MACHEREY-NAGEL

Application Note 10/2022 · Chromatography

Determination of Per- and Polyfluoroalkyl Substances from water samples according to ISO 21675:2019

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Application benefits

- Successful determination of short- and long-chain Per- and Polyfluoroalkyl Substances from water samples
- High recovery rates were achieved with a special SPE column: CHROMABOND® PFAS, 120 mg, 3 mL
- Fast and sensitive HPLC analysis on a NUCLEODUR® PFAS column

MN products

REF 7300009
CHROMABOND® PFAS, 3 mL, 120 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: 306970
HPLC: 129390

Keywords

PFAS, short- and long-chain, water, weak anion exchanger, LC-MS/MS, Delay column

Introduction

According to the OECD, over 4730 per- and polyfluoroalkyl substances (PFAS) are currently known [1]. PFAS are a large group of man-made chemicals. Due to their physico-chemical properties, these compounds are used in a wide range of industries worldwide (e.g. textiles, household products, firefighting, automotive, food processing, construction, electronics). The distribution of PFAS from fire-foams into the environment is exemplarily shown in figure 1. They are mostly non-biodegradable and are therefore also called “forever chemicals”. These bio-accumulative pollutants are characterized by a linear aliphatic backbone, a high degree of fluorination, and often feature a carboxylic- or sulfonic- acid functionality. People can be exposed to PFAS in a variety of ways, including drinking water. But the exposure to PFAS can lead to adverse health effects. Many studies have examined possible connections between the level of per- and polyfluoroalkyl substances in the blood and adverse health effects in humans [2]. The research suggests that high levels of certain PFAS can lead to:

- Increased cholesterol level
- Reduced vaccine response in children
- Changes in liver enzymes
- Increased risk of high blood pressure or pre-eclampsia in pregnant women
- Small decreases in infant birth weight
- Increased risk of kidney or testicular cancer

People can be exposed to PFAS in a variety of ways, including drinking water. In September 2020, the European Food Safety Authority (EFSA) published a report on the risk to human health related to the presence of perfluoroalkyl substances in food [3]. The focus of the report concerns the intake of four PFAS:

- Perfluorooctanesulfonic acid (PFOS)
- Perfluorooctanoic acid (PFOA)
- Perfluorononanoic acid (PFNA)
- Perfluorohexanesulfonic acid (PFHxS)

A tolerable weekly intake (TWI) of 4.4 nanograms (ng) per kilogram (kg) of bodyweight per week is discussed as the sum of these compounds.

In recent years PFAS analysis has established itself worldwide. To protect human health, many guidelines and method proposals have been issued and published by regulatory authorities around the world, including ISO 21675:2019.

In this application note, a SPE method according to ISO 21675:2019 using the CHROMBOND® PFAS is presented. High recovery rates with very good reproducibility are achieved. Finally, the extracts are analyzed using HPLC-MS/MS on a NUCLEODUR® PFAS column.

Determination of Per- and Polyfluoroalkyl Substances from water samples according to ISO 21675:2019

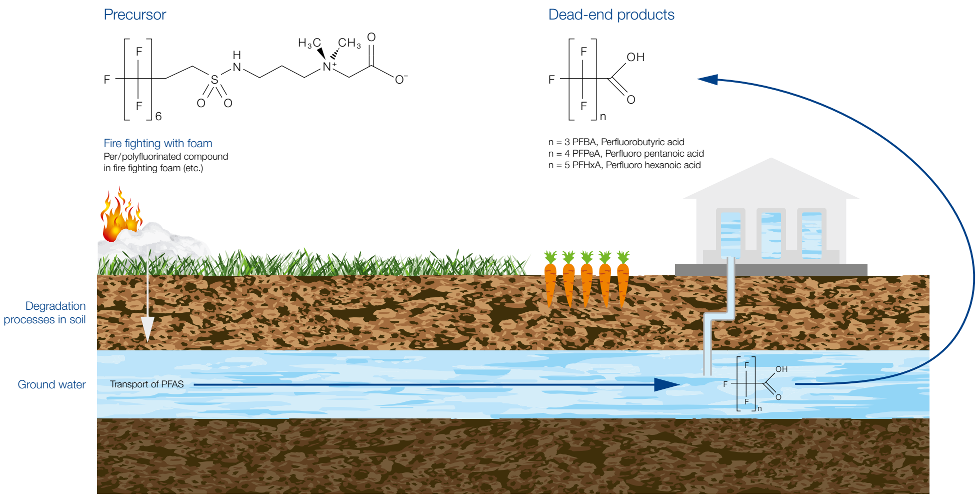

Figure 1: Distribution of PFAS from fire-foams into the environment-examples.

Sample pretreatment

MN Appl. No. 306970

Solid phase extraction according to ISO 21675:2019

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. The pH value of the sample shall be adjusted to the pH value of 3 with acetic acid or ammonia solution, if necessary.
2. Add the spiking solution containing the internal standard substances to the water sample in the sample bottle (adding 0.5 ng of each) and mix thoroughly by shaking.

Column: CHROMABOND® PFAS, 3 mL 120 mg (REF 730009)

Conditioning: Add 4 ml of 0.1 % NH_3 in methanol solution, 4 ml of methanol and 4 ml of water to the cartridge.

Sample application: Add 200 mL water sample with a flow rate of 3 ml/min to 6 ml/min to the cartridge. (Do not let the sorbent material in the cartridge go dry and ensure it is immersed in water at all times.)

Bottle Rinse: Rinse the wall of sample bottle and reservoir column with a volume of methanol which corresponds to at least 0.5 % of original sample volume. This aliquot of methanol is collected and used as elution solvent for sample extraction.

Washing step 1: Add 4 ml of water and 4 ml of acetate buffer (0.025 mol/l, pH 4) solution to the cartridge and discard the eluate.

Elution: Add 4 ml of methanol, followed by 4 ml of 0.1 % NH_3 in methanol solution with a flow rate of 3 ml/min and collect into the sample tube, separately. (Neutral substances such as FOSA, *N*-MeFOSA and *N*-EtFOSA elute with methanol. Anionic substances such as PFOS and PFOA elute with ammonia/methanol.)

Eluent exchange of methanol eluate: Evaporate the eluate with a gentle stream of nitrogen gas to a final volume of 0.5 ml. Add 0.5 mL of methanol (spiked with internal standard).

Eluent exchange of 0.1 % NH_3 in methanol eluate: 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. 129390

Chromatographic conditions

DELAY Colin	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	1 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	212.904	168.8	2.01
Perfluoro-*n*-pentanoic	PFPeA	2706-90-3	262.880	219.0	3.90
Perfluoro-*n*-butanesulfonic acid	PFBS	375-73-5	298.933	98.9	4.20
Perfluoro-*n*-hexanoic acid	PFHxA	307-24-4	312.911	268.8	5.40
Hexafluoropropylene oxide dimer acid	HFPO-DA	13252-13-6	284.991	168.7	5.77
Perfluoro-*n*-heptanoic acid	PFHpA	375-85-9	362.931	318.8	6.45
Perfluoro-*n*-hexanesulfonic acid	PFHxS	355-46-4	398.942	79.8	6.49
4,8-Dioxa-3*H*-perfluorononanoic acid	ADONA	919005-14-4	376.901	250.7	6.58
6:2 Fluorotelomer sulfonic acid	6:2 FTSA	27619-97-2	426.927	406.9	7.24
Perfluoro-*n*-heptanesulfonic acid	PFHpS	375-92-8	448.929	79.8	7.26
Perfluoro-*n*-octanoic acid	PFOA	335-67-1	412.910	369.0	7.26
Perfluoro-*n*-octanesulfonic acid	PFOS	1763-23-1	498.836	79.9	7.89
Perfluoro-*n*-nonanoic acid	PFNA	375-95-1	462.893	418.9	7.92
8:2 Fluorotelomer unsaturated carboxylic acid	FTUCA	70887-84-2	457.000	393.0	8.09
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	Cl-PF3ONS	73606-19-6	530.752	350.7	8.25
Perfluoro-*n*-decanoic acid	PFDA	335-76-2	512.841	468.9	8.49
8:2 Fluorotelomer sulfonic acid	8:2 FTSA	39108-34-4	526.000	506.8	8.50
N-methyl perfluorooctanesulfonamidoacetic acid	*N*-MeFOSAA	2355-31-9	569.801	418.9	8.78
Perfluorooctanesulfonamide	FOSA	754-91-6	497.870	77.8	8.84
Perfluoro-*n*-decanesulfonic	PFDS	335-77-3	598.790	79.9	8.90
Perfluoro-*n*-undecanoic acid	PFUnDA	2058-94-8	562.801	518.9	8.95
N-ethyl perfluorooctanesulfonamidoacetic acid	*N*-EtFOSAA	2991-50-6	583.809	418.8	9.02
Perfluoro-*n*-dodecanoic acid	PFDoDA	307-55-1	612.787	568.9	9.33
N-methyl perfluorooctanesulfonamide	*N*-MeFOSA	31506-32-8	512.000	169.0	9.55
Perfluoro-*n*-tridecanoic acid	PFTrDA	72629-94-8	662.767	618.9	9.66
N-ethyl perfluorooctanesulfonamide	*N*-EtFOSA	4151-50-2	526.000	169.0	9.80
Perfluoro-*n*-tetradecanoic acid	PFTeDA	376-06-7	712.774	668.8	9.94
8:2 Polyfluoroalkyl phosphate diester	8:2 diPAP	678-41-1	988.911	96.9	10.51
Perfluoro-*n*-octadecanoic acid	PFOcDA	16517-11-6	913.000	869.0	10.64
Perfluoro-*n*-hexadecanoic acid	PFHxDA	67905-19-5	813.000	769.0	10.80
Surrogates					
Perfluoro-($^{13}C_5$)pentanoic acid	M5PFPeA		267.970	222.9	3.92
Sodium perfluoro-(2,3,4–$^{13}C_3$)butanesulfonate	M3PFBS		301.887	98.9	4.22
Sodium 1*H*,1*H*,2*H*,2*H*-perfluoro(1,2–$^{13}C_2$)hexanesulfonate	M2–4:2FTS		328.971	81.0	5.26
Perfluoro-(1,2,3,4,6–$^{13}C_5$)hexanoic acid	M5PFHxA		317.998	272.8	5.40
Perfluoro-(1,2,3,4–$^{13}C_4$)heptanoic acid	M4PFHpA		366.953	321.8	6.45
Sodium perfluoro-(1,2,3–$^{13}C_3$)hexanesulfonate	M3PFHxS		401.901	79.9	6.50
Sodium 1*H*,1*H*,2*H*,2*H*-perfluoro(1,2–$^{13}C_2$)octanesulfonate	M2–6:2FTS		428.938	81.0	7.23
Perfluoro-($^{13}C_8$)octanoic acid	M8PFOA		420.952	376.0	7.27
Sodium perfluoro-($^{13}C_8$)octanesulfonate	M8PFOS		506.907	98.9	7.89
Perfluoro-($^{13}C_9$)nonanoic acid	M9PFNA		471.943	427.0	7.92
Perfluoro-(1,2,3,4,5,6–$^{13}C_6$)decanoic acid	M6PFDA		518.923	474.0	8.49
Sodium 1*H*,1*H*,2*H*,2*H*-perfluoro(1,2–$^{13}C_2$)decanesulfonate	M2–8:2FTS		528.935	80.9	8.50
N-methyl-d3 -perfluorooctanesulfonamidoacetic acid	d3-*N*-MeFOSAA		572.891	419.0	8.78
Perfluoro-($^{13}C_8$)octanesulfonamide	M8FOSA		505.978	77.9	8.84

Analyte	Abbreviation	CAS number	Q1 mass [Da]	Q3 mass [Da]	Retention time [min]
Perfluoro-(1,2,3,4,5,6,7–$^{13}C_7$)undecanoic acid	M7PFUdA		569.945	525.0	8.95
N-ethyl-d5 -perfluorooctanesulfonamidoacetic acid	d5-*N*-EtFOSAA		588.845	418.8	9.02
Perfluoro-(1,2–$^{13}C_2$)dodecanoic acid	MPFDoA		614.947	569.9	9.33
Perfluoro-(1,2–$^{13}C_2$)tetradecanoic acid	M2PFTeDA		714.936	670.0	9.94

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

Chromatogramms

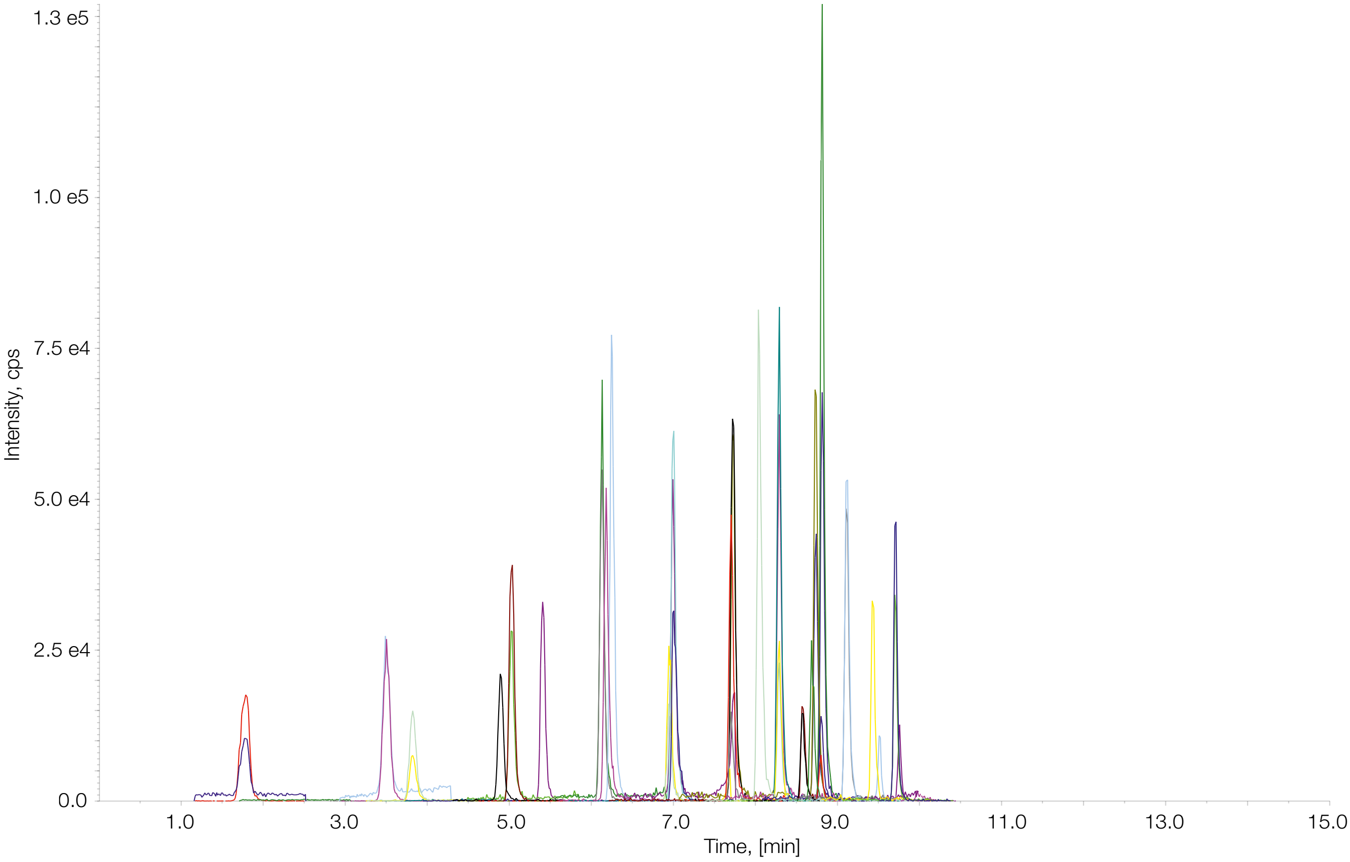

Figure 2: Chromatogram of a standard solution (concentration, β = 0.5 ng/mL)

Recovery rates

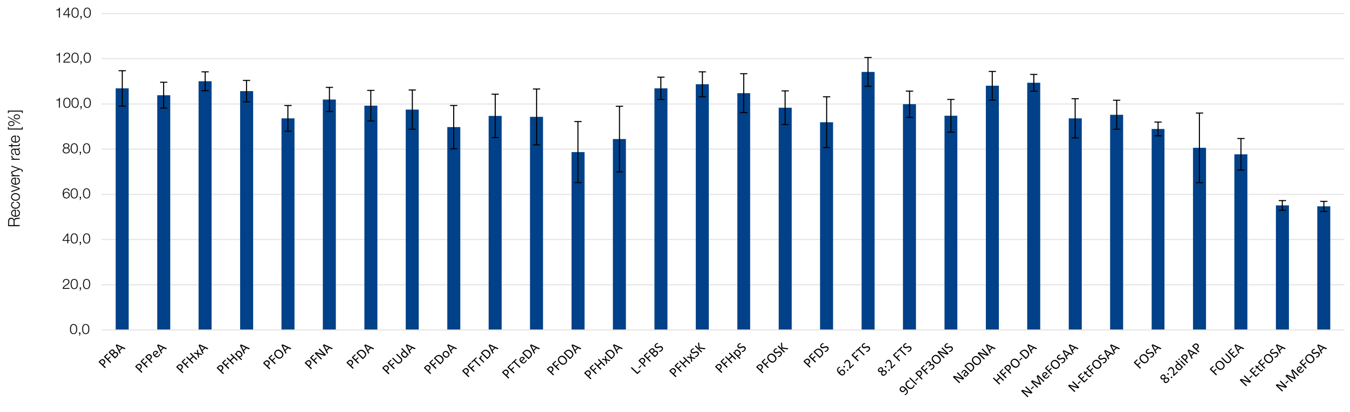

Figure 3: Recovery rate from water sample (concentration, β = 2 ng/L) (n=5)

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Abbreviation	Recovery rate (%) ± RSD (%)
PFBA	106.9 ± 7.8
PFPeA	103.9 ± 5.7
PFHxA	110.0 ± 4.2
PFHpA	105.7 ± 4.7
PFOA	93.6 ± 5.7
PFNA	102.0 ± 5.3
PFDA	99.2 ± 6.8
PFUdA	97.5 ± 8.7
PFDoA	89.7 ± 9.6
PFTrDA	94.7 ± 9.6
PFTeDA	94.3 ± 12.4
PFODA	78.7 ± 13.5
PFHxDA	84.4 ± 14.5
L-PFBS	106.9 ± 4.9
PFHxSK	108.7 ± 5.5
PFHpS	104.8 ± 8.6
PFOSK	98.3 ± 7.5
PFDS	91.9 ± 11.2
6:2 FTS	114.2 ± 6.4
8:2 FTS	99.9 ± 5.8
9Cl-PF3ONS	94.8 ± 7.2
NaDONA	108.0 ± 6.3
HFPO-DA	109.4 ± 3.7
N-MeFOSAA	93.6 ± 8.7
N-EtFOSAA	95.2 ± 6.4
FOSA	88.9 ± 3.1
8:2diPAP	80.6 ± 15.4
FOUEA	77.7 ± 7.0
N-EtFOSA	55.1 ± 2.2
N-MeFOSA	54.7 ± 2.2

Table 3: Recovery rates for the presented SPE method using CHROMABOND® PFAS, 120 mg, 3 mL (n=5).

Conclusion

This application note presents the reliable and successful determination of 30 PFAS from drinking water according to ISO 21675:2019 from drinking water. By using a double-layer SPE column, CHROMABOND® PFAS, it was possible to achieve high recovery rates and good reproducibility. By the combination of different SPE sorbents in a multi-layer column it is possible to use various interaction types like ionic, hydrophobic, hydrogen bonds and dipole-dipole interactions for the enrichment of a broad spectrum of PFAS. In this way, a SPE method could be developed applying the strength of two sorbents. As a result most of the PFAS show recovery rates between 80 % to 100 %.

The two-step elution, (first methanol and second 0.1 % ammonium hydroxide in methanol) was chosen to improve recovery rates of neutral substances such as FOSA, *N*-MeFOSA and *N*-EtFOSA.

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

References

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