Analysis of Per- and Polyfluoroalkyl Substances in Aqueous Samples with CHROMABOND® WAX according to EPA Method 533

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Application benefits

- Successful determination of 25 Per- and Polyfluoroalkyl Substances from water samples according to EPA Method 533
- High recovery rates were achieved with a CHROMABOND® WAX SPE Column
- Fast and sensitive HPLC analysis on a NUCLEODUR® PFAS column

MN products

REF 7300011
CHROMABOND® WAX, 6 mL, 150 mg

REF 760666.20
EC 100/2 NUCLEODUR® PFAS, 3 µm

REF 760673.20
EC 50/2 NUCLEODUR® PFAS Delay

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 x 32.0 mm, 0.3 mL, inner cone, PP transparent

MN application numbers

SPE: 306960
HPLC: 129380

Keywords

EPA Method 533, PFAS, WAX, weak anion exchanger, water, LC-MS/MS, Delay column

Introduction

In December 2019, the United States Environmental Protection Agency (US EPA) has published a method for the analysis of per- and polyfluoroalkyl substances (PFAS) [1]. The method was developed to target “short chain” PFAS (none greater than C12), including perfluorinated acids, sulfonates, fluorotelemers, and poly/perfluorinated ether carboxylic acids. This “short chain” PFAS could not be analyzed using 537.1 due to physicochemical properties. 25 PFAS should be analyzed using a solid phase extraction (SPE) and a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method in drinking water. Method 533 requires SPE cartridge containing weak anion exchange, mixed-mode polymeric sorbent (polymeric backbone and a diamino ligand) and a particle size of approximately 33 µm. The SPE sorbent must have a pKa above 8 so that it remains positively charged during extraction. The use of 200 mg sorbents is recommended for the extraction of 100 mL samples.

In this application note, a solid phase extraction (SPE) using the CHROMBOND® WAX coupled with a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method 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® PFAS column.

Requirements EPA Method 533	Specifications of CHROMABOND® WAX
Weak anion exchange	pass
Mixed-mode polymeric sorbent (polymeric backbone and a diamino ligand)	pass
Particle size approximately 33 µm	pass
pKa above 8	pass
Low blind level of PFAS	pass

Table 1: Matching of EPA 533 requirements with specifications of CHROMABOND® WAX.

Analysis of PFAS in Aqueous Samples with CHROMABOND WAX according to EPA Method 533

Sample pretreatment

MN Appl. No. 306960

Solid phase extraction according to EPA 533

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. Add ammonium acetate (1.0 g/L) to the sample. Ammonium acetate will sequester free chlorine to form chloramine.
2. Verify that the sample containing 1 g/L ammonium acetate has a pH between 6.0 and 8.0. Acetic acid may be added as needed to adjust the pH.
3. Add 20 µL organic standard solution* (β = 12.5 ng/mL in methanol for each compound) to the 250 mL water sample.

* Contains native and isotopically labeled per- and polyfluoroalkyl substances

Column: CHROMABOND® WAX, 6 mL 150 mg (REF 7300011)

Conditioning:

Rinse each cartridge with 10 mL methanol, 10 mL of aqueous 0.1 M phosphate buffer. Close the valve and add 2–3 mL of phosphate buffer (pH 7.0)* to the cartridge reservoir and fill the remaining volume with reagent water.

* Mix 500 mL of 0.1 M dibasic sodium phosphate with approximately 275 mL of 0.1 M monobasic sodium phosphate. Verify that the solution pH is approximately 7.0

Sample application:

Attach the reservoir cartridges, turn on the vacuum, and begin adding the 250 mL water sample with a flow rate of 5 mL/min to the cartridge. Do not allow the cartridge to run dry before all the sample has passed through.

Sample bottle and cartridge rinse:

After the entire sample has passed through the cartridge, rinse the sample bottles and the transfer cartridges with aliquots of 1 g/L ammonium acetate in water and draw each aliquot through the SPE columns. Add 1 mL of methanol to the sample bottle and draw through the transfer cartridge and SPE cartridge. Draw air or nitrogen through the cartridge for 5 min at high vacuum (15–20 in. Hg).

Sample bottle and cartridge elution:

Rinse the sample bottles and the transfer cartridge with 5 mL of methanol with 2% ammonium hydroxide (v/v) and elute the analytes from the cartridges by pulling the 5 mL of methanol with 2% ammonium hydroxide (v/v) through the SPE column. Use a low vacuum such that the solvent exits the cartridge in a dropwise fashion. Repeat sample bottle rinse and cartridge elution with a second 5 mL aliquot of methanol with 2% ammonium hydroxide (v/v).

Eluent exchange:

Evaporate eluate to dryness at 40 °C under a stream of nitrogen and dissolve residue in 0.5 mL methanol.

Analysis by HPLC-MS / MS

MN Appl. No. 129380

Chromatographic conditions

DELAY Column	EC 50/2 NUCLEODUR® PFAS Delay (REF 760673.20)
Column	EC 100/2 NUCLEODUR® PFAS, 3 µm (REF 760666.20)
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-3-methoxypropanoic acid	PFMPA	377-73-1	229.00	85.00	1.96
Perfluoro-*n*-butanoic acid	PFBA	375-22-4	212.90	168.80	2.01
Perfluoro-4-methoxybutanoic acid	PFMBA	863090-89-5	279.00	85.00	3.64
Perfluoro-*n*-pentanoic	PFPeA	2706-90-3	262.88	219.00	3.90
Perfluoro(2-ethoxyethane)sulfonic acid	PFEESA	113507-82-7	315.00	135.00	4.14
Perfluoro-*n*-butanesulfonic acid	PFBS	375-73-5	298.93	98.90	4.20
Nonafluoro-3,6-dioxaheptanoic acid	NFDHA	151772-58-6	295.00	201.00	4.48
1*H*,1*H*, 2*H*, 2*H*-Perfluorohexane sulfonic acid	4:2FTS	757124-72-4	326.94	306.90	5.27
Perfluoro-*n*-hexanoic acid	PFHxA	307-24-4	312.91	268.80	5.40
Perfluoropentansulfonic acid	PFPeS	2706-91-4	348.85	80.00	5.54
Hexafluoropropylene oxide dimer acid	HFPO-DA	13252-13-6	284.99	168.70	5.77
Perfluoro-*n*-heptanoic acid	PFHpA	375-85-9	362.93	318.80	6.45
Perfluoro-*n*-hexanesulfonic acid	PFHxS	355-46-4	398.94	79.80	6.49
4,8-Dioxa-3*H*-perfluorononanoic acid	ADONA	919005-14-4	376.90	250.70	6.58
1*H*,1*H*, 2*H*, 2*H*-Perfluorooctane sulfonic acid	6:2FTS	27619-97-2	426.93	406.90	7.24
Perfluoro-*n*-heptanesulfonic acid	PFHpS	375-92-8	448.93	79.80	7.26
Perfluoro-*n*-octanoic acid	PFOA	335-67-1	412.91	369.00	7.26
Perfluoro-*n*-octanesulfonic acid	PFOS	1763-23-1	498.84	79.90	7.89
3-Perfluoroheptyl propanoic acid	7:3FTCA	812-70-4	441.00	317.00	8.00
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	9Cl-PF3ONS	73606-19-6	530.75	350.70	8.25
Perfluoro-*n*-decanoic acid	PFDA	335-76-2	512.84	468.90	8.49
1*H*,1*H*, 2*H*, 2*H*-Perfluorodecane sulfonic acid	8:2FTS	39108-34-4	526.00	506.80	8.50
Perfluoro-*n*-undecanoic acid	PFUnDA	2058-94-8	562.80	518.90	8.95
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF3OUdS	763051-92-9	630.74	451.00	9.15
Perfluoro-*n*-dodecanoic acid	PFDoDA	307-55-1	612.79	568.90	9.33
Surrogates					
Perfluoro-(2,3,4-$^{13}C_3$)butanoic acid	M3PFBA		216.00	172.00	1.91
Perfluoro-($^{13}C_4$)butanoic acid	M4PFBA		216.94	171.90	2.01
Sodium perfluoro-(2,3,4-$^{13}C_3$)butanesulfonate	M3PFBS		301.89	98.90	4.22
Sodium 1*H*,1*H*,2*H*,2*H*-perfluoro(1,2-$^{13}C_2$)hexanesulfonate	M2-4:2FTS		328.97	81.00	5.26
Tetrafluoro-2-heptafluoropropoxy-$^{13}C_3$-propanoic acid	M3HPFO-DA		287.00	169.00	5.44
Perfluoro-(1,2,3,4-$^{13}C_4$)heptanoic acid	M4PFHpA		366.95	321.80	6.45
Sodium perfluoro-(1,2,3-$^{13}C_3$)hexanesulfonate	M3PFHxS		401.90	79.90	6.50
Perfluoro-(1,2,3,4-$^{13}C_4$)octanoic acid	MPFOA		417.00	372.00	7.08
Sodium 1*H*,1*H*,2*H*,2*H*-perfluoro(1,2-$^{13}C_2$)octanesulfonate	M2-6:2FTS		428.94	81.00	7.23
Perfluoro-($^{13}C_8$)octanoic acid	M8PFOA		420.95	376.00	7.27
Perfluoro-(1,2,3,4-$^{13}C_4$)octanesulfonic acid	MPFOS		503.00	99.00	7.78
Sodium perfluoro-($^{13}C_8$)octanesulfonate	M8PFOS		506.91	98.90	7.89
Perfluoro-($^{13}C_9$)nonanoic acid	M9PFNA		471.94	427.00	7.92
Perfluoro-(1,2,3,4,5,6-$^{13}C_6$)decanoic acid	M6PFDA		518.92	474.00	8.49
Sodium 1*H*,1*H*,2*H*,2*H*-perfluoro(1,2-$^{13}C_2$)decanesulfonate	M2-8:2FTS		528.94	80.90	8.50
Perfluoro-(1,2,3,4,5,6,7-$^{13}C_7$)undecanoic acid	M7PFUdA		569.95	525.00	8.95

Table 2: MRM transitions and retention times of native PFAS and isotopically labeled PFAS analytical standards.

Analysis of PFAS in Aqueous Samples with CHROMABOND WAX according to EPA Method 533

Chromatogramms

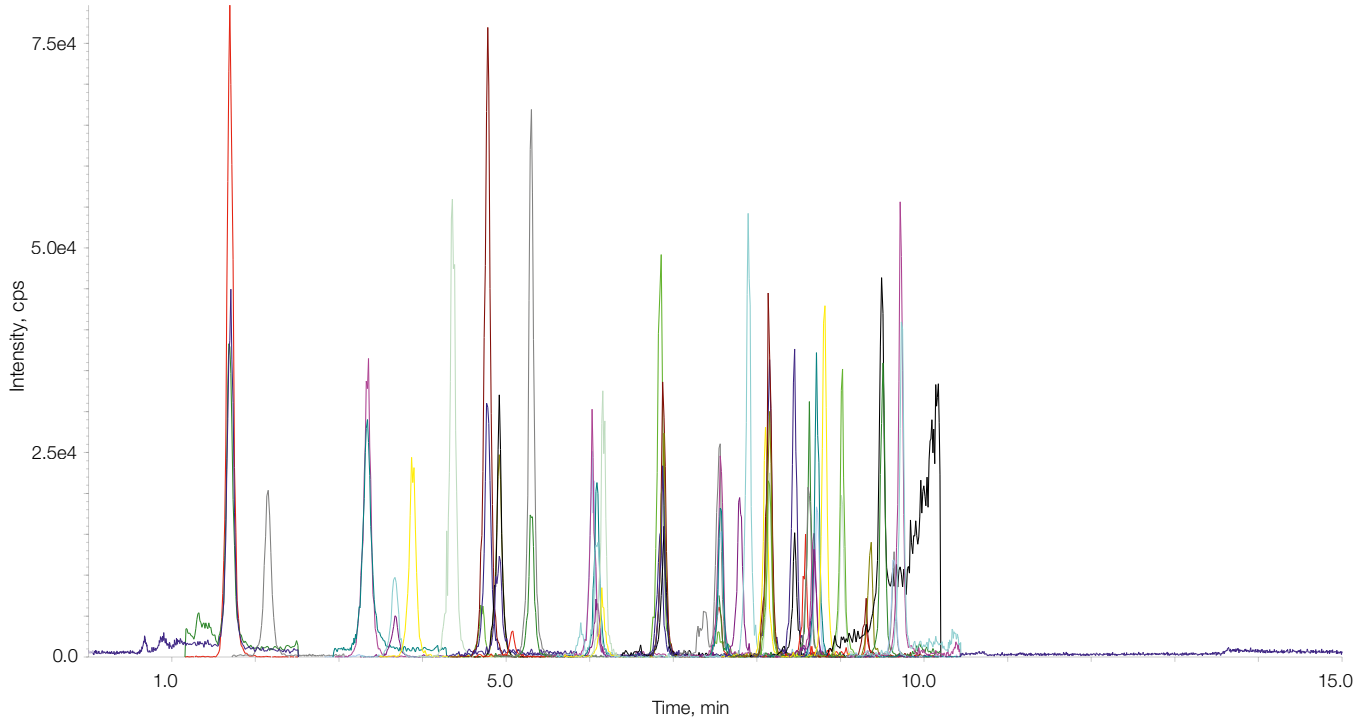

Figure 1: Chromatogram of a standard solution (concentration, β = 0.5 ng/mL)

Recovery rates

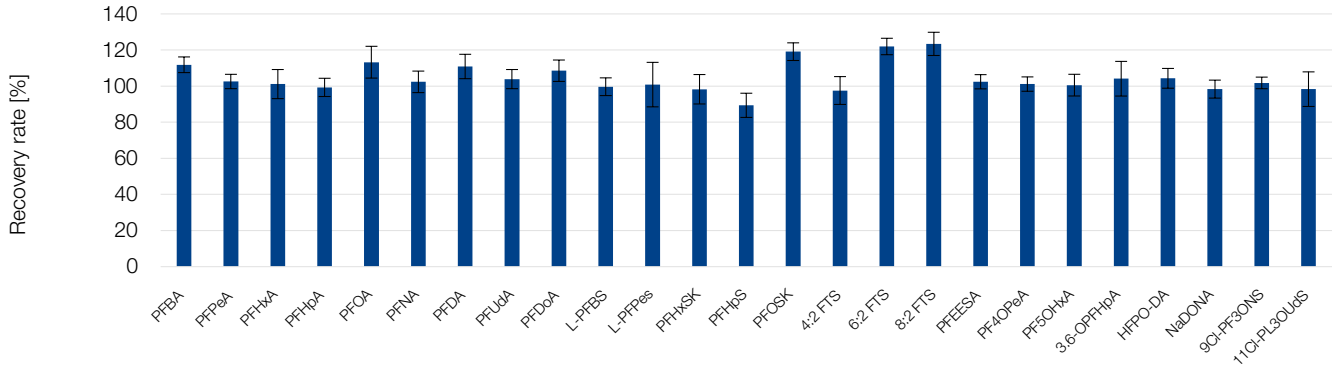

Figure 2: Recovery rate from water sample (concentration, β = 1 ng/L, n=5)

Analyte	Abbreviation	Recovery rate (%) ± RSD (%)
Perfluoro-3-methoxypropanoic acid	PFMPA	105 ± 2
Perfluoro-*n*-butanoic acid	PFBA	99 ± 4
Perfluoro-4-methoxybutanoic acid	PFMBA	95 ± 3
Perfluoro-*n*-pentanoic	PFPeA	100 ± 2
Perfluoro(2-ethoxyethane) sulfonic acid	PFEESA	94 ± 3
Perfluoro-*n*-butanesulfonic acid	PFBS	83 ± 4

Analyte	Abbreviation	Recovery rate (%) ± RSD (%)
Nonafluoro-3,6-dioxaheptanoic acid	NFDHA	84 ± 4
1*H*,1*H*, 2*H*, 2*H*-Perfluorohexane sulfonic acid	4:2FTS	73 ± 5
Perfluoro-*n*-hexanoic acid	PFHxA	95 ± 3
Perfluoropentansulfonic acid	PFPeS	69 ± 6
Hexafluoropropylene oxide dimer acid	HFPO-DA	100 ± 5

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Analyte	Abbreviation	Recovery rate (%) ± RSD (%)
Perfluoro-*n*-heptanoic acid	PFHpA	95 ± 3
Perfluoro-*n*-hexanesulfonic acid	PFHxS	87 ± 4
4,8-Dioxa-3*H*-perfluorononanoic acid	ADONA	82 ± 2
1*H*,1*H*, 2*H*, 2*H*-Perfluorooctane sulfonic acid	6:2FTS	74 ± 9
Perfluoro-*n*-heptanesulfonic acid	PFHpS	95 ± 4
Perfluoro-*n*-octanoic acid	PFOA	110 ± 10
Perfluoro-*n*-octanesulfonic acid	PFOS	83 ± 11
Perfluoro-*n*-nonanoic acid	PFNA	94 ± 15
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	9Cl-PF3ONS	70 ± 9
Perfluoro-*n*-decanoic acid	PFDA	80 ± 21
1*H*,1*H*, 2*H*, 2*H*-Perfluorodecane sulfonic acid	8:2FTS	75 ± 22
Perfluoro-*n*-undecanoic acid	PFUnDA	92 ± 30
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF3OUdS	61 ± 15
Perfluoro-*n*-dodecanoic acid	PFDoDA	72 ± 28
Surrogates		
Perfluoro-(2,3,4-$^{13}C_3$) butanoic acid	M3PFBA	86 ± 2
Perfluoro-($^{13}C_4$)butanoic acid	M4PFBA	85 ± 4
Sodium perfluoro-(2,3,4-$^{13}C_3$) butanesulfonate	M3PFBS	86 ± 3
Sodium 1*H*,1*H*,2*H*,2*H*-perfluoro(1,2-$^{13}C_2$) hexanesulfonate	M2-4:2FTS	84 ± 9
Tetrafluoro-2-heptafluoropropoxy-$^{13}C_3$-propanoic acid	M3HPFO-DA	99 ± 3
Perfluoro-(1,2,3,4-$^{13}C_4$) heptanoic acid	M4PFHpA	100 ± 2
Sodium perfluoro-(1,2,3-$^{13}C_3$) hexanesulfonate	M3PFHxS	84 ± 5
Perfluoro-(1,2,3,4-$^{13}C_4$) octanoic acid	MPFOA	111 ± 5
Sodium 1*H*,1*H*,2*H*,2*H*-perfluoro(1,2-$^{13}C_2$) octanesulfonate	M2-6:2FTS	127 ± 25
Perfluoro-($^{13}C_8$)octanoic acid	M8PFOA	97 ± 3
Perfluoro-(1,2,3,4-$^{13}C_4$) octanesulfonic acid	MPFOS	80 ± 3
Sodium perfluoro-($^{13}C_8$) octanesulfonate	M8PFOS	92 ± 2
Perfluoro-($^{13}C_9$)nonanoic acid	M9PFNA	89 ± 1
Perfluoro-(1,2,3,4,5,6-$^{13}C_6$)decanoic acid	M6PFDA	84 ± 2
Sodium 1*H*,1*H*,2*H*,2*H*-perfluoro(1,2-$^{13}C_2$) decanesulfonate	M2-8:2FTS	94 ± 20
Perfluoro-(1,2,3,4,5,6,7-$^{13}C_7$) undecanoic acid	M7PFUdA	80 ± 5

Table 3: Recovery rates for the presented SPE method using CHROMABOND® WAX, 150 mg, 6 mL (n=5).

Conclusion

This application note presents the reliable and successful determination of 25 PFAS according to EPA method 533 from drinking water. By using the SPE column, CHROMABOND® WAX, it was possible to achieve high recovery rates with good reproducibility. CHROMABOND® WAX was optimized for PFAS analysis and provides various 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. Most of the PFAS show recovery rates between 80 % to 110 %. The identification and the quantification of PFAS in food were finally carried out by ESI mass spectrometry on a NUCLEODUR® PFAS column.

References

[1] METHOD 533: DETERMINATION OF PER- AND POLYFLUOROALKYL SUBSTANCES IN DRINKING WATER BY ISOTOPE DILUTION ANION EXCHANGE SOLID PHASE EXTRACTION AND LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY; December 2019.