MACHEREY-NAGEL
Chromatography application note

MN application note 05/2021

PFAS analysis in water matrices by simple sample preparation followed by liquid chromatography/tandem mass spectrometry (LC-MS/MS) analysis according to EPA 8327

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Abstract

This application note describes the analysis of selected per- and polyfluoroalkyl substances (PFAS) in water matrices by simple sample preparation, followed with liquid chromatography/tandem mass spectrometry (LC-MS/MS) analysis. Water samples are prepared by diluting 1:1 with methanol, and then acidifying with acetic acid (pH ~3–4), and analyzing on a NUCLEODUR® PFAS HPLC column by LC-MS/MS.

Introduction

Per- and polyfluoroalkyl substances (PFAS) are a group of highly stable synthetic organic compounds used in a wide variety of industrial and commercial applications 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 anthropogenic pollutants are characterized by a linear aliphatic backbone, a high degree of fluorination and often feature a carboxylic or sulfonic acid functionality.

They strongly bioaccumulate and have become ubiquitous throughout the global environment. In the global press, they are often referred to as "Forever Chemicals". There is also evidence that exposure to PFAS can lead to adverse human health effects.

To protect environment and human health, authorities in the US have published variety of laws and regulations [1]:

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

Therefore, authorities like the EPA (Environmental Protection Agency) have additionally published an action plan for identifying and for understanding PFAS, approaches to addressing current PFAS contamination, for preventing future contamination, and for effectively communicating with the public about PFAS [2]. Currently, US EPA is developing a method for the analysis of PFAS in environmental waters using LC-MS/MS [3].

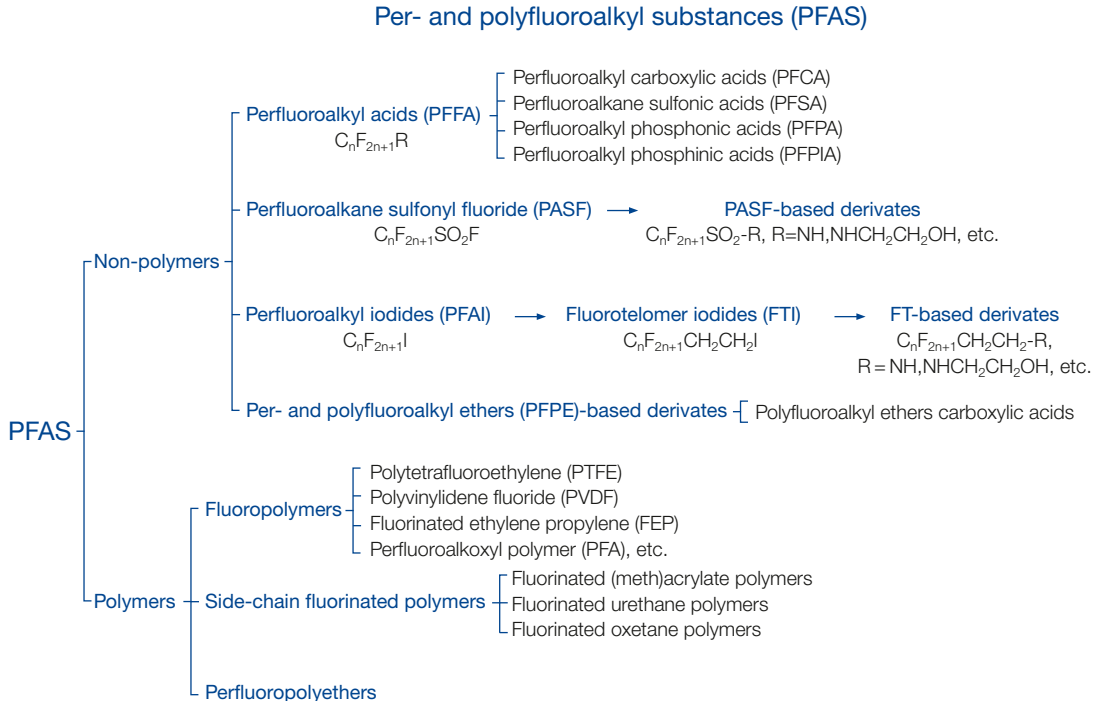

Figure 1: Classes of per- and polyfluoroalkyl substances (PFAS).

PFAS analysis in water matrices according to EPA 8327

Sample pretreatment for water samples according to Method 3512

Sample dilution

- Dilute sample 50:50 with MeOH and 0.1 % acetic acid, spiked with isotopically labeled surrogates
- Shake for 2 min
- Fill up sample solution in polypropylene vials with fluorine-free polyimide septum.

Analysis by HPLC-MS / MS

Chromatographic conditions

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:	in 4.5 min from 5.0 % B to 95 % B, hold 95 % B for 0.5 min, in 0.05 min to 5.0 % B, hold 5.0 % B for 1.45 min
Flow rate:	0.33 mL/min
Temperature:	40 °C
Injection volume:	20 µL

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	Classes of per- and polyfluoroalkyl substances	Abbreviation	CAS Number	Q_1 Mass [Da]	Q_3 Mass Quantifier [Da]	Q_3 Mass Qualifier [Da]	Retention time [min]
Perfluorobutanesulfonic acid	PFAS sulfonic acids	PFBS	375-73-5	298.9	98.9	79.8	3.98
Perfluoropentanesulfonic acid	PFAS sulfonic acids	PFPeS	2706-91-4	348.8	80.0	98.9	4.26
Perfluorohexanesulfonic acid	PFAS sulfonic acids	PFHxS	355-46-4	398.9	79.8	98.9	4.55
Perfluoroheptanesulfonic acid	PFAS sulfonic acids	PFHpS	375-92-8	448.9	79.8	98.9	4.77
Perfluorooctanesulfonic acid	PFAS sulfonic acids	PFOS	1763-23-1	498.8	79.9	98.9	4.97
Perfluorononanesulfonic acid	PFAS sulfonic acids	PFNS	68259-12-1	548.8	79.9	98.9	5.14
Perfluorodecanesulfonic acid	PFAS sulfonic acids	PFDS	335-77-3	598.8	79.9	98.9	5.28
1H,1H, 2H, 2H-Perfluorohexane sulfonic acid	PFAS sulfonic acids	4:2FTS	757124-72-4	326.9	306.9	80.8	4.20
1H,1H, 2H, 2H-Perfluorooctane sulfonic acid	PFAS sulfonic acids	6:2FTS	27619-97-2	426.9	406.9	80.8	4.77
1H,1H, 2H, 2H-Perfluorodecane sulfonic acid	PFAS sulfonic acids	8:2FTS	39108-34-4	526.8	506.8	80.8	5.17
Perfluorobutanoic acid	PFAS carboxylic acids	PFBA	375-22-4	212.9	168.8	N/A	3.08
Perfluoropentanoic acid	PFAS carboxylic acids	PFPeA	2706-90-3	262.9	219.0	N/A	3.79
Perfluorohexanoic acid	PFAS carboxylic acids	PFHxA	307-24-4	312.9	268.8	118.9	4.23
Perfluoroheptanoic acid	PFAS carboxylic acids	PFHpA	375-85-9	362.9	318.8	168.9	4.55
Perfluorooctanoic acid	PFAS carboxylic acids	PFOA	335-67-1	412.9	369.0	168.9	4.79
Perfluorononanoic acid	PFAS carboxylic acids	PFNA	375-95-1	462.9	418.9	219.0	4.99
Perfluorodecanoic acid	PFAS carboxylic acids	PFDA	335-76-2	512.8	468.9	268.8	5.16
Perfluoroundecanoic acid	PFAS carboxylic acids	PFUdA	2058-94-8	562.8	518.9	268.8	5.31
Perfluorododecanoic acid	PFAS carboxylic acids	PFDoA	307-55-1	612.8	568.9	168.9	5.44
Perfluorotridecanoic acid	PFAS carboxylic acids	PFTrDA	72629-94-8	662.8	618.9	168.9	5.55
Perfluorotetradecanoic acid	PFAS carboxylic acids	PFTeDA	376-06-7	712.8	668.8	168.9	5.65

PFAS analysis in water matrices according to EPA 8327

Analyte	Classes of per- and polyfluoroalkyl substances	Abbreviation	CAS Number	Q_1 Mass [Da]	Q_3 Mass Quantifier [Da]	Q_3 Mass Qualifier [Da]	Retention time [min]
N-ethylperfluoro-1-octanesulfonamidoacetic acid	PFAS sulfonamides and sulfonamidoacetic acids	*N*-EtFOSAA	2991-50-6	583.8	418.8	482.8	5.33
N-methylperfluoro-1-octanesulfonamidoacetic acid	PFAS sulfonamides and sulfonamidoacetic acids	*N*-MeFOSAA	2355-31-9	569.8	418.9	482.8	5.25
Perfluoro-1-octanesulfonamide	PFAS sulfonamides and sulfonamidoacetic acids	FOSA	754-91-6	497.9	77.8	N/A	5.32
Sodium perfluoro-1-[2,3,4–$^{13}C_3$]-butanesulfonate	PFAS sulfonic acids	M3PFBS	N/A	301.9	98.9	N/A	3.98
Sodium perfluoro-1-[1,2,3–$^{13}C_3$]-hexanesulfonate	PFAS sulfonic acids	M3PFHxS	N/A	401.9	79.9	N/A	4.55
Sodium perfluoro-1-[$^{13}C_8$]-octanesulfonate	PFAS sulfonic acids	M8PFOS	N/A	506.9	98.9	N/A	4.97
Sodium 1H,1H,2H,2H-perfluoro-1-[1,2–$^{13}C_2$]-hexane sulfonate (4:2)	PFAS sulfonic acids	M2–4:2FTS	N/A	329.0	81.0	N/A	4.20
Sodium 1H,1H,2H,2H-perfluoro-1-[1,2–$^{13}C_2$]-octane sulfonate (6:2)	PFAS sulfonic acids	M2–6:2FTS	N/A	428.9	81.0	N/A	4.77
Sodium 1H,1H,2H,2H-perfluoro-1-[1,2–$^{13}C_2$]-decane sulfonate (8:2)	PFAS sulfonic acids	M2–8:2FTS	N/A	528.9	80.9	N/A	5.17
Perfluoro-*n*-[$^{13}C_4$]butanoic acid	PFAS carboxylic acids	MPFBA	N/A	216.9	171.9	N/A	3.08
Perfluoro-*n*-[$^{13}C_5$]pentanoic acid	PFAS carboxylic acids	M5PFPeA	N/A	268.0	222.9	N/A	3.79
Perfluoro-*n*-[1,2,3,4,6–$^{13}C_5$]hexanoic acid	PFAS carboxylic acids	M5PFHxA	N/A	318.0	272.8	N/A	4.23
Perfluoro-*n*-[1,2,3,4–$^{13}C_4$]heptanoic acid	PFAS carboxylic acids	M4PFHpA	N/A	367.0	321.8	N/A	4.55
Perfluoro-*n*-[$^{13}C_8$]octanoic acid	PFAS carboxylic acids	M8PFOA	N/A	421.0	376.0	N/A	4.79
Perfluoro-*n*-[$^{13}C_9$]nonanoic acid	PFAS carboxylic acids	M9PFNA	N/A	471.9	427.0	N/A	4.99
Perfluoro-*n*-[1,2,3,4,5,6–$^{13}C_6$] decanoic acid	PFAS carboxylic acids	M6PFDA	N/A	518.9	474.0	N/A	5.16
Perfluoro-*n*-[1,2,3,4,5,6,7–$^{13}C_7$] undecanoic acid	PFAS carboxylic acids	M7PFUnA	N/A	569.9	525.0	N/A	5.31
Perfluoro-*n*-[1,2–$^{13}C_2$]dodecanoic acid	PFAS carboxylic acids	MPFDoA	N/A	614.9	569.9	N/A	5.44
Perfluoro-*n*-[1,2–$^{13}C_2$]tetradecanoic acid	PFAS carboxylic acids	M2PFTeDA	N/A	714.9	670.0	N/A	5.65
N-methyl-d_3 -perfluoro-1-octanesulfonamidoacetic acid	PFAS sulfonamides and sulfonamidoacetic acids	d_3-NMeFOSAA	N/A	572.9	419.0	N/A	5.25
N-ethyl-d_5 -perfluoro-1-octanesulfonamidoacetic acid	PFAS sulfonamides and sulfonamidoacetic acids	d_5-NEtFOSAA	N/A	588.8	418.8	N/A	5.33
Perfluoro-1-[$^{13}C_8$]octanesulfonamide	PFAS sulfonamides and sulfonamidoacetic acids	M8FOSA	N/A	506.0	77.9	N/A	5.32

Table 1: MRM transitions and retention times of PFAS analytes according to EPA 8327.

PFAS analysis in water matrices according to EPA 8327

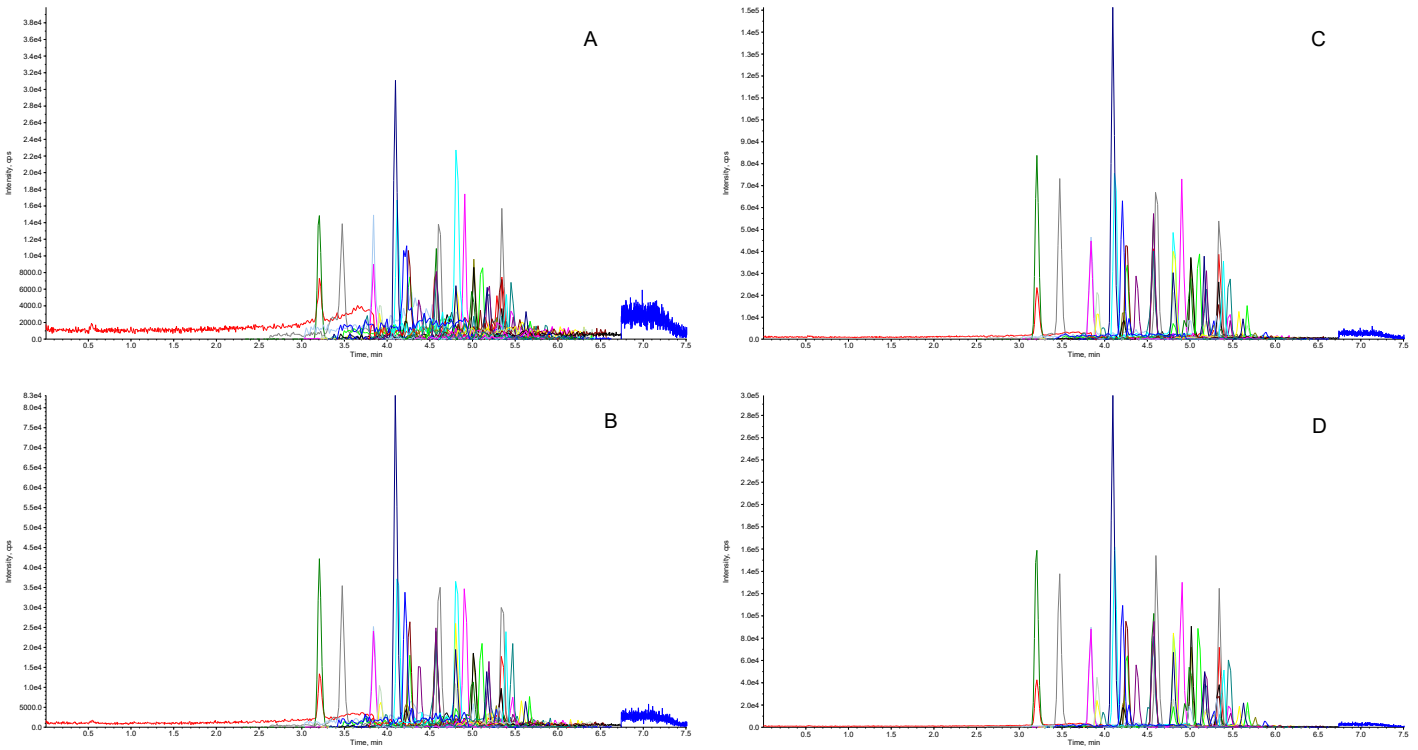

Figure 2: Chromatograms of PFAS spiked samples according to EPA 8327 on NUCLEODUR® PFAS EC 50 x 2 mm column (A: β = 10.0 ng/L for each compound, B: β = 25.0 ng/L for each compound, C: β = 50.0 ng/L for each compound, D: β = 100.0 ng/L for each compound).

Installation and advantages of a NUCLEODUR® PFAS Delay column

A big challenge in the analysis of PFAS in very low concentration ranges is the contamination of the analysis systems. PFAS contamination of HPLC equipment is well known. For handling these contaminations from, e.g. solvent bottle caps, PTFE tubing, filters, and consumables, usage of an isolator column is an option to reduce the background PFAS contamination from interfering with the PFAS of interest that are separated with the analytical column. Figure 4 shows the effectiveness of the NUCLEODUR® PFAS Delay column by impeding the instrument PFOA contamination from the sample by 0.3 minutes (RT 4.81 min PFOA from sample, RT 5.14 min PFOA from LC system).

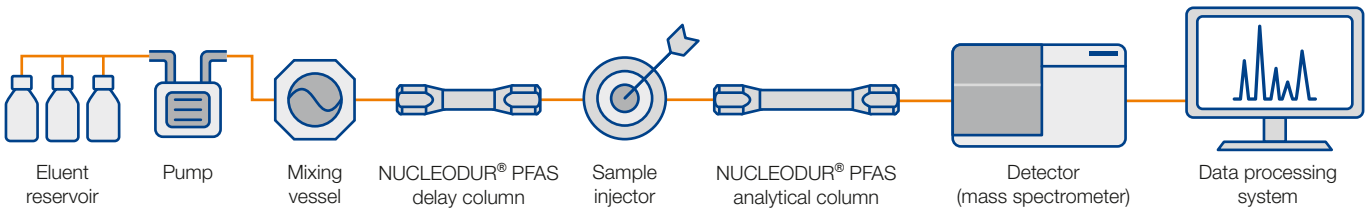

Figure 3: Installation and usage of NUCLEODUR® PFAS Delay column.

PFAS analysis in water matrices according to EPA 8327

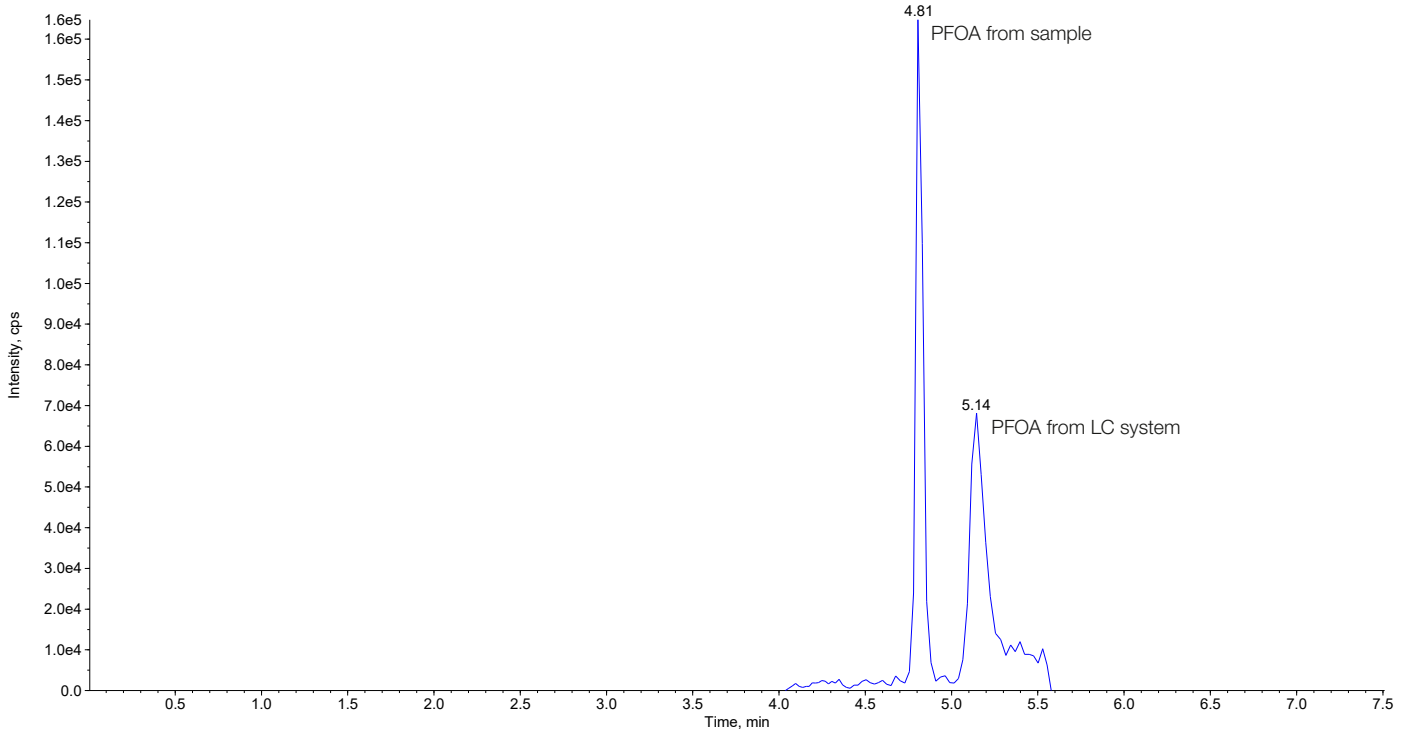

Figure 4: Impeding of PFOA instrument contamination with a NUCLEODUR® PFAS Delay column.

Calibration Ranges and LLOQ

The linearity of the presented method was determined with standard solutions of each PFAS including the surrogates at different concentration levels from 5, 10, 25, 50, 75, 100 to 200 ng/L. The limit of quantification was estimated by fulfilling a S/N value higher than 10.

Requirements:

- Calibration curve has $R^2 > 0.99$
- The calibration standards should include the analyte concentration level

Abbreviation	LLOQ [ng/L]	Calibration Ranges [ng/L]	R^2
PFBS	10	5–200	0.99783
PFPeS	10	5–200	0.99570
PFHxS	10	5–200	0.99845
PFHpS	10	5–200	0.99885
PFOS	10	5–200	0.99882
PFNS	10	5–200	0.99697
PFDS	10	5–200	0.99357
4:2FTS	10	5–200	0.99432
6:2FTS	10	5–200	0.99476
8:2FTS	10	5–200	0.99534
PFBA	10	5–200	0.99801
PFPeA	10	5–200	0.99874
PFHxA	10	5–200	0.99651
PFHpA	10	5–200	0.99750
PFOA	10	5–200	0.99880
PFNA	20	5–200	0.99549
PFDA	20	5–200	0.99909
PFUdA	20	5–200	0.99573
PFDoA	20	5–200	0.99641
PFTrDA	20	5–200	0.99109
PFTeDA	20	5–200	0.99606
N-EtFOSAA	10	5–200	0.99759
N-MeFOSAA	10	5–200	0.99694
FOSA	10	5–200	0.99874
MPFBA	10	5–200	0.99812
M3PFBS	10	5–200	0.99807
M5PFHxA	10	5–200	0.99775
M4PFHpA	10	5–200	0.99818
M3PFHxS	10	5–200	0.99560
M8PFOA	10	5–200	0.99964
M9PFNA	10	5–200	0.99709
M8PFOS	10	5–200	0.99911
M6PFDA	10	5–200	0.99742
M7PFUnA	10	5–200	0.99907
d_3-NMeFOSAA	10	5–200	0.99818
d_5-NEtFOSAA	10	5–200	0.99353
MPFDoA	10	5–200	0.99703
M2PFTA	10	5–200	0.99698
M2–4:2FTS	10	5–200	0.99422
M2–6:2FTS	10	5–200	0.99699
M2–8:2FTS	10	5–200	0.99574
M8FOSA	10	5–200	0.99708
M5PFPeA	10	5–200	0.99752

Table 2: Lower limit of quantification, calibration ranges and correlation for the presented method for drinking water.

PFAS analysis in water matrices according to EPA 8327

Recovery rates

The recovery rates of PFAS were achieved with sample solutions of each PFAS including the surrogates at different concentration levels from 10, 25, 50 to 100 ng/L.

Requirements:

- Mean recovery for presented spiking levels is within 70 to 130 %.
- RPDs from at least three replicates are ≤ 30 %.

Analyte abbreviation	Recovery sample (concentration 10 ng/L) in %	Standard deviation RPD [%]	Recovery sample (concentration 25 ng/L) in %	Standard deviation RPD [%]	Recovery sample (concentration 50 ng/L) in %	Standard deviation RPD [%]	Recovery sample (concentration 100 ng/L) in %	Standard deviation RPD [%]
PFBS	122.0	5.1	105.0	7.4	94.5	7.0	109.6	2.0
PFPes	93.0	17.0	117.9	9.6	87.3	0.3	97.7	7.7
PFHxS	112.2	13.6	118.4	10.8	90.8	6.2	100.5	2.9
PFHpS	122.8	2.7	111.9	9.8	87.1	7.8	95.5	5.4
PFOS	97.0	22.3	107.0	11.4	90.0	3.3	98.1	5.3
PFNS	78.0	15.6	78.7	13.4	88.1	6.8	85.6	5.4
PFDS	53.9	39.7	86.5	9.0	103.4	9.7	104.7	4.1
4:2FTS	91.3	12.7	118.3	4.6	94.2	3.1	98.6	4.3
6:2FTS	64.3	26.5	109.3	15.5	89.8	12.6	84.0	3.5
8:2FTS	N/A	N/A	113.8	8.0	75.0	15.5	77.5	7.5
PFBA	92.0	10.0	125.8	7.3	106.1	3.5	107.5	1.8
PFPeA	93.5	13.7	99.5	2.8	93.9	4.3	98.5	1.8
PFHxA	118.4	7.2	123.0	1.7	94.0	8.3	106.4	6.9
PFHpA	87.8	14.4	108.3	12.4	91.9	6.0	98.6	4.1
PFOA	82.5	7.3	115.5	10.1	88.4	11.0	92.7	4.7
PFNA	137.2	22.7	90.8	0.7	94.0	10.1	96.7	6.0
PFDA	94.1	17.5	87.1	3.7	87.6	8.8	85.5	6.4
PFUdA	N/A	N/A	121.0	5.5	80.8	6.1	114.6	4.5
PFDoA	N/A	N/A	91.1	22.1	83.7	2.9	106.1	10.2
PFTrDA	N/A	N/A	113.9	12.9	97.0	11.2	104.3	2.8
PFTeDA	N/A	N/A	104.0	17.7	79.5	20.0	89.1	9.8
N-EtFOSAA	N/A	N/A	106.0	18.1	79.4	2.7	109.4	4.5
N-MeFOSAA	N/A	N/A	112.8	3.2	72.4	10.0	109.7	7.7
FOSA	N/A	N/A	98.8	6.2	73.0	3.8	100.6	2.5
M3PFBS	95.3	12.9	116.4	3.3	83.7	7.9	94.7	2.2
M3PFHxS	101.1	9.4	106.5	13.3	90.6	7.1	105.4	6.1
M8PFOS	73.9	14.6	87.4	13.0	85.6	0.6	93.7	3.6
M2–4:2FTS	118.5	32.9	102.2	19.6	100.0	12.6	107.2	13.5
M2–6:2FTS	114.9	15.9	106.0	19.6	94.5	11.8	104.8	6.0
M2–8:2FTS	45.9	6.7	67.1	45.1	75.6	10.9	72.6	6.8
M4PFBA	100.4	1.2	122.5	1.2	96.9	1.4	104.2	0.9
M5PFPeA	97.9	6.5	112.1	2.9	89.1	4.6	97.9	1.9
M5PFHxA	95.0	0.7	116.8	5.5	86.4	5.3	97.2	1.6
M4PFHpA	103.2	21.9	114.3	5.6	87.7	3.9	99.6	4.4
M8PFOA	108.9	2.9	128.6	7.9	88.9	4.6	99.7	1.4
M9PFNA	71.2	21.5	100.7	4.0	83.8	15.3	97.3	2.2
M6PFDA	87.4	25.8	93.0	9.4	78.4	11.5	78.8	7.7
M7PFUnA	79.0	5.7	76.4	2.1	85.3	12.2	75.5	9.0
MPFDoA	67.3	14.4	72.9	15.1	83.0	6.1	81.2	4.2
M2PFTeDA	23.3	32.0	84.1	24.2	77.3	11.4	83.0	9.5
d_3-NMeFOSAA	73.6	12.7	86.1	6.3	76.2	15.5	83.6	11.4
d_5-NEtFOSAA	87.1	5.6	75.8	14.9	77.4	12.3	82.3	8.9
M8FOSA	50.2	18.6	83.7	2.5	78.0	4.0	80.8	4.0

Table 3: Recovery rates for the presented method for drinking water.

PFAS analysis in water matrices according to EPA 8327

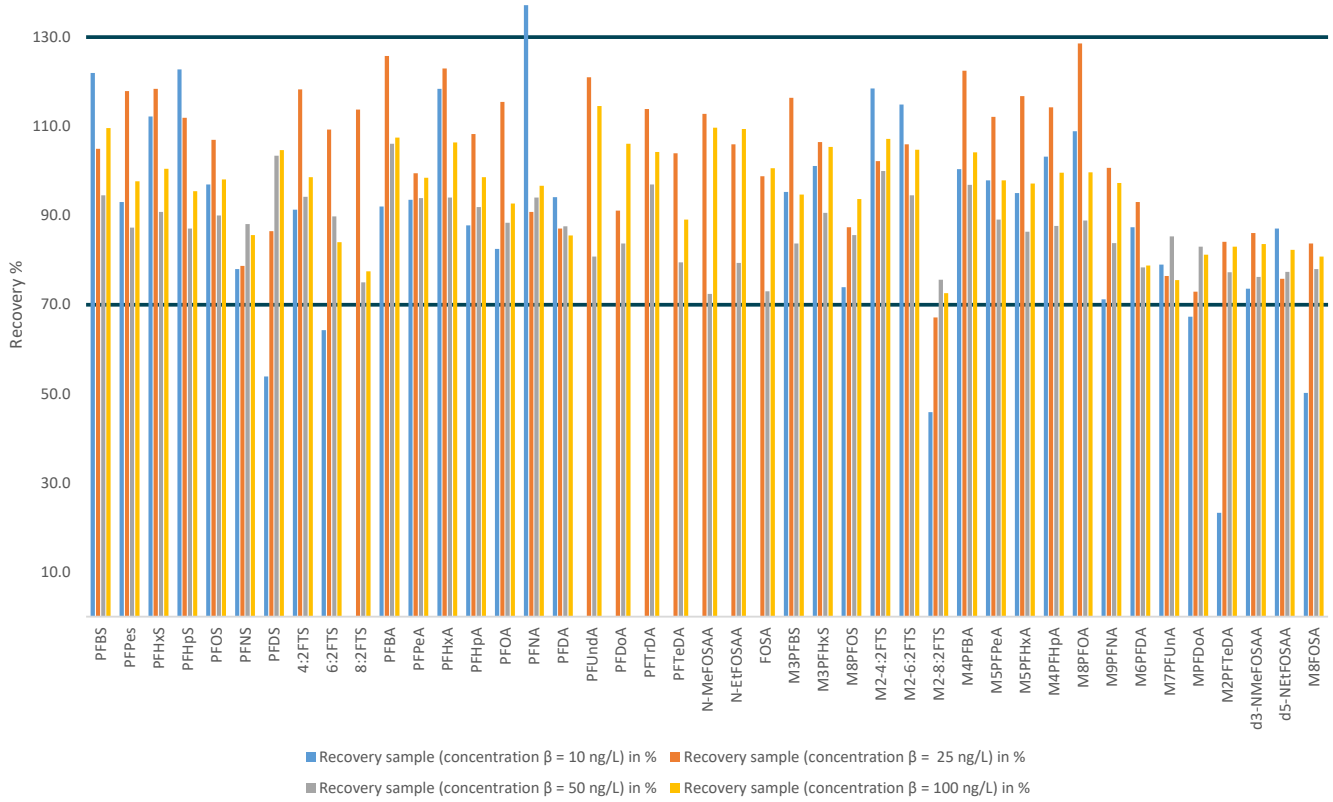

Figure 5: Recovery rates of PFAS according to EPA 8327 method for drinking water.

Conclusion

This application note presents the reliable and successful determination of per- and polyfluoroalkyl substances (PFAS) from water according to EPA method 8327. The use of direct aqueous injection eliminates the need for costly and time consuming sample preparation techniques for non-difficult sample matrices.

Most of the PFAS show recovery rates between 70 % and 130 %. This results were determined with sample solutions of spiked samples including native and isotope labeled analytes at different concentration levels from 10, 25, 50 to 100 ng/L. To improve results, a large injection volume, more than 20 µL, could be applied.

The chromatographic results were achieved by using NUCLEODUR® PFAS HPLC column. This phase is specially suitable for the analysis of PFAS compounds. It shows high retention for polar PFAS, good peak shapes, high MS intensity and excellent batch-to-batch reproducibility. PFC background contamination 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 HPLC products allow a reliable and successful determination of per- and polyfluoroalkyl substances (PFAS) from water according to EPA method 8327.

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 823R$_1$8004, February 2019, www.epa.gov/pfas.

[3] Method 8327: PER- AND POLYFLUOROALKYL SUBSTANCES (PFAS) USING EXTERNAL STANDARD CALIBRATION AND MULTIPLE REACTION MONITORING (MRM) LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY (LC-MS/MS), Revision 0, June 2019.

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 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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