Determination of PFAS from food according to FDA Method C-010.02

MACHEREY-NAGEL application department · Dr. H. R. Wollseifen, T. Kretschmer, L. Emmerich

Application benefits

- Successful determination of PFAS from food according to FDA Method C-010.01
- High recovery rates and an effective dSPE clean-up were achieved with a special QuEChERS mix
- Fast and sensitive HPLC analysis on a NUCLEODUR® PFAS column

MN products

REF 730223
CHROMABOND® polypropylene (PP) centrifuge tubes, 50 mL, empty

REF 7300008
CHROMABOND® QuEChERS Mix L, Clean-up, 15 mL Centrifuge tubes

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,c. hole,Sili. w./Polyimide orange,1.0mm,fluorine-free

REF 702009
Screw neck vial, N 9, 11.6x32.0 mm, 0.3 mL, inner cone, PP tr.

MN application numbers

SPE: 306840
HPLC: 129000

Keywords

PFAS, food, milk, bread, vegetables, LC-MS/MS, FDA Method C-010.02

Introduction

Per- and polyfluoroalkyl substances (PFAS) are a large group of man-made chemicals and are used in a variety of industries around the world (e. g. textiles, household products, fire-fighting, automotive, food processing, construction, electronics). These bioaccumulative pollutants are characterized by a linear aliphatic backbone, a high degree of fluorination and often feature a carboxylic or sulfonic acid functionality. The exposure to PFAS may lead to adverse health effects. People can be exposed to PFAS in different ways, including food. These substances are most often found in drinking water, fish, fruit, eggs, and egg products.

The FDA classified PFAS as food contact substances because of their potential to migrate into food and therefore regulates them as food additives [1]. In September 2020, the European Food Safety Authority (EFSA) published a report about the risk to human health related to the presence of perfluoroalkyl substances in food [2]. The report includes a tolerable weekly intake (TWI) of 4.4 nanograms (ng) per kilogram (kg) of bodyweight per week as the sum of four PFAS

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

To protect human health, the exposure of the levels of PFAS along the food chain must be investigated more intensively. Therefore, there is need for more sensitive analytical methods for PFAS in food of animal and plant-based origin. This work presents the analysis of PFAS from food according to FDA Method C-010.02 [3]. It shows high recoveries using a modified QuEChERS extraction technique from food samples. The extracts are finally analyzed by HPLC-MS/MS on a NUCLEODUR® PFAS column.

Sample pretreatment

MN Appl. No. 306840

Extraction

1. Weigh amount of sample and LC/MS grade water based on table 1 and commodity type into an empty 50 mL centrifuge tube (REF 730223)
2. Add 0.1 mL of internal standard solution (0.1 µg/mL each compound in methanol) and 0.1 mL of native standard solution (0.1 µg/mL each compound in methanol) for determining recovery rate
3. Shake the mixture for 1 min
4. Add 9.8 mL acetonitrile and 150 µL formic acid
5. Shake the mixture for 1 min
6. Add CHROMABOND QuEChERS Mix XII (REF 730648)
7. Shake the mixture for 1 min
8. Centrifuge the mixture for 5 min at 4500 rpm at 5 °C

Clean-up

1. Transfer 6 mL supernatant to a 15 mL centrifuge tube, which is prefilled with CHROMABOND® QuEChERS Mix L (REF 7300008)
2. Shake for 1 minute
3. Centrifuge again for 5 min at 4500 rpm at 5 °C
4. Supernatant is ready to be analyzed by LC-MS/MS

Sample matrix	Amount of sample used (g)	Water added (mL)	CH_3CN added (mL)
Milk	5	5	10
Curd cheese	1	5	10
Bread	5	15	10
Brussel sprouts	5	5	10
Spinach	5	5	10
Egg	5	5	10

Table 1: Sample preparation conditions based on food commodity type

Analysis by HPLC-MS / MS

MN Appl. No. 129000

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	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

Large portfolio of QuEChERS mixes

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Find more information here:
https://www.mn-net.com/quechers

MRM transitions

Abbreviation	Analyte	CASRN	Retention Time (min)	Q1 Mass (Da)	Q3 Mass (Da)
PFBA	Perfluorobutanoic acid	375-22-4	2.01	212.904	168.8
PFPeA	Perfluoropentanoic acid	2706-90-3	3.90	262.880	219.0
HFPO-DA	2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy) propanoic acid (GenX)	13252-13-6	5.77	284.991	168.7
FBSA	Perfluorobutanesulfonamide	30334-69-1	4.50	298.050	77.9
L-PFBS	Perfluorobutanesulfonic acid	375-73-5	4.20	298.933	98.9
PFHxA	Perfluorohexanoic acid	307-24-4	5.40	312.911	268.8
4:2 FTS	Sodium 1H,1H,2H,2H-perfluorohexanesulfonate	757124-72-4	5.27	326.940	306.9
L-PFPes	Perfluoropentanesulfonic acid	2706-91-4	5.54	348.845	80.0
PFHpA	Perfluoroheptanoic acid	375-85-9	6.45	362.931	318.8
NaDONA	Sodium dodecafluoro-3H-4,8-dioxanonanoate	919005-14-4	6.58	376.901	250.7
FHxSA	Perfluorohexanesulfonamide	41997-13-1	7.35	397.998	77.9
PFHxSK	Perfluorohexanesulfonic acid	355-46-4	6.49	398.942	79.8
PFOA	Perfluorooctanoic acid	335-67-1	7.26	412.910	369.0
6:2 FTS	Sodium 1H,1H,2H,2H-perfluorooctanesulfonate	27619-97-2	7.24	426.927	406.9
PFHpS	Perfluoroheptanesulfonic acid	375-92-8	7.26	448.929	79.8
PFNA	Perfluorononanoic acid	375-95-1	7.92	462.893	418.9
FOSA	Perfluorooctanesulfonamide	754-91-6	8.84	497.870	77.8
PFOSK	Perfluorooctanesulfonic acid	1763-23-1	7.89	498.836	79.9
PFDA	Perfluorodecanoic acid	335-76-2	8.49	512.841	468.9
8:2 FTS	Sodium 1H,1H,2H,2H-perfluorodecanesulfonate	39108-34-4	8.50	526.821	506.8
9Cl-PF3ONS	Potassium 9-chlorohexadecafluoro-3-oxanonane-1-sulfonate	756426-58-1	8.25	530.752	350.7
L-PFNS	Sodium perfluorononanesulfonate	98789-57-2	8.45	548.808	79.9
PFUdA	Perfluoroundecanoic acid	2058-94-8	8.95	562.801	518.9
N-MeFOSAA	*N*-methylperfluorooctanesulfonamidoacetic acid	2355-31-9	8.78	569.801	418.9
N-EtFOSAA	*N*-ethylperfluorooctanesulfonamidoacetic acid	2991-50-6	9.02	583.809	418.8
PFDS	Sodium perfluorodecanesulfonate	2806-15-7	8.90	598.790	79.9
PFDoA	Perfluorododecanoic acid	307-55-1	9.33	612.787	568.9
11Cl-PL3OUdS	11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	763051-92-9	9.15	630.738	451.0
PFTrDA	Perfluorotridecanoic acid	72629-94-8	9.66	662.767	618.9
PFTeDA	Perfluorotetradecanoic acid	376-06-7	9.94	712.774	668.8
M4PFBA	Perfluoro-($^{13}C_4$)butanoic acid		2.01	216.935	171.9
M5PFPeA	Perfluoro-($^{13}C_5$)pentanoic acid		3.92	267.970	222.9
M3PFBS	Sodium perfluoro-(2,3,4–$^{13}C_3$)butanesulfonate		4.22	301.887	98.9
M5PFHxA	Perfluoro-(1,2,3,4,6–$^{13}C_5$)hexanoic acid		5.40	317.998	272.8
M2–4:2FTS	Sodium 1H,1H,2H,2H-perfluoro(1,2–$^{13}C_2$) hexanesulfonate		5.26	328.971	81.0
M4PFHpA	Perfluoro-(1,2,3,4–$^{13}C_4$)heptanoic acid		6.45	366.953	321.8
M3PFHxS	Sodium perfluoro-(1,2,3–$^{13}C_3$)hexanesulfonate		6.50	401.901	79.9
M8PFOA	Perfluoro-($^{13}C_8$)octanoic acid		7.27	420.952	376.0
M2–6:2FTS	Sodium 1H,1H,2H,2H-perfluoro(1,2–$^{13}C_2$) octanesulfonate		7.23	428.938	81.0
M9PFNA	Perfluoro-($^{13}C_9$)nonanoic acid		7.92	471.943	427.0
M8FOSA	Perfluoro-($^{13}C_8$)octanesulfonamide		8.84	505.978	77.9
M8PFOS	Sodium perfluoro-($^{13}C_8$)octanesulfonate		7.89	506.907	98.9
M6PFDA	Perfluoro-(1,2,3,4,5,6–$^{13}C_6$)decanoic acid		8.49	518.923	474.0

Abbreviation	Analyte	CASRN	Retention Time (min)	Q1 Mass (Da)	Q3 Mass (Da)
M2–8:2FTS	Sodium 1H,1H,2H,2H-perfluoro(1,2–$^{13}C_2$) decanesulfonate		8.50	528.935	80.9
M7PFUdA	Perfluoro-(1,2,3,4,5,6,7–$^{13}C_7$)undecanoic acid		8.95	569.945	525.0
d3-*N*-MeFOSAA	*N*-methyl-d_3 -perfluorooctanesulfonamidoacetic acid		8.78	572.891	419.0
d5-*N*-EtFOSAA	*N*-ethyl-d_5 -perfluorooctanesulfonamidoacetic acid		9.02	588.845	418.8
MPFDoA	Perfluoro-(1,2–$^{13}C_2$)dodecanoic acid		9.33	614.947	569.9
M2PFTeDA	Perfluoro-(1,2–$^{13}C_2$)tetradecanoic acid		9.94	714.936	670.0

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

Chromatogramms of sample extracts

Figure 1: a

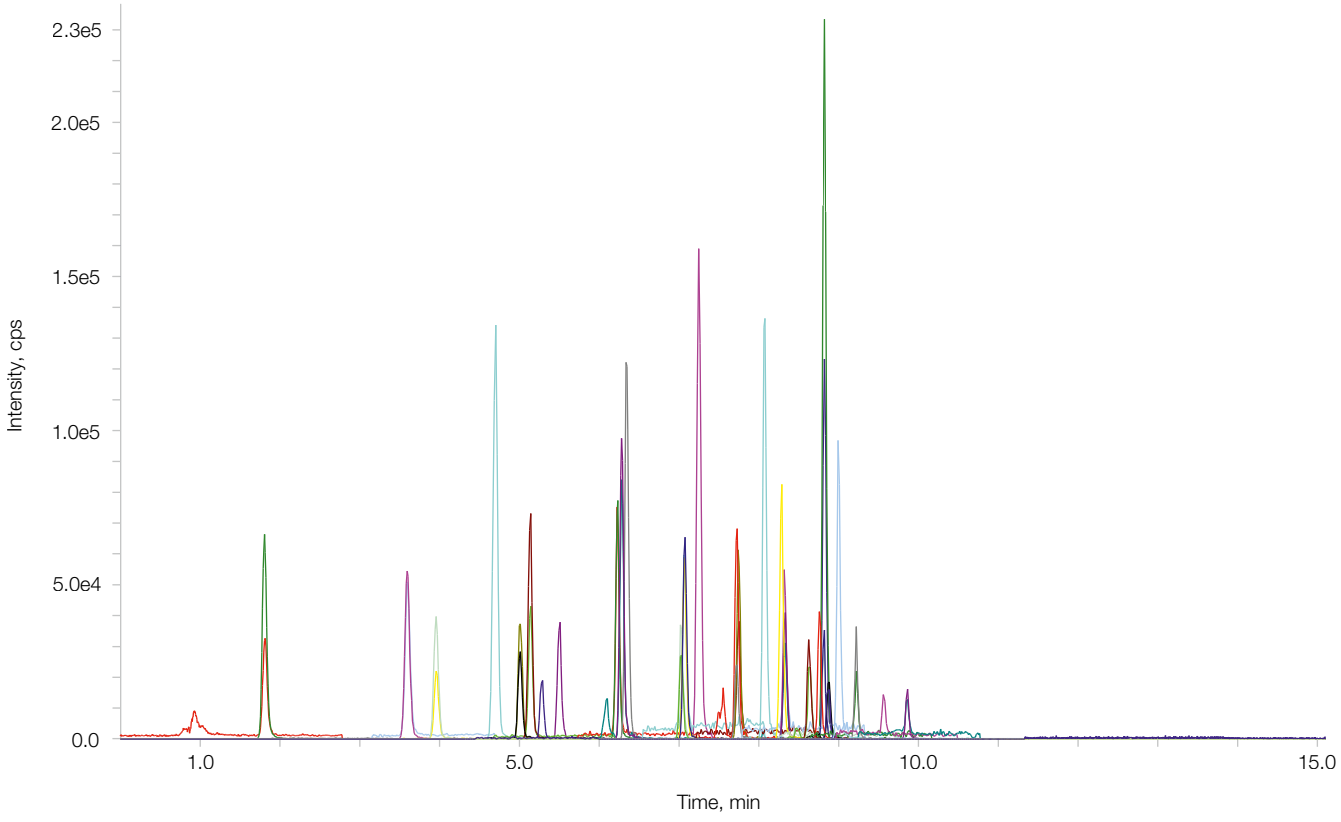

Figure 1: b

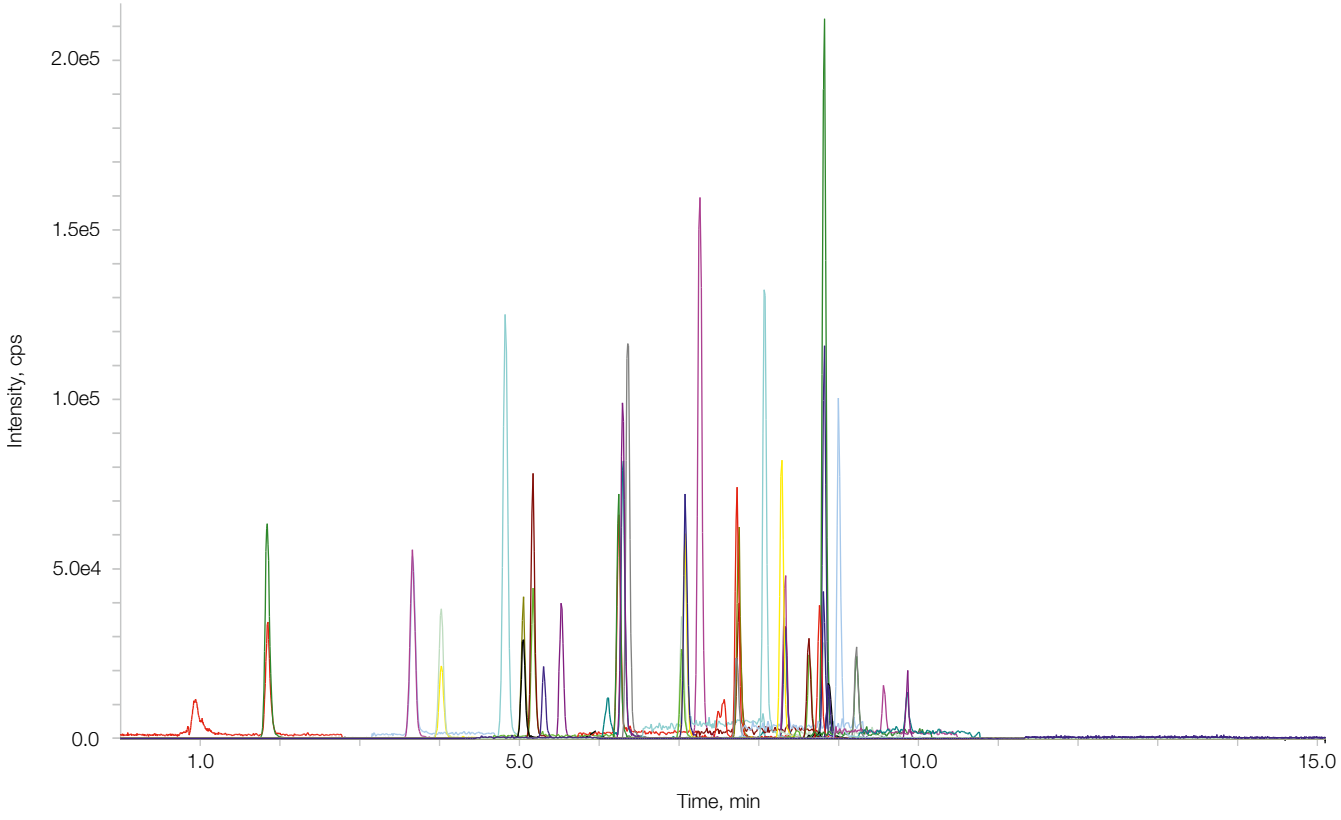

Figure 1: c

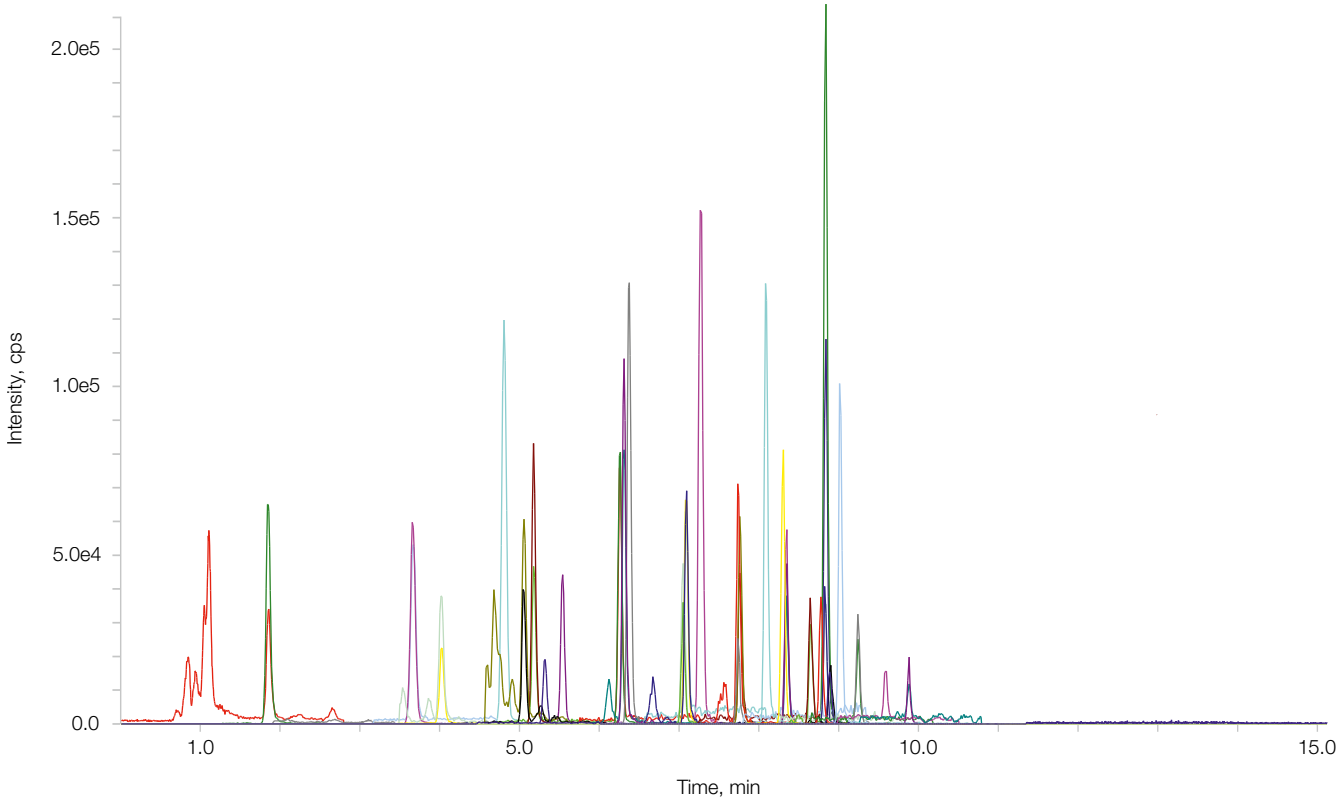

Figure 1: d

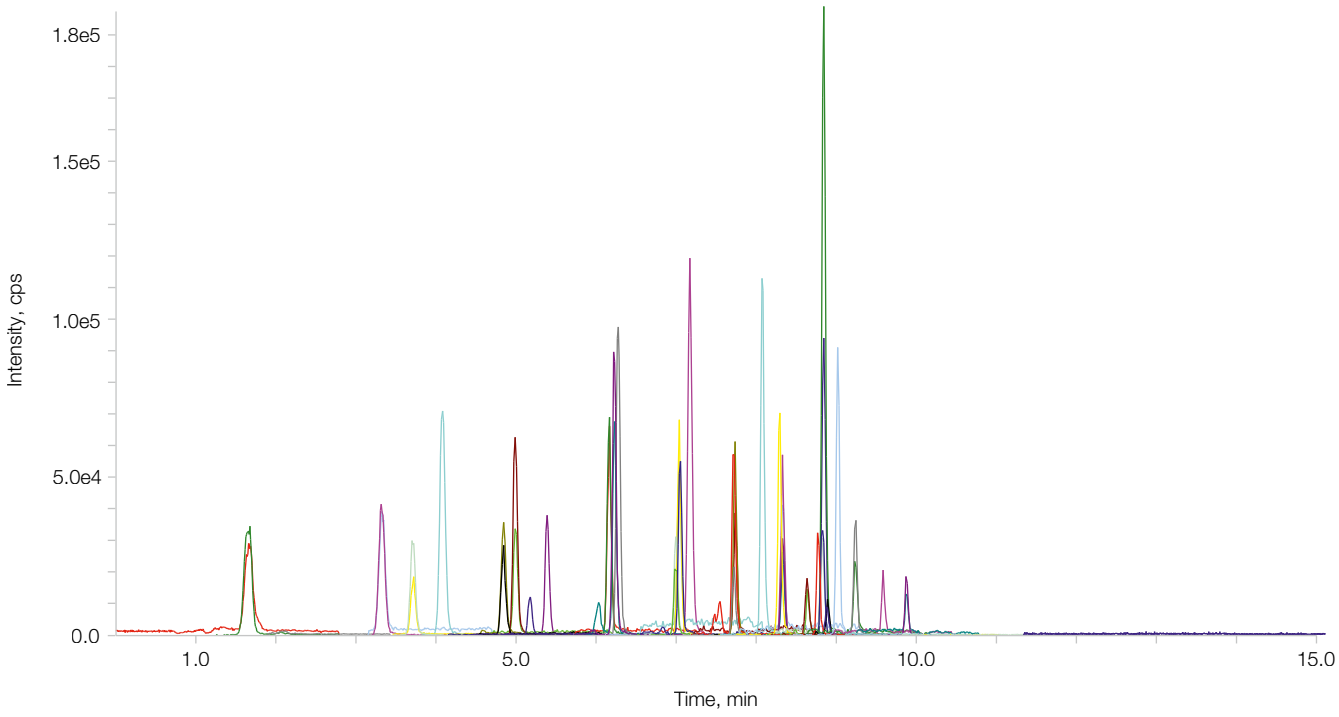

Figure 1: e

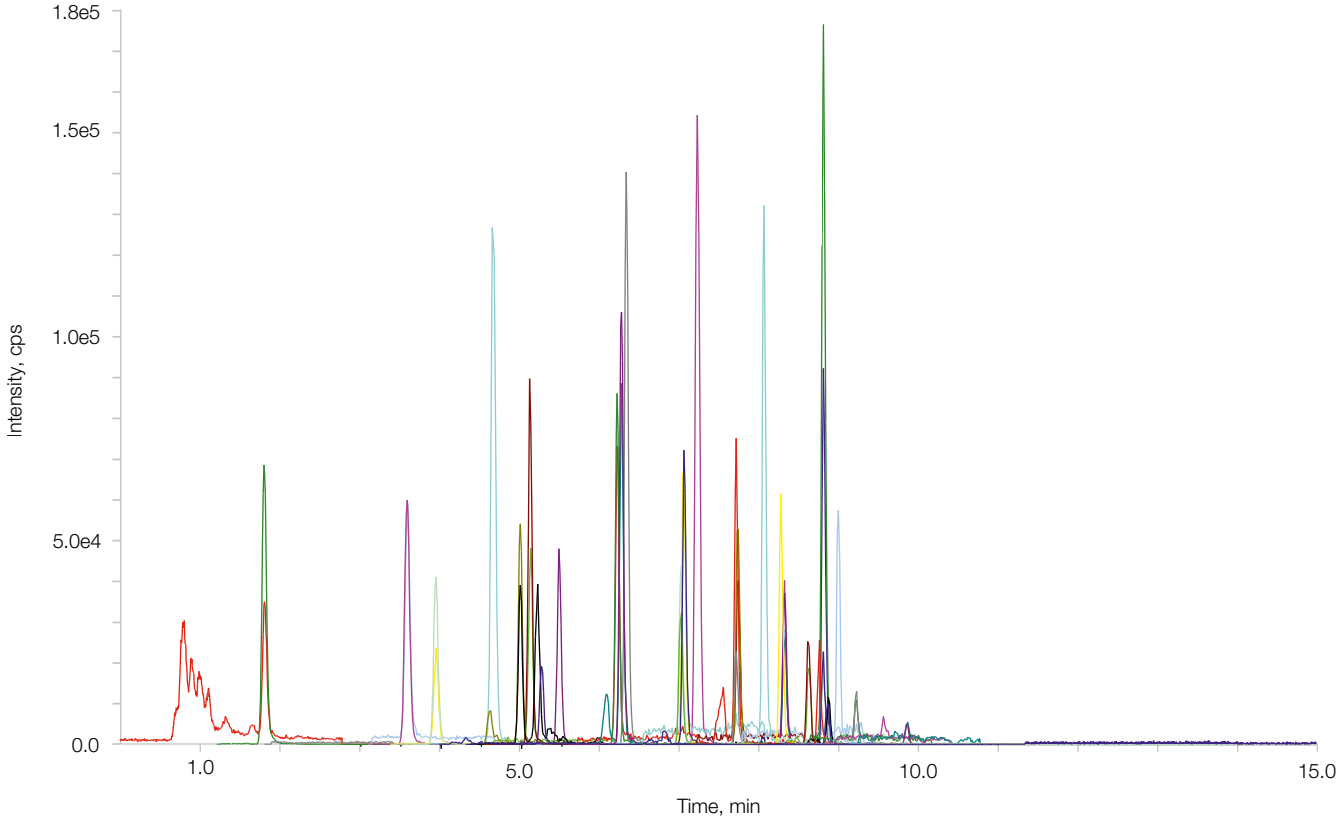

Figure 1: f

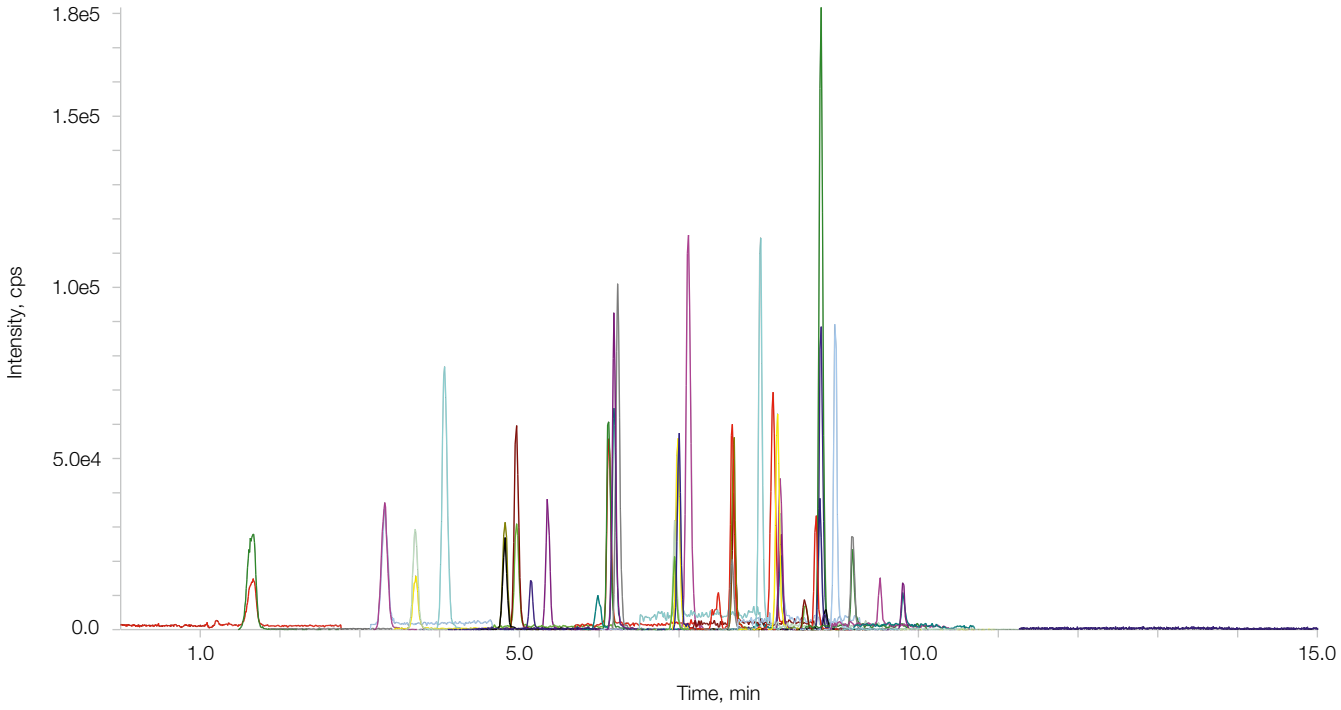

Figure 1: Chromatogram of samples: a = milk, b= curd cheese, c = brussels sprouts, d = spinach, e = bread, f = egg.

Recovery rates

Abbreviation	Recovery rate from milk [%], [n=5]	Recovery rate from curd cheese [%], [n=5]	Recovery rate from brussels sprouts [%], [n=5]	Recovery rate from bread [%], [n=5]	Recovery rate from spinach [%], [n=5]	Recovery rate from egg [%], [n=5]
PFBA	86.2 ± 2.2	88.0 ± 2.5	84.6 ± 2.5	85.1 ± 2.2	66.5 ± 1.7	77.6 ± 3.1
PFPeA	88.5 ± 2.4	91.2 ± 3.6	93.1 ± 3.6	98.1 ± 1.0	83.2 ± 2.5	77.2 ± 2.6
PFHxA	90.0 ± 2.8	89.7 ± 2.9	94.2 ± 2.9	97.0 ± 2.4	83.2 ± 1.9	77.2 ± 3.7
PFHpA	88.9 ± 3.1	88.3 ± 3.4	97.9 ± 3.4	98.7 ± 3.1	87.9 ± 2.7	80.8 ± 2.8
PFOA	84.7 ± 5.8	88.2 ± 2.3	100.0 ± 2.3	94.8 ± 1.7	84.7 ± 5.1	83.6 ± 5.2
PFNA	81.2 ± 4.2	89.5 ± 9.2	97.0 ± 9.2	85.0 ± 2.7	84.7 ± 4.9	75.9 ± 3.4
PFDA	88.9 ± 9.1	86.8 ± 8.8	100.1 ± 8.8	73.6 ± 7.5	87.7 ± 3.7	83.8 ± 6.1
L-PFBS	91.6 ± 2.0	94.5 ± 4.2	92.7 ± 4.2	96.6 ± 2.0	91.1 ± 2.5	94.6 ± 2.3
L-PFPes	93.7 ± 1.7	95.5 ± 1.5	91.3 ± 1.5	98.5 ± 0.9	85.8 ± 5.4	94.4 ± 2.1
PFHxSK	92.6 ± 1.5	93.6 ± 1.5	94.4 ± 1.5	98.2 ± 1.7	92.2 ± 1.4	95.8 ± 0.9
PFHpS	94.9 ± 1.3	95.9 ± 1.2	92.4 ± 1.2	95.2 ± 2.8	83.6 ± 3.0	92.8 ± 1.0
PFOSK	91.6 ± 1.9	94.9 ± 2.8	93.7 ± 2.8	87.9 ± 1.7	89.7 ± 3.2	91.0 ± 3.6
9Cl-PF3ONS	95.0 ± 2.2	97.0 ± 1.4	94.7 ± 1.4	91.5 ± 2.7	94.6 ± 1.8	98.3 ± 0.8
11Cl-PL3OUdS	93.6 ± 1.9	96.5 ± 2.9	94.8 ± 2.9	56.9 ± 3.3	92.7 ± 2.2	94.9 ± 2.2
HFPO-DA	86.4 ± 4.7	89.8 ± 3.4	92.5 ± 3.4	102.0 ± 2.2	161.7 ± 2.2	155.9 ± 1.3
NaDONA	89.7 ± 1.9	90.6 ± 0.9	94.9 ± 0.9	102.1 ± 1.2	85.0 ± 1.3	82.6 ± 1.7
M4PFBA	85.2 ± 1.9	83.2 ± 1.5	84.4 ± 1.5	86.7 ± 0.7	84.3 ± 1.7	75.1 ± 1.8
M5PFPeA	87.1 ± 2.8	85.2 ± 1.6	90.8 ± 1.6	94.2 ± 0.7	84.8 ± 0.7	78.2 ± 3.1
M5PFHxA	89.4 ± 2.3	85.6 ± 1.8	94.1 ± .18	98.1 ± 1.7	88.6 ± 2.2	81.7 ± 4.8
M4PFHpA	87.1 ± 3.8	84.6 ± 3.0	95.9 ± 3.0	99.1 ± 4.0	91.2 ± 3.1	83.9 ± 1.6
M8PFOA	81.2 ± 3.7	80.4 ± 3.4	92.4 ± 3.4	92.3 ± 1.5	89.8 ± 4.3	84.5 ± 3.0
M9PFNA	85.6 ± 4.7	84.3 ± 7.7	94.3 ± 7.7	86.2 ± 6.6	91.9 ± 3.9	81.9 ± 6.2

Abbreviation	Recovery rate from milk [%], [n=5]	Recovery rate from curd cheese [%], [n=5]	Recovery rate from brussels sprouts [%], [n=5]	Recovery rate from bread [%], [n=5]	Recovery rate from spinach [%], [n=5]	Recovery rate from egg [%], [n=5]
M6PFDA	88.2 ± 4.7	89.4 ± 4.2	103.7 ± 4.2	75.0 ± 6.7	97.9 ± 3.0	85.0 ± 3.6
M3PFBS	94.2 ± 2.8	93.0 ± 1.8	89.5 ± 1.8	98.0 ± 1.4	95.4 ± 2.5	96.4 ± 1.8
M3PFHxS	91.5 ± 3.0	90.8 ± 2.7	91.0 ± 2.7	97.1 ± 0.9	94.8 ± 0.9	95.0 ± 1.7
M8PFOS	88.3 ± 3.5	85.3 ± 1.7	85.3 ± 1.7	81.0 ± 1.9	91.4 ± 4.0	92.9 ± 2.9
PFUdA	87.8 ± 9.9	93.6 ± 7.5	104.9 ± 7.5	53.2 ± 4,6	79.5 ± 6.7	74.2 ± 6.5
PFDoA	75.5 ± 12.9	77.7 ± 9.6	83.7 ± 9.6	34.6 ± 14.7	85.9 ± 10.0	79.5 ± 10.8
PFTrDA	86.5 ± 7.7	99.1 ± 9.9	103.6 ± 9.9	31.8 ± 8.2	86.8 ± 8.7	72.8 ± 13.7
PFTeDA	71.7 ± 5.7	81.5 ± 3.5	75.4 ± 3.5	22.9 ± 5.7	81.7 ± 6.0	62.6 ± 10.0
L-PFNS	94.5 ± 1.7	96.9 ± 1.0	93.1 ± 1.0	68.6 ± 4.6	95.3 ± 4.1	98.4 ± 4.6
PFDS	88.0 ± 3.3	92.6 ± 2.0	87.2 ± 2.0	51.3 ± 4.0	85.7 ± 3.5	87.9 ± 3.9
4:2 FTS	71.7 ± 3.4	71.5 ± 2.8	92.9 ± 2.8	88.4 ± 3.1	65.9 ± 5.4	65.5 ± 3.2
6:2 FTS	79.8 ± 3.9	78.1 ± 1.2	95.3 ± 1.2	97.1 ± 3.7	75.7 ± 2.9	70.5 ± 2.0
8:2 FTS	77.0 ± 3.7	76.4 ± 3.6	88.5 ± 3.6	75.0 ± 4.6	75.2 ± 3.3	71.5 ± 4.9
N-MeFOSAA	93.4 ± 2.7	96.0 ± 2.9	104.3 ± 2.9	74.9 ± 4.7	49.0 ± 8.6	33.7 ± 4.5
N-EtFOSAA	89.0 ± 4.5	89.7 ± 3.8	92.6 ± 3.8	53.7 ± 1.9	48.8 ± 4.5	33.5 ± 8.2
FBSA	99.6 ± 2.3	100.9 ± 1.3	98.2 ± 1.3	102.8 ± 2.1	88.0 ± 1.7	91.6 ± 1.6
FHxSA	96.7 ± 2.1	97.9 ± 1.1	96.1 ± 1.1	98.2 ± 1.1	94.3 ± 1.2	99.0 ± 1.2
FOSA	96.0 ± 2.0	97.1 ± 3.0	95.2 ± 3.0	75.4 ± 1.6	91.3 ± 5.3	93.7 ± 4.3
M7PFUdA	88.0 ± 9.4	83.9 ± 11.5	106.6 ± 11.5	56.0 ± 5.8	90.1 ± 2.8	84.6 ± 3.9
MPFDoA	85.8 ± 4.8	81.7 ± 4.2	93.7 ± 4.2	38.6 ± 10.2	92.3 ± 7.7	80.8 ± 6.8
M2PFTeDA	87.2 ± 7.9	91.4 ± 3.8	93.2 ± 3.8	27.4 ± 7.8	85.2 ± 3.4	70.3 ± 8.8
M2–4:2FTS	71.7 ± 4.5	68.7 ± 3.3	93.7 ± 3.3	90.8 ± 3.8	68.3 ± 2.8	64.2 ± 4.1
M2–6:2FTS	75.9 ± 5.5	75.1 ± 2.0	93.5 ± 2.0	94.2 ± 1.1	78.0 ± 5.5	72.6 ± 3.7
M2–8:2FTS	79.1 ± 6.2	75.8 ± 3.6	88.1 ± 3.6	75.5 ± 6.5	81.3 ± 4.7	74.7 ± 2.4
d_3-*N*-MeFOSAA	93.4 ± 2.1	88.0 ± 3.0	100.5 ± 3.0	73.2 ± 5.2	54.3 ± 8.3	33.2 ± 6.9
d_5-*N*-EtFOSAA	86.0 ± 5.7	85.4 ± 7.2	91.5 ± 7.2	52.8 ± 4.7	49.0 ± 7.8	36.6 ± 10.3
M8FOSA	95.1 ± 0.5	92.3 ± 1.4	93.8 ± 1.4	73.9 ± 1.9	93.5 ± 2.3	92.9 ± 1.6

Table 3: Recovery rates for the presented dSPE clean-up using CHROMABOND® QuEChERS Mix L.

Matrix Reduction

Figure 2:

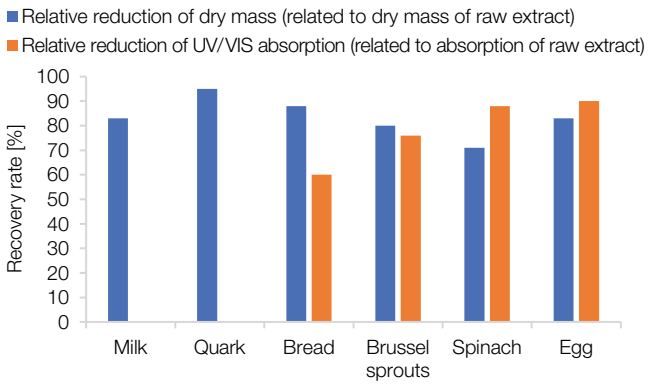

Figure 2: The relative matrix reduction (UV/VIS) was measured with the *NANOCOLOR*® Advance Spectrophotometer at a wavelength of 340–800 nm.

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Conclusion

This application note presents the reliable and successful determination of 30 PFAS from several matrices of animal and plant-based origin according to regulative recommendations of FDA Method C-010.02. By using CHROMABOND® QuEChERS Mix L, it was possible to achieve high recovery rates for PFAS from six food matrices with good reproducibility.

The used QuEChERS clean-up mix shows effective matrix reduction. The high amount of Primary Secondary Amine removes organic and fatty acids, sugars and anthocyanin pigments. Figure 2 shows that the amounts of chlorophyll and sterols are effectively reduced in matrices like spinach by Graphitized Carbon Black.

This work shows an accurate and robust method for a range of 30 PFAS compounds of varying chemistry from different food samples. The identification and the quantification of PFAS in food were finally carried out by ESI mass spectrometry on a NUCLEODUR® PFAS column.

References

[1] Inventory of Food Contact Substances Listed in 21 CFR

[2] EFSA Journal 2020;18(9):6223 — Risk to human health related to the presence of perfluoroalkyl substances in food

[3] FDA Method C-010.02 - Determination of 16 Perfluoroalkyl and Polyfluoroalkyl Substances (PFAS) in Food using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)

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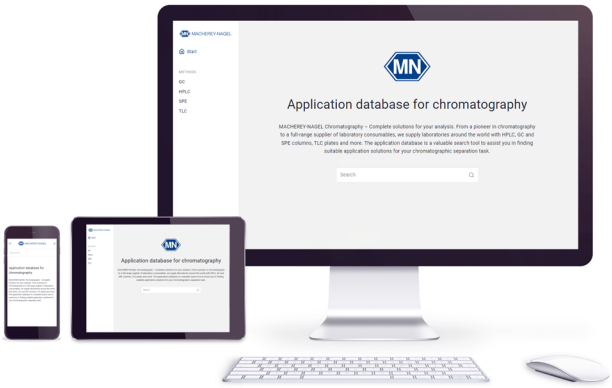

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