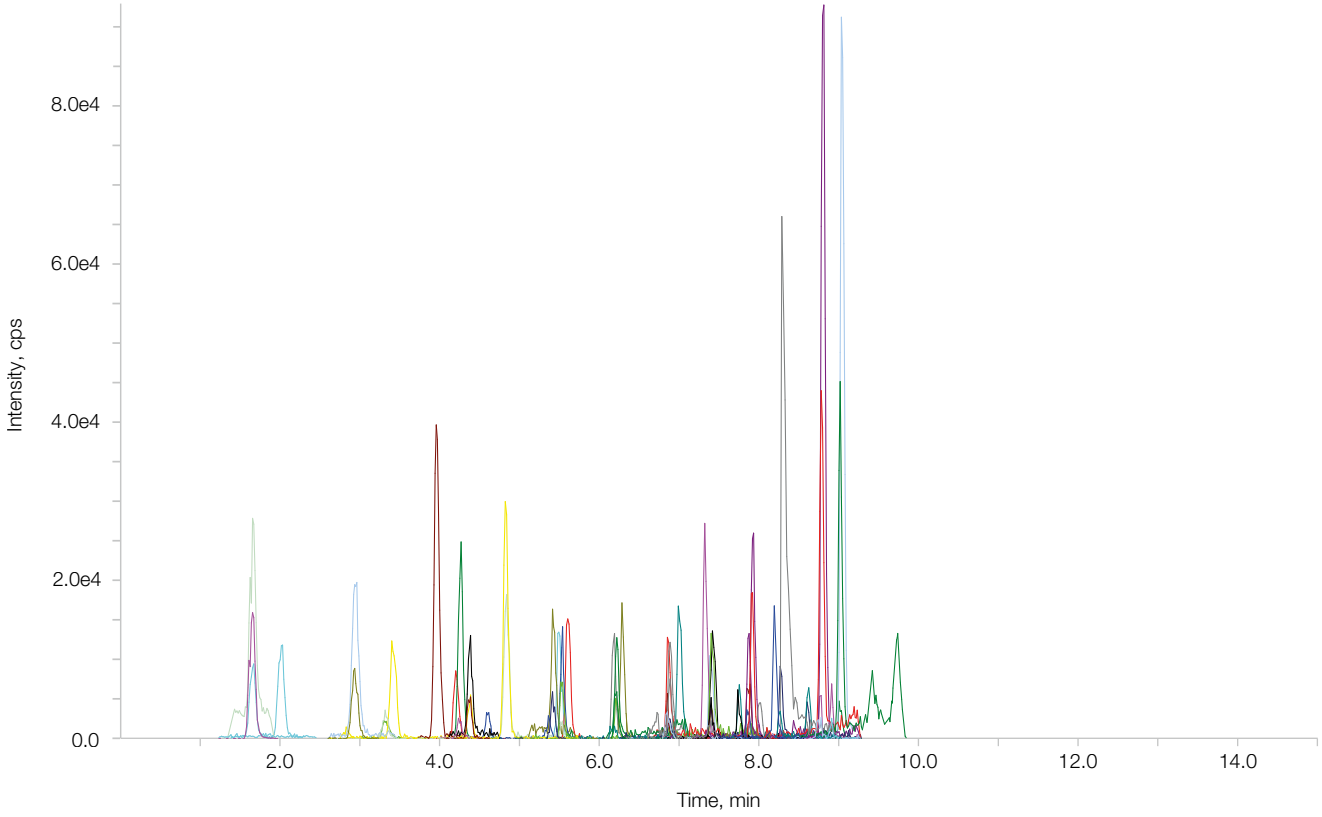

Figure 2: Chromatogram of a standard solution (concentration, β = 0.15 ng/mL)

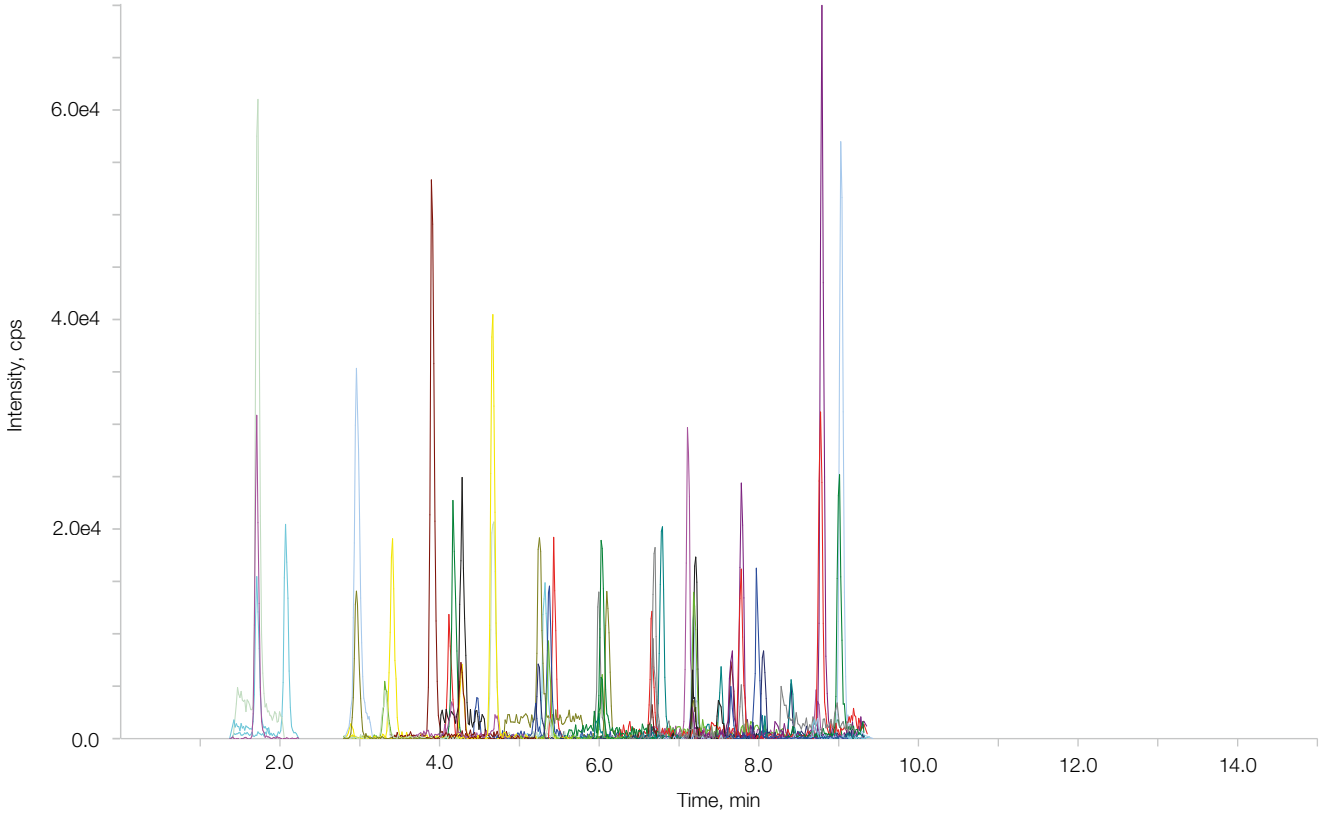

Figure 3: Chromatogram of a non-potable water sample (concentration, ß = 1.5 ng/L)

Analyte	Abbreviation	Recovery rate (%) ± RSD (%)
Perfluoro-3-methoxypropanoic acid	PFMPA	100.5 ± 5.6
Perfluoro-*n*-butanoic acid	PFBA	90.1 ± 15.5
3-Perfluoropropyl propanoic acid	3:3FTCA	115.1 ± 8.5
Perfluoro-4-methoxybutanoic acid	PFMBA	100.8 ± 7.1
Perfluoro-*n*-pentanoic	PFPeA	96.1 ± 3.7
Perfluoro(2-ethoxyethane)sulfonic acid	PFEESA	96.6 ± 5.2
Perfluoro-*n*-butanesulfonic acid	PFBS	79.1 ± 2.1
Nonafluoro-3,6-dioxaheptanoic acid	NFDHA	99.7 ± 5.2
1H,1H, 2H, 2H-Perfluorohexane sulfonic acid	4:2FTS	109.1 ± 7.1
Perfluoro-*n*-hexanoic acid	PFHxA	85.1 ± 5.1
Perfluoropentansulfonic acid	PFPeS	95.9 ± 9.7
Hexafluoropropylene oxide dimer acid	HFPO-DA	95.2 ± 5.9
2H,2H,3H,3H-Perfluorooctanoic acid	5:3FTCA	92.3 ± 5.5
Perfluoro-*n*-heptanoic acid	PFHpA	91.7 ± 4.3
Perfluoro-*n*-hexanesulfonic acid	PFHxS	95.0 ± 6.9
4,8-Dioxa-3H-perfluorononanoic acid	ADONA	81.8 ± 3.3
1H,1H, 2H, 2H-Perfluorooctane sulfonic acid	6:2FTS	93.1 ± 6.1
Perfluoro-*n*-heptanesulfonic acid	PFHpS	93.3 ± 5.8
Perfluoro-*n*-octanoic acid	PFOA	89.6 ± 7.3
Perfluoro-*n*-octanesulfonic acid	PFOS	88.4 ± 7.3
Perfluoro-*n*-nonanoic acid	PFNA	101.3 ± 1.6
3-Perfluoroheptyl propanoic acid	7:3FTCA	97.8 ± 5.7
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	9Cl-PF3ONS	94.6 ± 5.6
Perfluorononanesulfonic acid	PFNS	92.8 ± 3.1
Perfluoro-*n*-decanoic acid	PFDA	98.1 ± 4.1
1H,1H, 2H, 2H-Perfluorodecane sulfonic acid	8:2FTS	97.7 ± 4.7
N-methyl perfluorooctanesulfonamidoacetic acid	*N*-MeFOSAA	93.5 ± 9.2
Perfluorooctanesulfonamide	FOSA	98.4 ± 6.2
Perfluoro-*n*-decanesulfonic	PFDS	90.3 ± 9.0
Perfluoro-*n*-undecanoic acid	PFUnDA	72.7 ± 3.6
N-ethyl perfluorooctanesulfonamidoacetic acid	*N*-EtFOSAA	93.4 ± 8.6
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF3OUdS	96.5 ± 4.5
Perfluoro-*n*-dodecanoic acid	PFDoDA	108.1 ± 2.8
N-methyl perfluorooctanesulfonamide	*N*-MeFOSA	85.0 ± 12.2
Perfluoro-*n*-tridecanoic acid	PFTrDA	85.7 ± 4.7
Perfluorododecanesulfonic acid	PFDoS	84.1 ± 9.8
N-ethyl perfluorooctanesulfonamide	*N*-EtFOSA	68.7 ± 13.9
N-methyl perfluorooctanesulfonamidoethanol	*N*-MeFOSE	95.1 ± 2.1
Perfluoro-*n*-tetradecanoic acid	PFTeDA	92.5 ± 6.6
N-ethyl perfluorooctanesulfonamidoethanol	*N*-EtFOSE	98.9 ± 3.7

Table 2: Recovery rates for the presented SPE method using CHROMABOND® WAX / GCB, 200 mg / 50 mg, 6 mL.

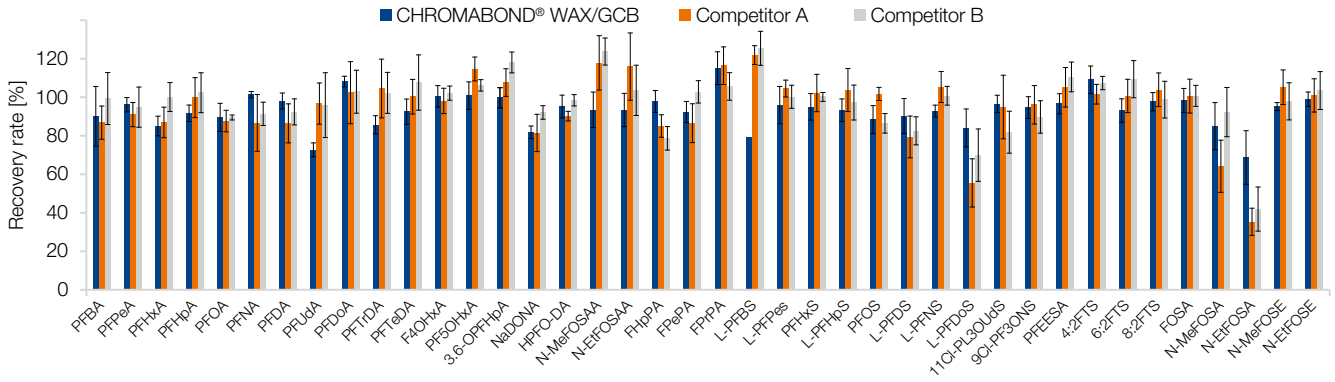

Figure 4: Recovery rates for the presented SPE method using CHROMABOND® WAX / GCB, 200 mg / 50 mg, 6 mL.

Conclusion

This application note presents the reliable and successful determination of 40 PFAS according to EPA 1633 from non-potable water. By using a dual-layer SPE column, CHROMABOND® WAX / GCB, it was possible to achieve high recovery rates with good reproducibility for various PFAS. The sorbent of the upper layer, CHROMABOND® WAX, was optimized for PFAS analysis and provides various strong ionic interaction types like ionic, hydrophobic, hydrogen bonds and dipole-dipole interactions for the enrichment of a broad spectrum of PFAS. The sorbent is specially recommended for PFAS analysis because of its very low blind value levels.

The results showed that the bilayer dual-phase SPE cartridge CHROMABOND® WAX / GCB could successfully substitute the combination of a weak anion exchange (WAX) SPE cartridge and a dispersive Solid Phase extraction (dSPE) with a graphitized carbon black (GCB) in a powder format. The short contact time between GCB layer and the sample using a stacked cartridge prevents a loss of PFAS by adsorption. Further laboratory tests showed that there was no loss of PFAS even over a wide concentration range. As expected, the method presented showed advantages in terms of handling and processing time.

The identification and the quantification of PFAS in samples extracts were finally carried out by ESI mass spectrometry on a NUCLEODUR® Phenyl-Hexyl column.

References

[1] Method 1633, Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS, January 2024.

[2] US Environmental Protection Agency. Clean Water Act Analytical Methods: CWA Analytical Methods for Per – and Polyfluorinated Alkyl Substances (PFAS). https://www.epa.gov/cwa-methods/cwa-analytical-methods-and-polyfluorinated-alkyl-substances-pfas Accessed 15.11.2024.

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