MACHEREY-NAGEL
Chromatography application note

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PFAS Analysis According to EPA 533

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Abstract

This application note describes the determination of per- and polyfluoroalkyl substances (PFAS) according to EPA directive 533. It demonstrates the enrichment of PFAS using the SPE column CHROMABOND® HR-XAW and subsequent chromatographic separation on a NUCLEODUR® PFAS analytical HPLC column.

Introduction

Per- and polyfluoroalkyl substances (PFAS) are a group of anthropogenic chemicals widely used as additives in consumer products like fire-fighting foam, fiber coating, cookware, paper finishing, food packaging (e. g. pizza cartons, paper cups), building material, (e. g. water resistant lacquer). These persistent and bioaccumulative, anthropogenic pollutants are characterized by a linear aliphatic backbone, a high degree of fluorination and often feature a carboxylic or sulfonic acid functionality. PFAS present entail numerous analytical challenges, including their widespread presence in a variety of environmental samples, occurrence of isomers for some compounds and precursor transformations that may occur during preservation and storage of the samples. There is also evidence that exposure to PFAS can lead to adverse human health effects.

This is the reason why authorities in the US published variety of laws and regulations to protect public health and the environment [1]:

- Safe Drinking Water Act
- Toxic Substances Control Act (TSCA)
- Comprehensive Environmental Response, Compensation and Liability Act
- Clean Air Act

To protect environment and human health, the environmental protection agency (EPA) has published an action plan for identifying and for understanding PFAS e.g. new approaches to address current PFAS contamination, to prevent future contamination and to effectively communicate with the public about PFAS [2].

There is a need for robust and fast analytical methods to ensure accurate quantitation of low levels. Therefore, EPA recommends two methods to detect PFAS from drinking water by solid phase extraction (SPE) and liquid chromatography/tandem mass spectrometry (LC/MS/MS) [3, 4].

This work presents the analysis of PFAS from drinking water according to directive EPA method 533. It shows high recoveries using a weak anion exchanger based on a polystyrene-divinylbenzene copolymer (PS /DVB) in SPE columns for the enrichment of PFAS from water samples. These mixed-mode SPE phases successfully combine several interactions like ionic, hydrophobic and π–π. The extracts are finally analyzed by HPLC-MS/MS.

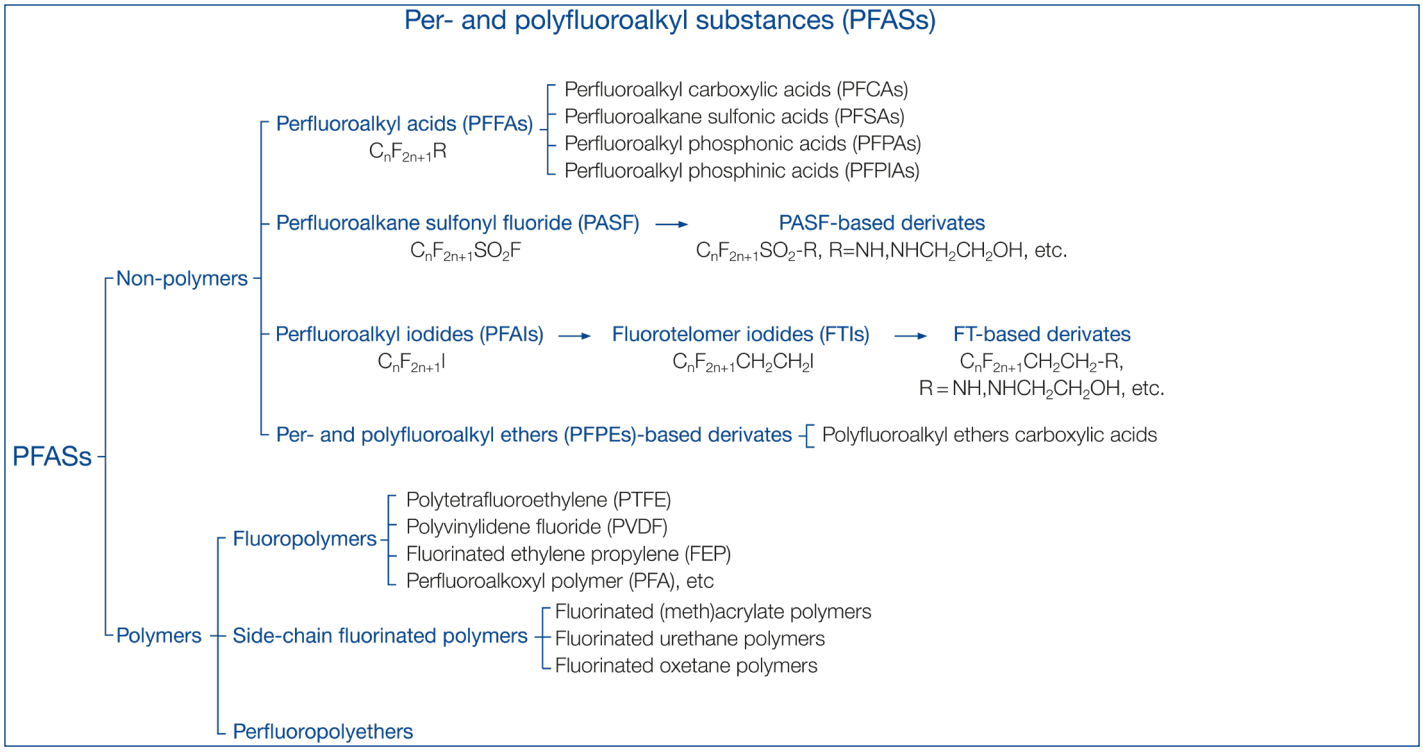

Figure 1: Classes of per- and polyfluoroalkyl substances (PFAS).

PFAS Analysis According to EPA 533

Sample pretreatment for solid phase extraction (SPE)

- Samples are preserved, collected and stored as presented in polypropylene bottles.
- Add ammonium acetate (1.0 g/L) to the sample. Ammonium acetate will sequester free chlorine to form chloramine.
- 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.
- Add 20 µL organic standard solution* (β = 25.0 ng/mL in methanol for each compound) to the 250 mL water sample.
  * Contains native and isotopically labeled per- and polyfluoroalkyl substances.

Solid phase extraction

Column:	CHROMABOND® HR- XAW, 85 µm, 6 mL, 500 mg, (REF 730745) CHROMABOND® HR-XAW, 45 µm, 3 mL, 200 mg, (REF 730748P45)
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 inches 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

Chromatographic conditions:

Delay column:	EC 50/2 NUCLEODUR® PFAS Delay , 5 µm (REF 760673.20)
Analytical 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 1.0 min, in 8 min from 40 % B to 95 % B, hold 95 % B for 3.0 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
Sample solution:	Mixture of PFAS in methanol, concentration 1 ng/mL for each compound

MS conditions :

AB Sciex QTRAP 5500

Acquisition mode:	SRM	Ion spray voltage:	– 4500 V
Interface:	ESI	Temperature:	400 °C
Polarity:	negative	Ion source gas 1:	50 psig
Curtain gas:	30 psig	Ion source gas 2:	60 psig
Collision Gas:	medium	Detection window:	60 s

PFAS Analysis According to EPA 533

MRM transitions

Abbreviation	Analyte	CASRN	Q_1 Mass (DA)	Q_3 Mass (DA)	RT (min)
NFDHA	Nonafluoro-3,6-dioxaheptanoic acid	151772-58-6	201.027	85.0	5.19
PFBA	Perfluorobutanoic acid	375-22-4	212.904	168.8	2.01
PFMPA	Perfluoro-3-methoxypropanoic acid	377-73-1	228.933	85.0	2.64
PFPeA	Perfluoropentanoic acid	2706-90-3	262.880	219.0	3.90
PFMBA	Perfluoro-4-methoxybutanoic acid	863090-89-5	279.184	84.9	7.97
HFPO-DA	Hexafluoropropylene oxide dimer acid	13252-13-6	284.991	168.7	5.77
PFBS	Perfluorobutanesulfonic acid	375-73-5	298.933	98.9	4.20
PFHxA	Perfluorohexanoic acid	307-24-4	312.911	268.8	5.40
PFEESA	Perfluoro(2-ethoxyethane)sulfonic acid	113507-82-7	315.118	135.1	4.85
4:2FTS	1H,1H, 2H, 2H-Perfluorohexane sulfonic acid	757124-72-4	326.940	306.9	5.27
PFPeS	Perfluoropentanesulfonic acid	2706-91-4	348.845	80.0	5.54
PFHpA	Perfluoroheptanoic acid	375-85-9	362.931	318.8	6.45
ADONA	4,8-Dioxa-3H-perfluorononanoic acid	919005-14-4	376.901	250.7	6.58
PFHxS	Perfluorohexanesulfonic acid	355-46-4	398.942	79.8	6.49
PFOA	Perfluorooctanoic acid	335-67-1	412.910	369.0	7.26
6:2FTS	1H,1H, 2H, 2H-Perfluorooctane sulfonic acid	27619-97-2	426.927	406.9	7.24
PFHpS	Perfluoroheptanesulfonic acid	375-92-8	448.929	79.8	7.26
PFNA	Perfluorononanoic acid	375-95-1	462.893	418.9	7.92
PFOS	Perfluorooctanesulfonic acid	1763-23-1	498.836	79.9	7.89
8:2FTS	1H,1H, 2H, 2H-Perfluorodecane sulfonic acid	39108-34-4	526.821	506.8	8.50
PFDA	Perfluorodecanoic acid	335-76-2	512.841	468.9	8.49
9Cl-PF_3ONS	9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acd	756426-58-1	530.752	350.7	8.25
PFUnA	Perfluoroundecanoic acid	2058-94-8	562.801	518.9	8.95
PFDoA	Perfluorododecanoic acid	307-55-1	612.787	568.9	9.33
11Cl-PF_3OUdS	11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	763051-92-9	630.738	451.0	9.15

Table 1: MRM transitions and retention times of PFAS according to EPA 533.

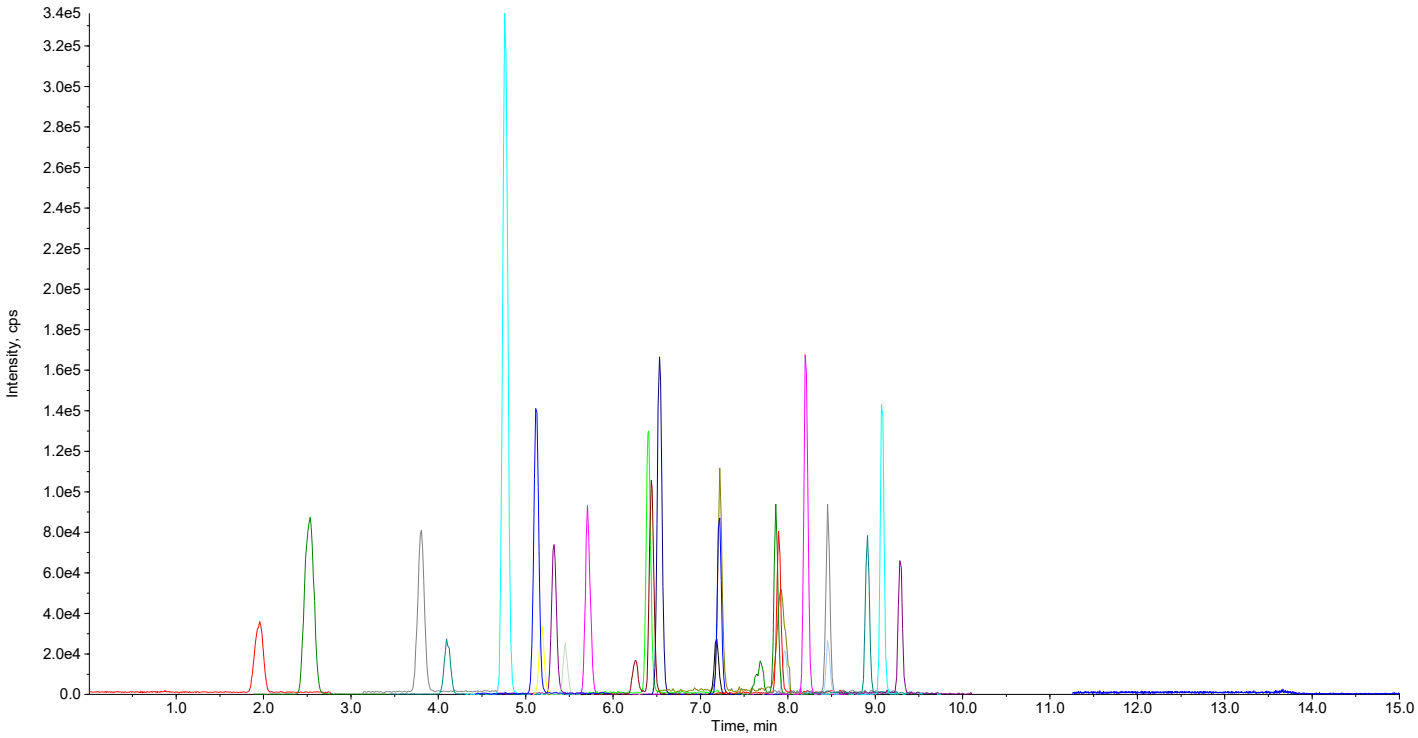

Figure 2: Chromatogram of PFAS according to EPA 533 on NUCLEODUR® PFAS EC 100/2 mm column (β = 1.0 ng/mL for each compound).

PFAS Analysis According to EPA 533

Recovery rates

Native Analyte	Abbreviation	CHROMABOND® HR-XAW, 85 µm, 6 mL/500 mg		CHROMABOND® HR-XAW, 45 µm, 3 mL/200 mg	
		Recovery %	RSD %	Recovery %	RSD %
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF_3OUdS	91.9	6.5	98.6	4.3
1H,1H, 2H, 2H-Perfluorohexane sulfonic acid	4:2FTS	96.7	2.5	99.2	5.8
1H,1H, 2H, 2H-Perfluorooctane sulfonic acid	6:2FTS	60.5	5.1	55.1	2.4
1H,1H, 2H, 2H-Perfluorodecane sulfonic acid	8:2FTS	79.8	7.4	83.4	8.7
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acd	9Cl-PF_3ONS	100.4	5.4	95.2	5.8
4,8-Dioxa-3H-perfluorononanoic acid	ADONA	98.5	3.4	104.5	2.7
Perfluoro-1-octanesulfonamide	FOSA	56.9	6.8	57.0	4.0
Hexafluoropropylene oxide dimer acid	HFPO-DA	99.9	5.5	107.7	1.0
N-ethyl perfluorooctanesulfonamidoacetic acid	NEtFOSAA	84.5	17.2	101.2	1.7
Nonafluoro-3,6-dioxaheptanoic acid	NFDHA	100.1	8.4	115.6	13.0
N-methyl perfluorooctanesulfonamidoacetic acid	NMeFOSAA	83.0	14.7	100.3	8.1
Perfluorobutanoic acid	PFBA	67.7	6.8	81.9	3.2
Perfluorobutanesulfonic acid	PFBS	102.4	5.3	102.5	3.3
Perfluorodecanoic acid	PFDA	89.9	3.0	94.7	6.0
Perfluorododecanoic acid	PFDoA	79.9	5.3	77.0	12.3
Perfluoro-1-decanesulfonate	PFDS	95.1	9.2	88.5	2.0
Perfluoro(2-ethoxyethane)sulfonic acid	PFEESA	96.8	4.7	101.5	4.9
Perfluoroheptanoic acid	PFHpA	98.1	4.9	99.9	3.4
Perfluoroheptanesulfonic acid	PFHpS	99.0	2.4	101.0	1.6
Perfluorohexanoic acid	PFHxA	98.3	2.8	96.0	3.1
Perfluorohexanesulfonic acid	PFHxS	103.4	6.2	100.5	4.4
Perfluoro-4-methoxybutanoic acid	PFMBA	94.5	2.4	92.1	1.6
Perfluoro-3-methoxypropanoic acid	PFMPA	94.4	14.3	82.2	6.3
Perfluorononanoic acid	PFNA	94.9	8.2	97.1	3.7
Perfluoro-1-nonanesulfonate	PFNS	100.5	3.4	87.3	0.9
Perfluorooctanoic acid	PFOA	95.3	5.7	82.5	7.5
Perfluorooctanesulfonic acid	PFOS	100.0	8.7	100.8	3.2
Perfluoropentanoic acid	PFPeA	91.6	4.4	88.6	5.1
Perfluoropentanesulfonic acid	PFPeS	102.7	4.6	96.1	6.9
Perfluorotetradecanoic acid	PFTA	87.1	17.9	98.6	1.0
Perfluorotridecanoic acid	PFTrDA	93.0	12.9	85.3	8.9
Perfluoroundecanoic acid	PFUnA	97.6	13.0	89.8	9.2
Perfluoro-1-butanesulfonamide	FBSA	46.5	27.1	80.1	9.3
Perfluoro-1-hexanesulfonamide	FHxSA	48.6	14.6	59.1	8.7

PFAS Analysis According to EPA 533

Internal Standards	Abbreviation	CHROMABOND® HR-XAW, 85 µm, 6 mL/500 mg		CHROMABOND® HR-XAW, 45 µm, 3 mL/200 mg	
		Recovery %	RSD %	Recovery %	RSD %
N-methyl-d_3 -perfluoro-1-octanesulfonamidoacetic acid	d3-NMeFOSAA	95.7	15.3	95.7	2.3
N-ethyl-d_5 -perfluoro-1-octanesulfonamidoacetic acid	d5-NEtFOSAA	85.8	14.0	104.7	5.8
Sodium 1H,1H,2H,2H-perfluoro-1-[1,2–$^{13}C_2$]- hexane sulfonate (4:2)	M2–4:2FTS	91.7	4.7	105.2	9.1
Sodium 1H,1H,2H,2H-perfluoro-1-[1,2–$^{13}C_2$]- octane sulfonate (6:2)	M2–6:2FTS	105.9	9.4	111.6	4.1
Sodium 1H,1H,2H,2H-perfluoro-1-[1,2–$^{13}C_2$]- decane sulfonate (8:2)	M2–8:2FTS	104.0	7.7	105.4	5.0
Perfluoro-*n*-[1,2–$^{13}C_2$]tetradecanoic acid	M2PFTA	86.3	20.4	100.8	4.4
Sodium perfluoro-1-[2,3,4–$^{13}C_3$]-butanesulfonate	M3PFBS	96.2	2.5	99.5	3.3
Sodium perfluoro-1-[1,2,3–$^{13}C_3$]-hexanesulfonate	M3PFHxS	98.7	3.2	96.1	7.5
Perfluoro-*n*-[1,2,3,4–$^{13}C_4$]heptanoic acid	M4PFHpA	95.6	3.0	94.3	4.3
Perfluoro-*n*-[1,2,3,4,6–$^{13}C_5$]hexanoic acid	M5PFHxA	96.0	3.2	93.4	3.6
Perfluoro-*n*-[$^{13}C_5$]pentanoic acid	M5PFPeA	97.8	3.3	91.2	2.6
Perfluoro-*n*-[1,2,3,4,5,6–$^{13}C_6$]decanoic acid	M6PFDA	92.3	7.1	80.7	9.1
Perfluoro-*n*-[1,2,3,4,5,6,7–$^{13}C_7$]undecanoic acid	M7PFUnA	97.9	7.1	91.0	3.1
Perfluoro-1-[$^{13}C_8$]octanesulfonamide	M8FOSA	56.0	6.1	53.3	2.4
Perfluoro-*n*-[$^{13}C_8$]octanoic acid	M8PFOA	90.7	4.6	85.3	5.7
Sodium perfluoro-1-[$^{13}C_8$]-octanesulfonate	M8PFOS	99.9	4.3	96.8	6.3
Perfluoro-*n*-[$^{13}C_9$]nonanoic acid	M9PFNA	92.5	4.1	84.7	8.3
Perfluoro-*n*-[1,2–$^{13}C_2$]dodecanoic acid	MPFDoA	93.7	12.1	85.1	3.1
Perfluoro-*n*-[$^{13}C_4$]butanoic acid	MPFBA	88.6	6.4	79.9	4.4

Table 2: Recovery rates for the presented SPE method for drinking water using SPE column, CHROMABOND® HR-XAW, 45 µm, 3 mL/200 mg and SPE column, CHROMABOND® HR-XAW, 85 µm, 6 mL/500 mg.

PFAS Analysis According to EPA 533

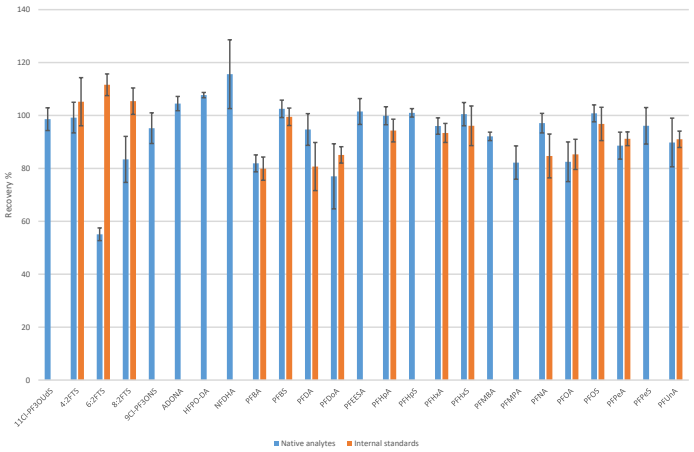

Figure 3: Recovery rates of PFAS according to EPA 533 method for drinking water using SPE column, CHROMABOND® HR-XAW, 45 µm, 3 mL/200 mg (spiked with 0.5 ng for each compound in 0.25 L water).

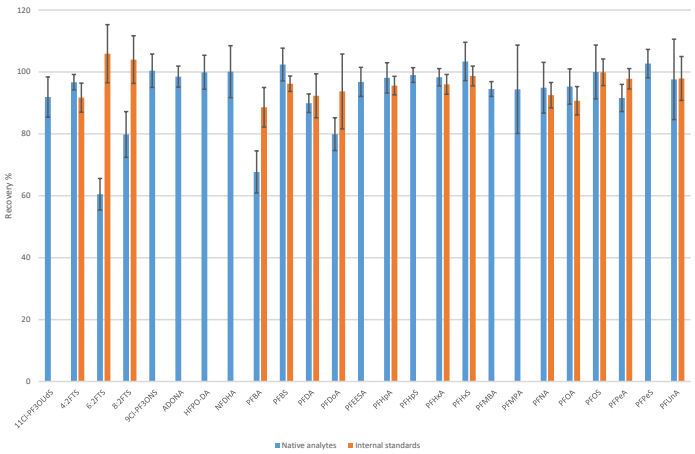

Figure 4: Recovery rates of PFAS according to EPA 533 method for drinking water using SPE column, CHROMABOND® HR-XAW, 85 µm, 6 mL/500 mg (spiked with 0.5 ng for each compound in 0.25 L water).

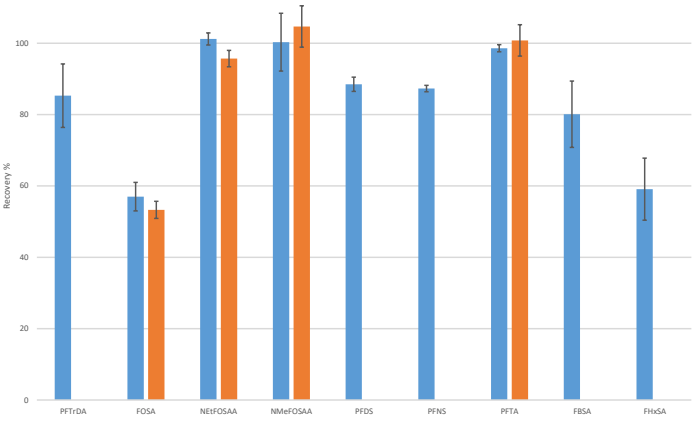

Figure 5: Recovery rates of further PFAS* according to EPA 533 method for drinking water using SPE column, CHROMABOND® HR-XAW, 45 µm, 3 mL/200 mg (spiked with 0.5 ng for each compound in 0.25 L water).
* not specified in EPA method 533

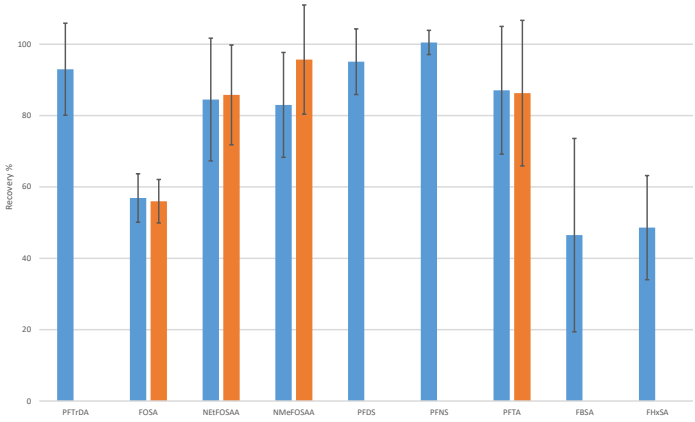

Figure 6: Recovery rates of further PFAS* according to EPA 533 method for drinking water using SPE column, CHROMABOND® HR-XAW, 85 µm, 6 mL/500 mg (spiked with 0.5 ng for each compound in 0.25 L water).
* not specified in EPA method 533

PFAS Analysis According to EPA 533

Conclusion

This application note presents the reliable and successful determination of per- and polyfluoroalkyl substances (PFAS) from drinking water according to EPA method 533. By using CHROMABOND® HR-XAW SPE columns, it was possible to achieve high recovery rates for PFAS from drinking water with good reproducibility.

The used SPE phase, a weak anion exchanger based on a polystyrene-divinylbenzene copolymer (PS / DVB), is excellently suited for the enrichment of PFAS from water samples. This mixed-mode SPE phase successfully combines the required retention modes, ion exchange and reversed phase.

The work shows that both tested SPE column dimensions, CHROMABOND® HR-XAW, 45 µm, 3 mL/200 mg and CHROMABOND® HR-XAW, 85 µm, 6 mL/500 mg, fulfill the analytical requirements of EPA method 533. Most of the PFAS show recovery rates between 80 % and 120 %. Further tests showed that good results could also be obtained for several other analytes with the before-mentioned SPE products. Due to the missing acid functionality, the recovery rates of the group of perfluorooctane sulfonamides, presented in figures 5 and 6, are smaller than the average. The use of SPE phases with stronger hydrophobic interaction could lead to better enrichment. We recommend to use CHROMABOND® PFAS for this issue.

The chromatographic separation of PFAS was performed by using NUCLEODUR® PFAS HPLC column. This phase is specially suitable for the analysis of PFAS compounds. It shows high retention for polar PFAS, high MS intensity and excellent batch-to-batch reproducibility. Contaminants from an LC system, especially PFOA, are separated from sample analytes by implementing an isolator column, NUCLEODUR® PFAS Delay.

In summary, the presented application shows that the utilized SPE and HPLC products allow a reliable and successful determination of per- and polyfluoroalkyl substances (PFAS) from drinking according to EPA method 533.

References

[1] United States Environmental Protection Agency, PFAS Laws and Regulations, https://www.epa.gov/pfas/pfas-laws-and-regulations

[2] EPA's Per- and Polyfluoroalkyl Substances (PFAS) Action Plan, EPA 823R18004, February 2019, www.epa.gov/pfas

[3] Method 537.1: Determination of Selected Per- and Polyfluorinated Alkyl Substances in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment, Washington, DC, 2018.

[4] 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. U.S. Environmental Protection Agency, Office of Research and Development, National Center for Environmental Assessment, Washington, DC, 2019.

Product information

The following MACHEREY-NAGEL products have been used in this application note:

REF 760673.20	EC 50/2 NUCLEODUR® PFAS Delay
REF 760666.20	EC 100/2 NUCLEODUR® PFAS
REF 730748P45	CHROMABOND® HR-XAW, 45µm, 3 mL, 200 mg
REF 730745	CHROMABOND® HR- XAW 85 µm, 6 mL, 500 mg
REF 730382	Reservoir columns, 70 mL with adaptor for 1, 3, 6 mL CHROMABOND® SPE PP columns
REF 702402	Screw closure, N 9, PP, blue, c. hole, Sili. w./ Polyimide orange,1.0mm,flourine-free
REF 702009	Screw neck vial, N 9, 11.6 x 32.0 mm, 0.3 mL, inner cone, PP tr.

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