

PFAS Analysis According to ISO 21675:2019 and to DIN 38407-42

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Abstract

This application note describes the determination of native per- and polyfluoroalkyl substances (PFAS) according to European directives ISO 21675:2019 and to DIN 38407-42. It demonstrates the chromatographic separation of PFAS using a NUCLEODUR® PFAS analytical HPLC column in combination with a NUCLEODUR® PFAS Delay column.

Introduction

Per- and Polyfluorinated Alkyl Substances (PFAS) are a group of anthropogenic chemicals that are known as highly stable and resistant to degradation. They have been manufactured since the 1940s and have been used in many consumer and industrial products (e.g. fire-fighting foam, textiles, food packaging, building materials and so on).

This broad use, appearance and their persistency leads to the fact that PFAS are now abundant in the environment. From industrial and private use, PFAS blaze their trail into soil, ground water, plants, animals and humans. Unfortunately, many of the PFAS are toxic and potentially harmful to humans. Therefore, monitoring these substances is important to investigate the risk to human health related to the presence of PFAS in food and feed [1]. This is the reason why authorities in the EU published a variety of laws and regulations. Well know methods for analyzing PFAS from drinking water are DIN

38414-14 and ISO 21675:2019 [2, 3]. They require robust and fast chromatographic separation of PFAS to ensure accurate quantitation of low levels.

This work presents the analysis of PFAS according to directives ISO 21675:2019 and to DIN 38407-42. It represents the chromatographic separation of PFAS using a NUCLEODUR® PFAS analytical HPLC column in combination with a NUCLEODUR® PFAS Delay column.



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Per- and polyfluoroalkyl substances (PFAS)

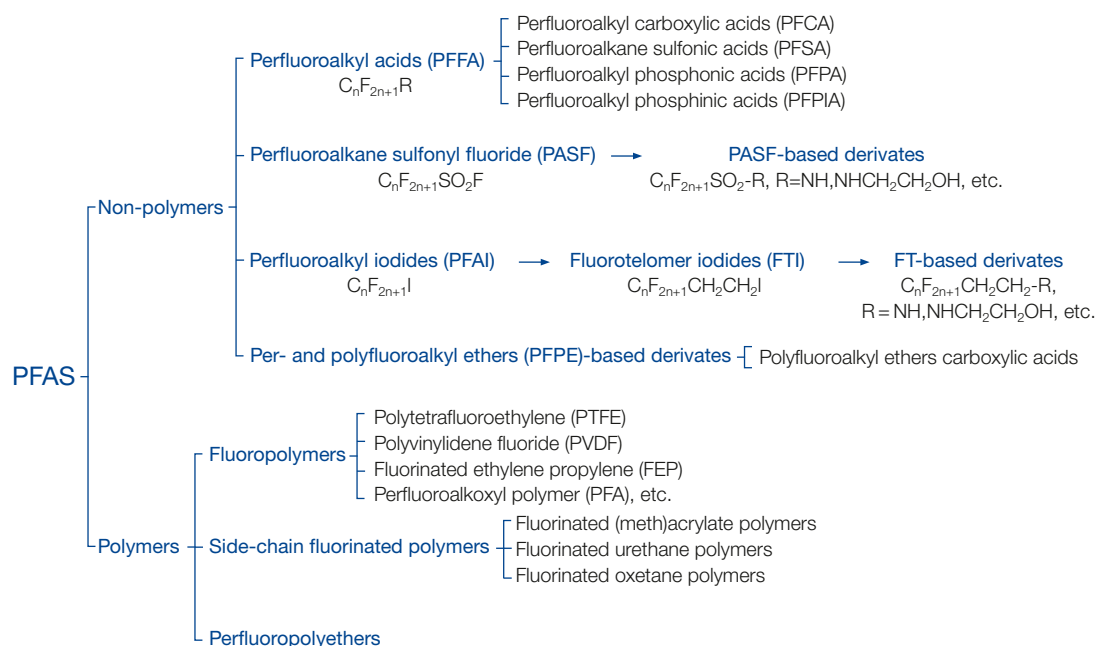


Figure 1: Classes of per- and polyfluoroalkyl substances (PFAS).

PFAS Analysis According to ISO 21675:2019 and to DIN 38407-42

Analysis by HPLC-MS/MS

A: Chromatographic conditions

NUCLEODUR® PFAS, EC 50 x 2 mm, 3 µm

Delay column: EC 50/2 NUCLEODUR® PFAS Delay , 5 µm (REF 760673.20)

Analytical column: EC 50/2 NUCLEODUR® PFAS, 3 µm (REF 760663.20)

Eluent A: 5 mM ammonium acetate in water

Eluent B: 5 mM ammonium acetate in methanol

Gradient: hold 40 % B for 0.5 min, in 4 min from 40 % B to 95 % B, hold 95 % B for 1.5 min, in 0.05 min to 40 % B, hold 40 % B for 1.45 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

B: Chromatographic conditions

NUCLEODUR® PFAS, EC 100 x 2 mm, 3 µm

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

MRM transitions

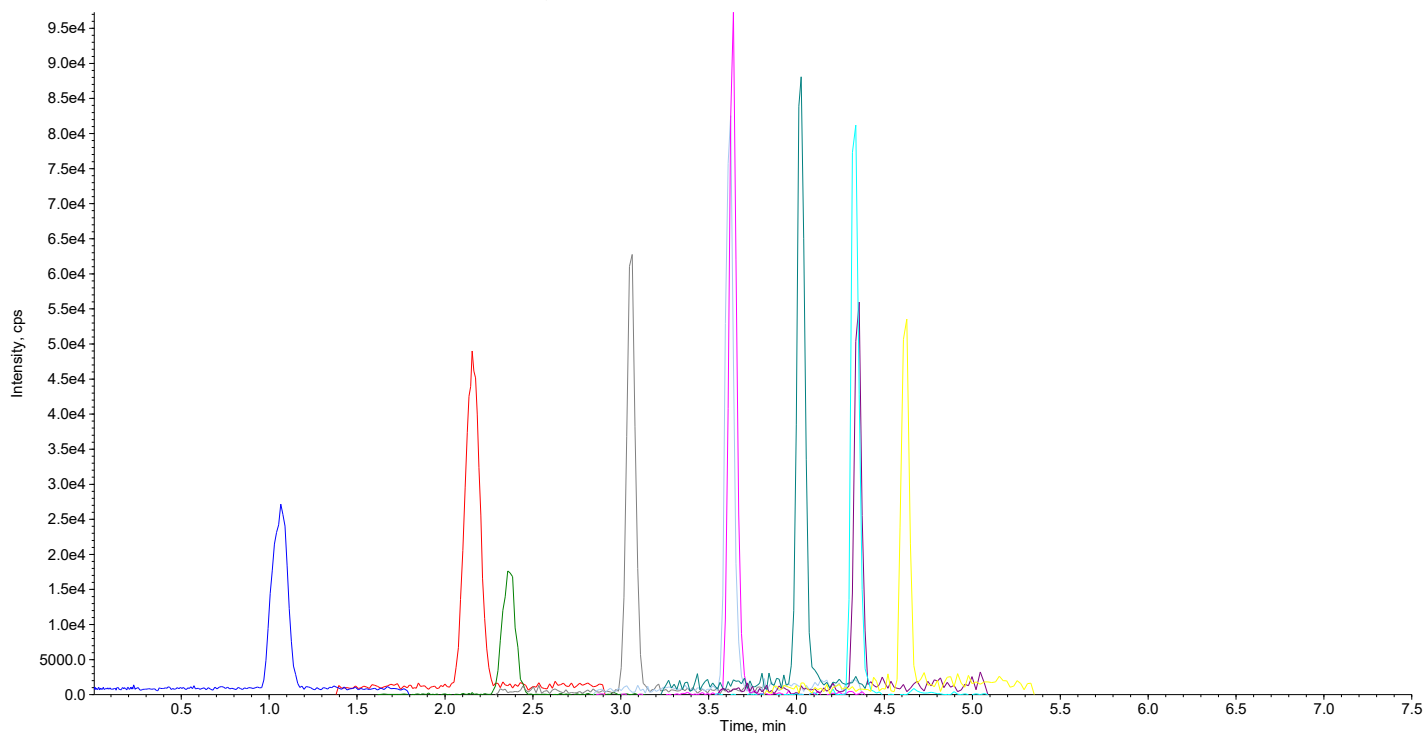
Analyte	Abbreviation	CAS number	Q ₁ mass [Da]	Q ₃ mass [Da]	Retention time [min] A	Retention time [min] B
Perfluoro- <i>n</i> -butanoic acid	PFBA	375-22-4	212.9	168.8	1.02	2.01
Perfluoro- <i>n</i> -butanesulfonic acid	PFBS	375-73-5	298.9	98.9	2.37	4.20
Perfluoro- <i>n</i> -decanoic acid	PFDA	335-76-2	512.8	468.9	4.58	8.49
Perfluoro- <i>n</i> -heptanoic acid	PFHpA	375-85-9	362.9	318.8	3.60	6.45
Perfluoro- <i>n</i> -hexanoic acid	PFHxA	307-24-4	312.9	268.8	3.05	5.40
Perfluoro- <i>n</i> -hexanesulfonic acid	PFHxS	355-46-4	398.9	79.8	3.62	6.49
Perfluoro- <i>n</i> -nonanoic acid	PFNA	375-95-1	462.9	418.9	4.31	7.92
Perfluoro- <i>n</i> -octanoic acid	PFOA	335-67-1	412.9	369.9	3.99	7.26
Perfluoro- <i>n</i> -octanesulfonic acid	PFOS	1763-23-1	498.8	79.9	4.30	7.89
Perfluoro- <i>n</i> -pentanoic acid	PFPeA	2706-90-3	262.9	219.0	2.14	3.90

Table 1: MRM transitions and retention times of PFAS according to DIN 38407-42.

PFAS Analysis According to ISO 21675:2019 and to DIN 38407-42

Analyte	Abbreviation	CAS number	Q ₁ mass [Da]	Q ₃ mass [Da]	Retention time [min] A	Retention time [min] B
6:2 Fluorotelomer sulfonic acid	6:2 FTSA	27619-97-2	426.9	406.9	3.99	7.24
8:2 Polyfluoroalkyl phosphate diester	8:2 diPAP	678-41-1	988.9	96.9	5.65	10.51
8:2 Fluorotelomer sulfonic acid	8:2 FTSA	39108-34-4	526.0	506.8	4.60	8.50
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	9Cl-PF ₃ ONS	73606-19-6	530.8	350.7	4.48	8.25
4,8-Dioxa-3H-perfluorononanoic acid	DONA	919005-14-4	376.9	250.7	3.66	6.58
Perfluorooctanesulfonamide	FOSA	754-91-6	497.9	77.8	4.80	8.84
8:2 Fluorotelomer unsaturated carboxylic acid	FTUCA	70887-84-2	457.0	393.0	4.43	8.09
Hexafluoropropylene oxide dimer acid	HFPO-DA	13252-13-6	285.0	168.7	3.26	5.77
<i>N</i> -ethyl perfluorooctanesulfonamide	<i>N</i> -EtFOSA	4151-50-2	526.0	169.0	5.37	9.80
<i>N</i> -ethyl perfluorooctanesulfonamidoacetic acid	<i>N</i> -EtFOSAA	2991-50-6	583.8	418.8	4.84	9.02
<i>N</i> -methyl perfluorooctanesulfonamide	<i>N</i> -MeFOSA	31506-32-8	512.0	169.0	5.24	9.55
<i>N</i> -methyl perfluorooctanesulfonamidoacetic acid	<i>N</i> -MeFOSAA	2355-31-9	569.8	418.9	4.71	8.78
Perfluoro- <i>n</i> -butanoic acid	PFBA	375-22-4	212.9	168.8	1.02	2.01
Perfluoro- <i>n</i> -butanesulfonic acid	PFBS	375-73-5	298.9	98.9	2.37	4.20
Perfluoro- <i>n</i> -decanoic acid	PFDA	335-76-2	512.8	468.9	4.58	8.49
Perfluoro- <i>n</i> -dodecanoic acid	PFDoDA	307-55-1	612.8	568.9	5.02	9.33
Perfluoro- <i>n</i> -decanesulfonic	PFDS	335-77-3	598.8	79.9	4.79	8.90
Perfluoro- <i>n</i> -heptanoic acid	PFHpA	375-85-9	362.9	318.8	3.60	6.45
Perfluoro- <i>n</i> -heptanesulfonic acid	PFHpS	375-92-8	448.9	79.8	4.00	7.26
Perfluoro- <i>n</i> -hexanoic acid	PFHxA	307-24-4	312.9	268.8	3.05	5.40
Perfluoro- <i>n</i> -hexadecanoic acid	PFHxDA	67905-19-5	813.0	769.0	5.57	10.80
Perfluoro- <i>n</i> -hexanesulfonic acid	PFHxS	355-46-4	398.9	79.8	3.62	6.49
Perfluoro- <i>n</i> -nonanoic acid	PFNA	375-95-1	462.9	418.9	4.31	7.92
Perfluoro- <i>n</i> -octanoic acid	PFOA	335-67-1	412.9	369.0	3.99	7.26
Perfluoro- <i>n</i> -octadecanoic acid	PFOcDA	16517-11-6	913.0	869.0	5.76	10.64
Perfluoro- <i>n</i> -octanesulfonic acid	PFOS	1763-23-1	498.8	79.9	4.30	7.89
Perfluoro- <i>n</i> -pentanoic acid	PFPeA	2706-90-3	262.9	219.0	2.14	3.90
Perfluoro- <i>n</i> -tetradecanoic acid	PFTeDA	376-06-7	712.8	668.8	5.34	9.94
Perfluoro- <i>n</i> -tridecanoic acid	PFTrDA	72629-94-8	662.8	618.9	5.19	9.66
Perfluoro- <i>n</i> -undecanoic acid	PFUnDA	2058-94-8	562.8	518.9	4.81	8.95

Table 2: MRM transitions and retention times of PFAS according to ISO 21675:2019.

Figure 2: Chromatogram of PFAS according to DIN 38407-42 on NUCLEODUR® PFAS EC 50 x 2 mm column ($\beta = 1.0$ ng/mL for each compound).

PFAS Analysis According to ISO 21675:2019 and to DIN 38407-42

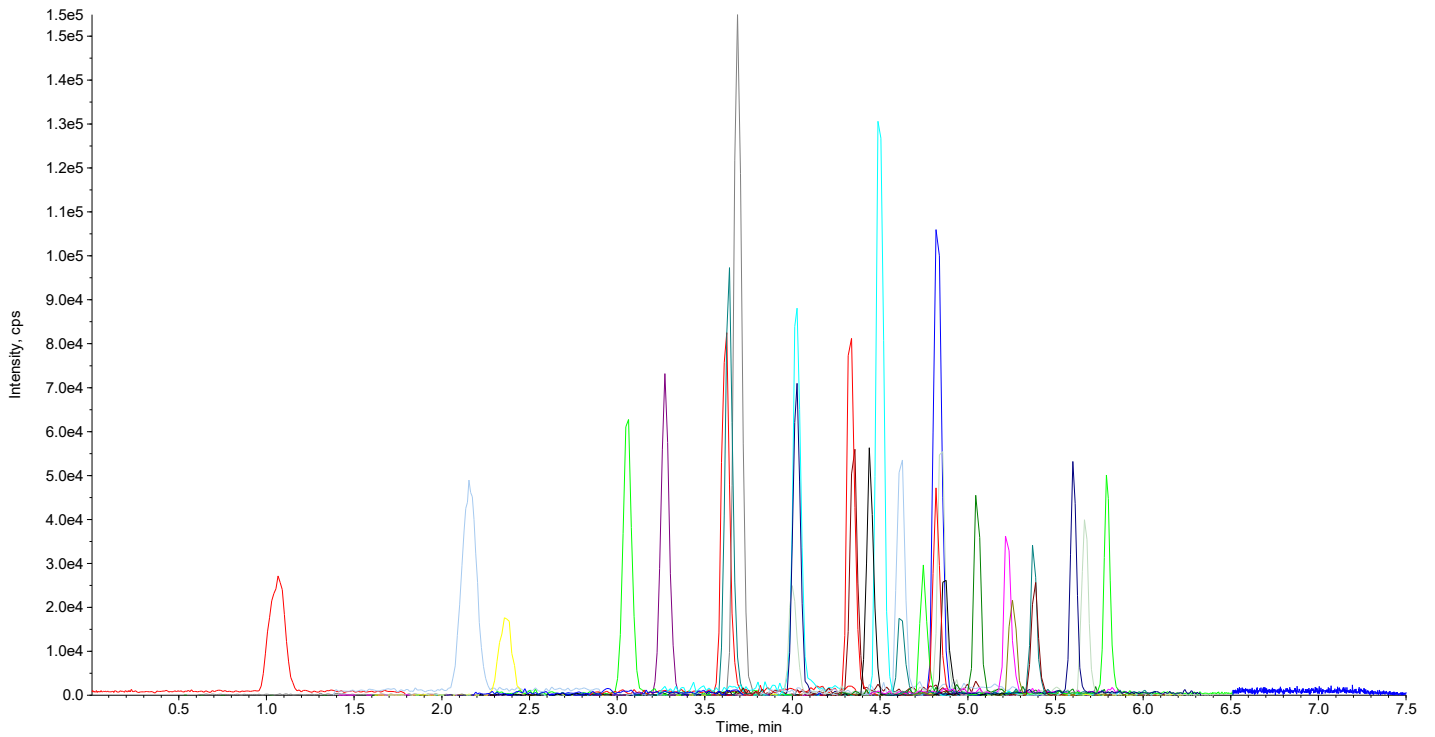


Figure 3: Chromatogram of PFAS according to ISO 21675:2019 on NUCLEODUR® PFAS EC 50 x 2 mm column ($\beta = 1.0$ ng/mL for each compound).

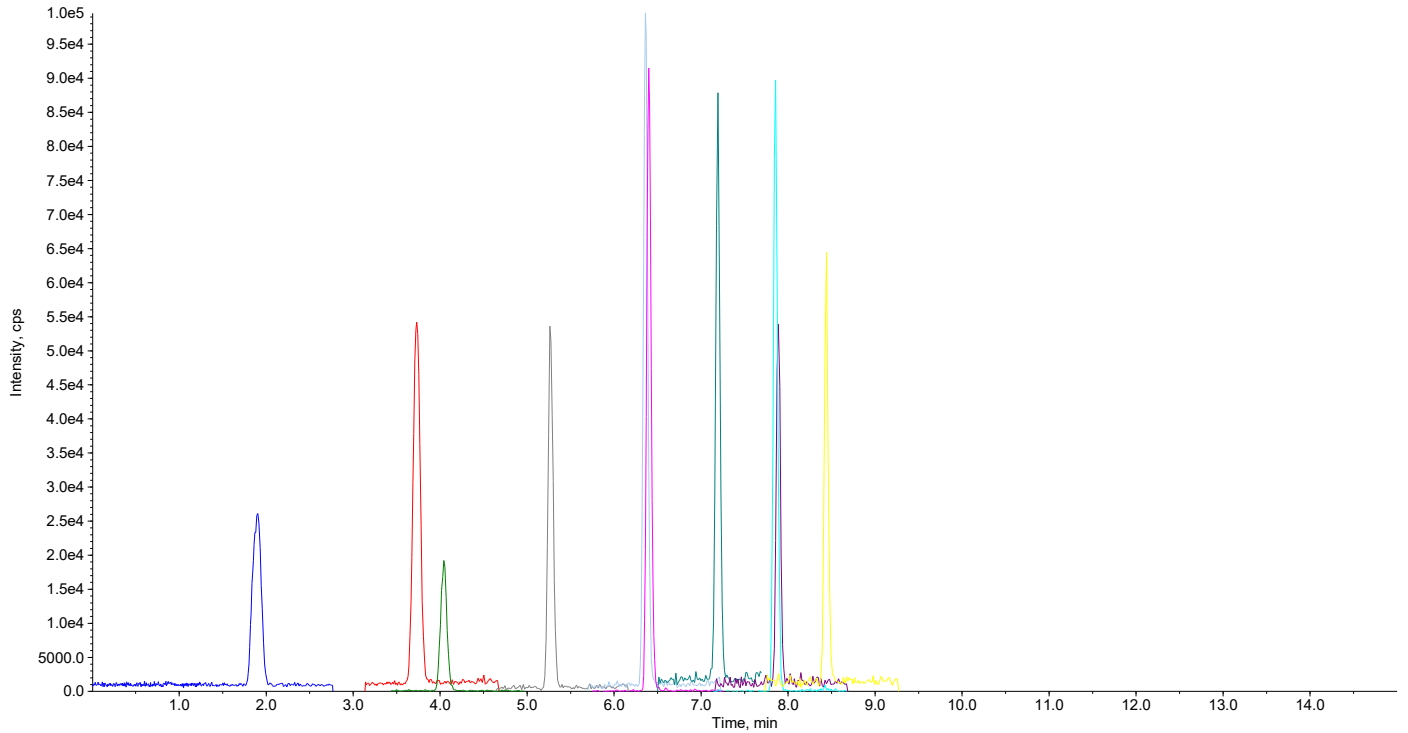


Figure 4: Chromatogram of PFAS according to EPA DIN 38407-42 on NUCLEODUR® PFAS EC 100 x 2 mm column ($\beta = 1.0$ ng/mL for each compound).

PFAS Analysis According to ISO 21675:2019 and to DIN 38407-42

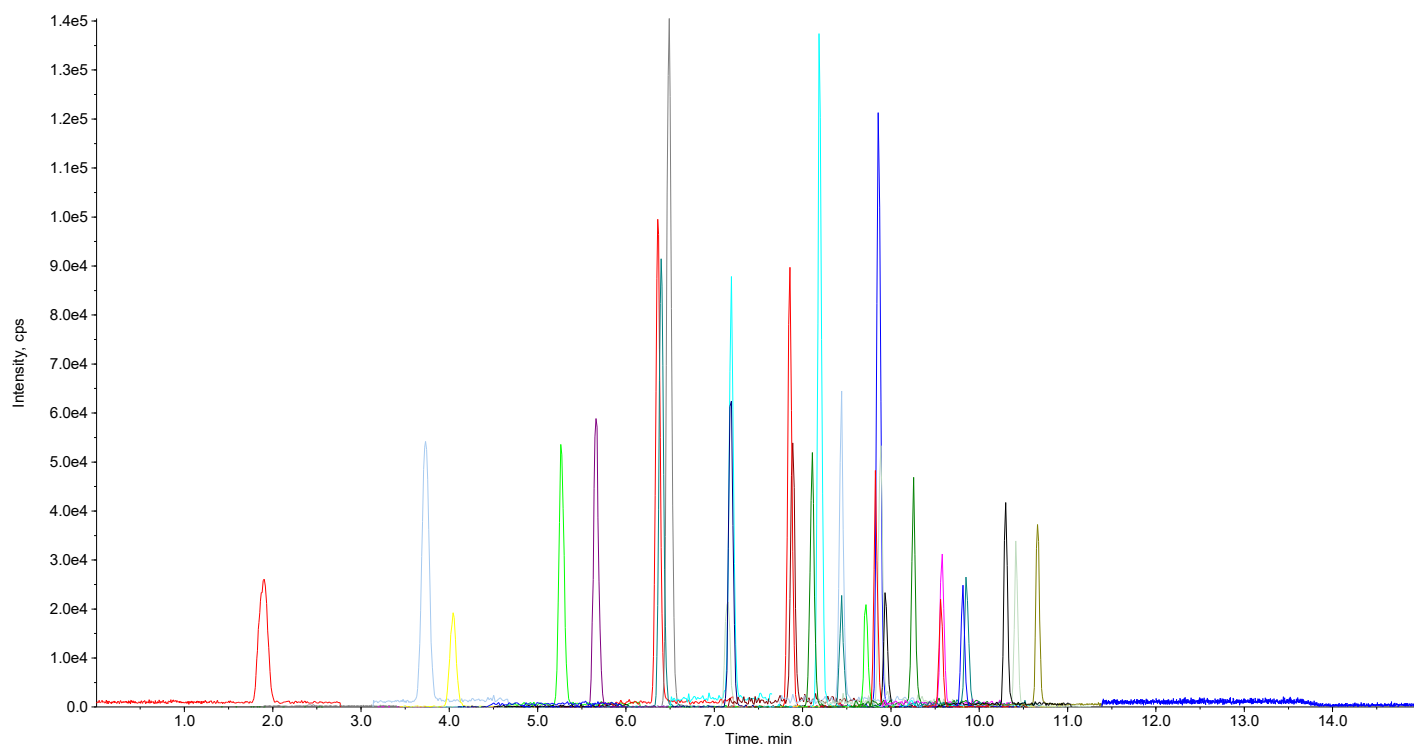


Figure 5: Chromatogram of PFAS according to ISO 21675:2019 on NUCLEODUR® PFAS EC 100 x 2 mm column ($\beta = 1.0$ ng/mL for each compound).

Separation of instrument contaminants by using a delay column

The prevalence of PFOA is commonly observed as an instrument materials contaminant. The following figures 6 and 7 show the benefits of using a delay column for the combinations A and B (see chromatographic conditions).

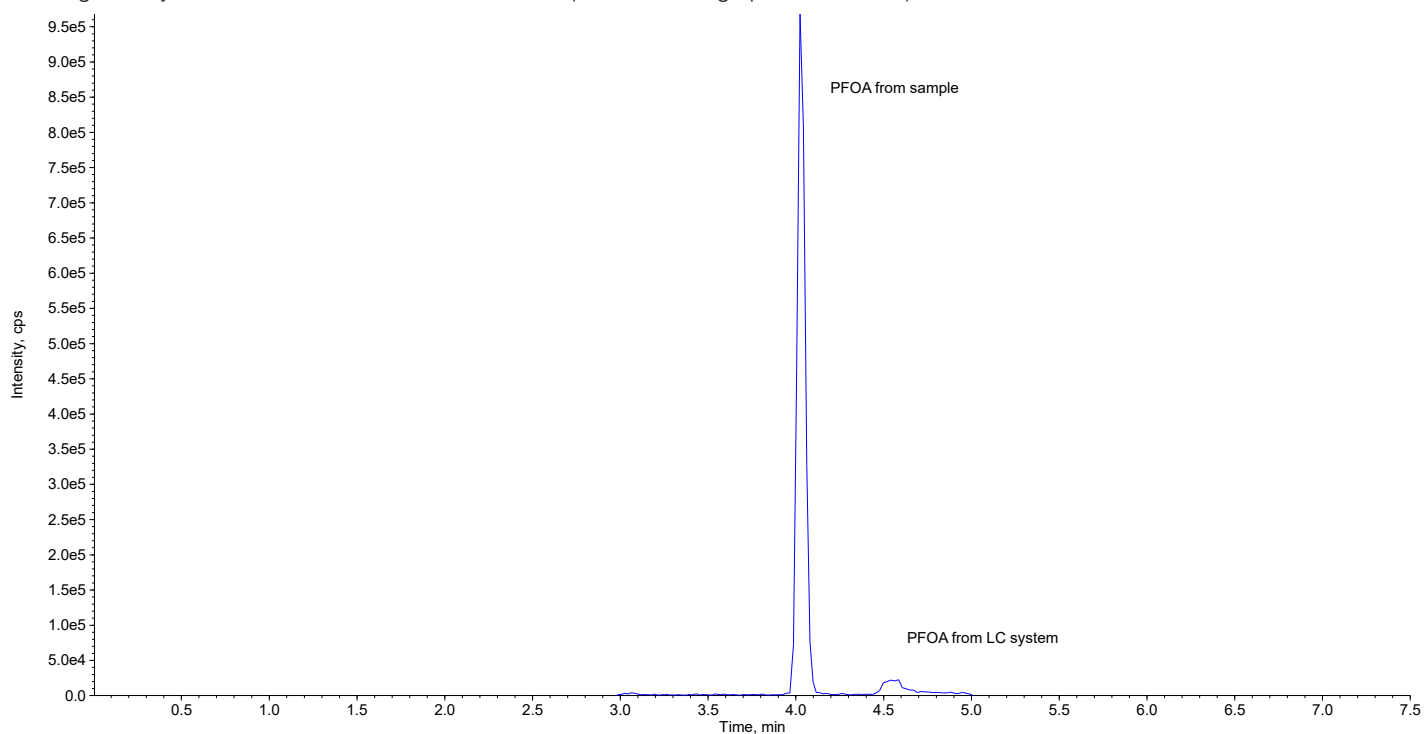


Figure 6: Chromatogram of PFOA resulted from the combination NUCLEODUR® PFAS Delay EC 50/2 mm column x NUCLEODUR® PFAS EC 50/2 mm column ($\beta = 10.0$ ng/mL in methanol). The chromatogram shows the effectiveness of the NUCLEODUR® PFAS Delay column by impeding the instrument PFOA contamination from the sample by 0.5 minutes (RT 4.02 min PFOA from sample, RT 4.55 min PFOA from LC system).

PFAS Analysis According to ISO 21675:2019 and to DIN 38407-42

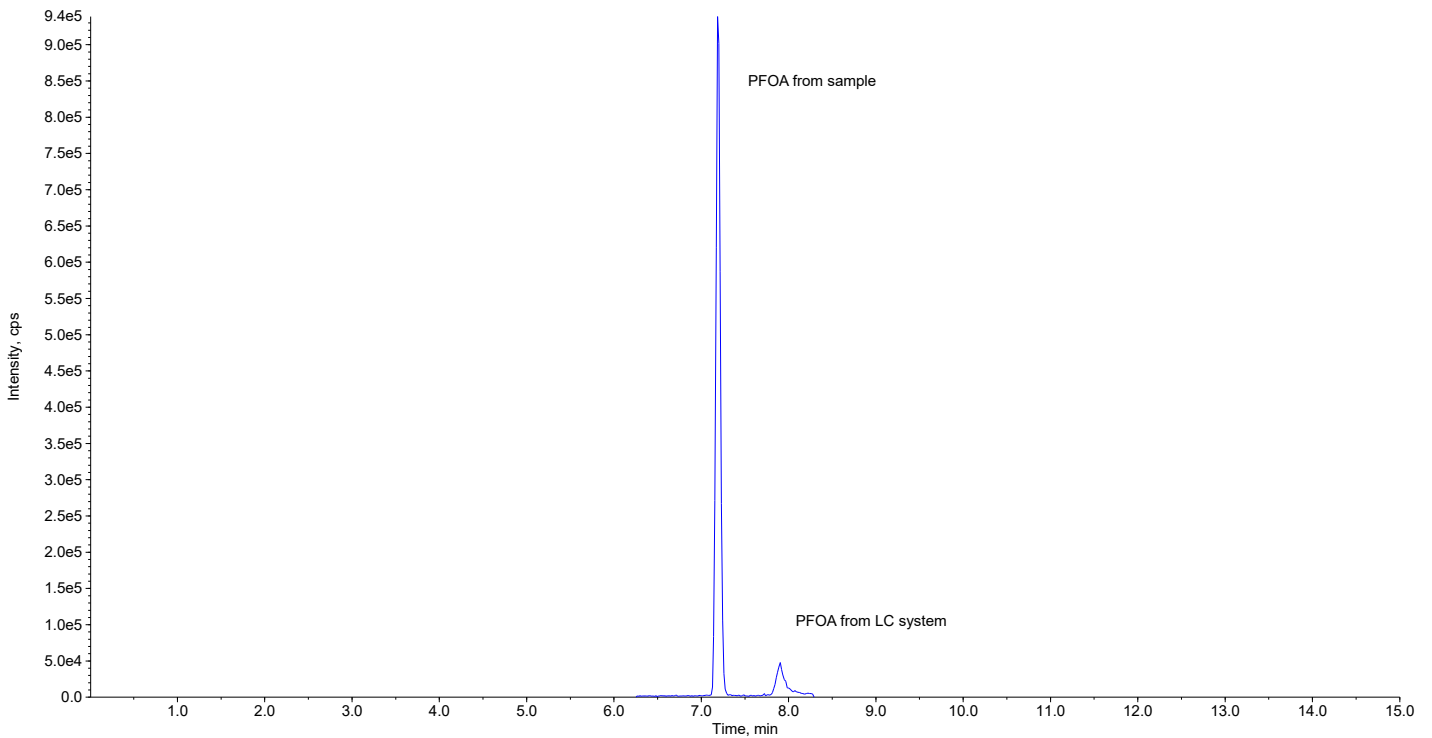


Figure 7: Chromatogram of PFOA resulted from the combination NUCLEODUR® PFAS Delay EC 50/2 mm column x NUCLEODUR® PFAS EC 100/2 mm column ($\beta = 10.0$ ng/mL in methanol). The chromatogram shows the effectiveness of the NUCLEODUR® PFAS delay column by impeding the instrument PFOA contamination from the sample by 0.7 minutes (RT 7.21 min PFOA from sample, RT 7.91 min PFOA from LC system).

Excursus: The way of PFAS from manufacturing over waste and consumer products to environment and human contamination

PFAS are emitted into the environment by different pathways. For example, exhaust air from industrial sources can contain PFAS and thus are dispersed into nearby ground and water bodies. Rain and snow, for example, can eventually carry them from the air into the soil and surface waters. Particle accumulation can even cause them to travel long distances through the air. PFAS are therefore also found far from industrial production sites and human living areas, such as in sediments from the Bering Sea to the Arctic [4, 5].

Through volatilization from products (evaporation from carpets or home textiles treated with soil-repellent agents) or from waterproofing sprays, indoor air can also be contaminated.

Soils can also be directly contaminated, for example by firefighting foams. With the uptake of PFAS from contaminated soils and waters in vegetation and their accumulation in fish, these substances enter the human food chain. Consequently, humans absorb PFAS from the environment through food, water or air.



PFAS Analysis According to ISO 21675:2019 and to DIN 38407-42

Batch-to-batch reproducibility

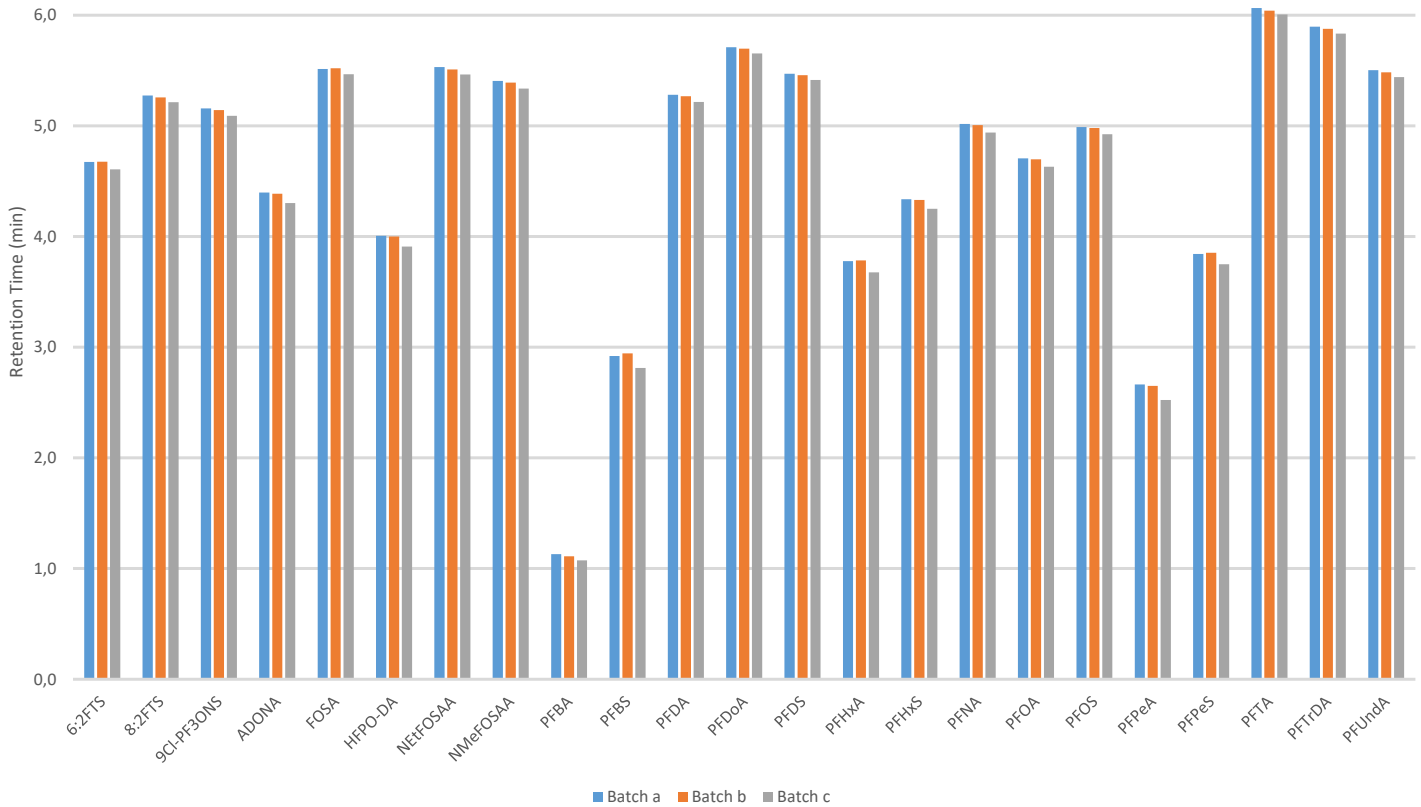


Figure 8: Comparison of retention times of PFAS on NUCLEODUR® PFAS EC 50 x 2 mm column using three batches (a-c).

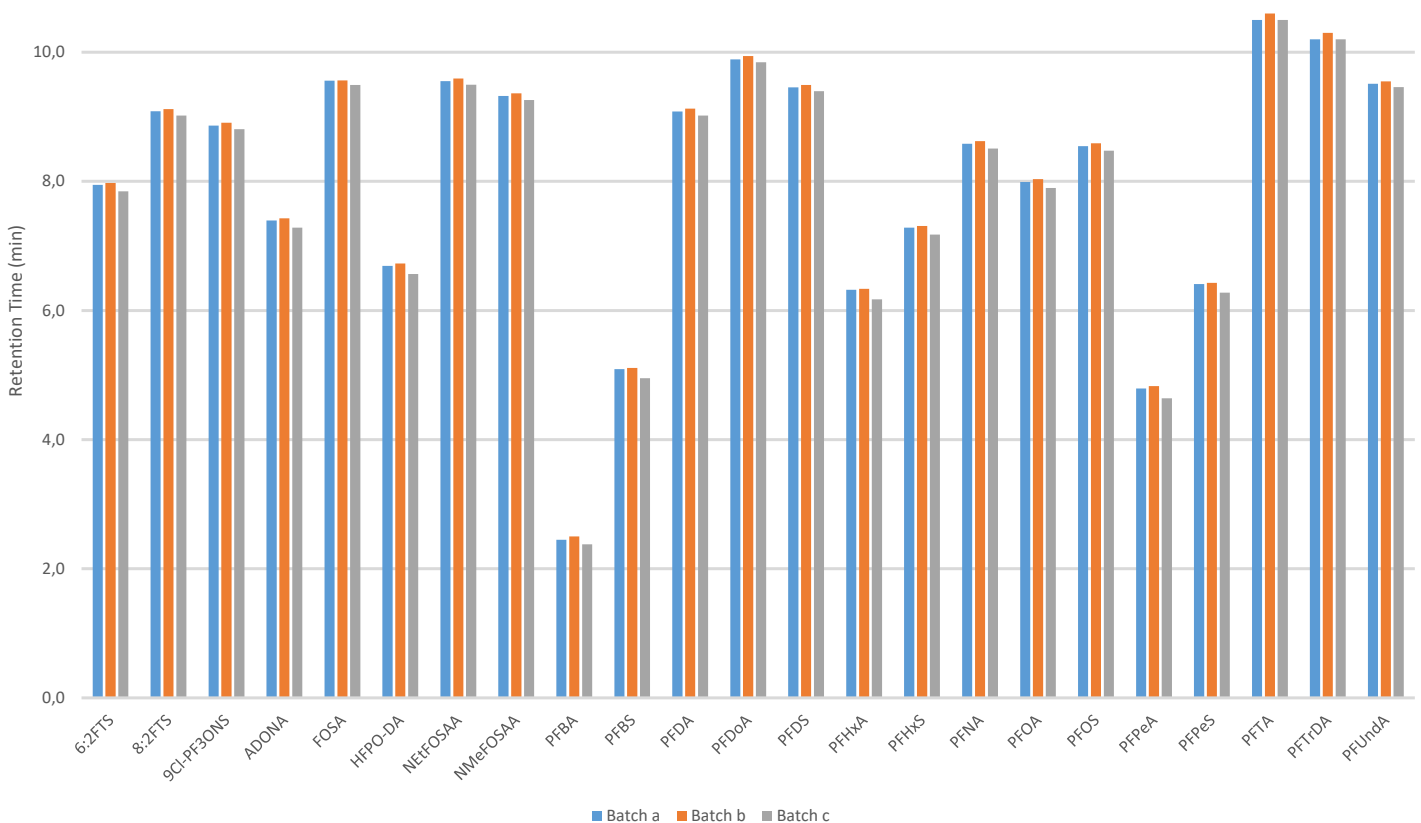


Figure 9: Comparison of retention times of PFAS on NUCLEODUR® PFAS EC 100 x 2 mm column using three batches (a-c).

PFAS Analysis According to ISO 21675:2019 and to DIN 38407-42

Conclusion

This application note shows the reliable and successful separation of per- and polyfluoroalkyl substances (PFAS) on optimized PFAS HPLC columns. Figures 2–5 show chromatograms of PFAS standard mixtures according to ISO 21675:2019 and to DIN 38407-42.

The recommended HPLC phase shows high retention for polar PFAS like PFBA and PBS and high MS intensity. Figure 8 and 9 present the excellent batch-to-batch reproducibility by comparing the retention times of several PFAS using three different batches. If the chromatographic issue is focused on the separation of linear and branched PFAS isomers NUCLEODUR® PFAS column should have a length of 100 mm. These facts point out that the NUCLEODUR® PFAS HPLC phase is especially well suited for the analysis of PFAS compounds.

The benefit using an isolator HPLC column for PFAS analysis is described in figure 6 and 7. The NUCLEODUR® PFAS delay column is able to impede the instrument contamination (especially PFOA) from the sample by 0.5 and 0.7 minutes respectively.

In summary, the presented application presents a quick and convenient method for chromatographic separation of PFAS analytes according to ISO 21675:2019 and to DIN 38407-42.

References

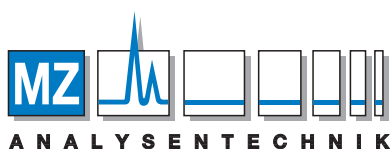
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- [4] Hanna Joeress, Zhiyong Xie, Charlotte C. Wagner, Wilken-Jon von Appen, Elsie M. Sunderland, and Ralf Ebinghaus: Transport of Legacy Perfluoroalkyl Substances and the Replacement Compound HFPO-DA through the Atlantic Gateway to the Arctic Ocean—Is the Arctic a Sink or a Source? Environ. Sci. Technol. 2020, Publication Date: July 29, 2020.
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Product information

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

REF 760673.20	EC 50/2 NUCLEODUR® PFAS Delay, 5 µm
REF 760666.20	EC 100/2 NUCLEODUR® PFAS, 3 µm
REF 760663.20	EC 50/2 NUCLEODUR® PFAS, 3 µm
REF 702402	Screw closure, N 9, PP, blue, center hole, silicone white/polyimide orange, 1.0 mm, fluorine-free
REF 702009	Screw neck vial, N 9, 11.6 x 32.0 mm, 0.3 mL, inner cone, PP transparent

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