Supelco®
Analytical Products

Application Note

LC-MS/MS Analysis of 16 PFAS in Milk using QuEChERS based on FDA method C-010.02

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Introduction

Per- and polyfluorinated alkyl substances (PFAS) are a class of compounds that have been widely used in commercial product applications over the past decades due to their versatile physical and chemical properties (e.g., water repellent, firefighting foams, cookware, food packaging). Owing to their chemical stability, these compounds are also widely present in our environment and have the potential to bioaccumulate in humans over time. Regulatory agencies such as the EPA and FDA have introduced limit values for certain substances and the development of analytical methods to avoid possible human health risks (such as low infant birth weights, cancer, and effects on the immune system).[1-4]

The U.S. Food and Drug Administration (FDA) has issued a methodology (C-010.02) for PFAS extraction from food samples applying a modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) extraction technique and further clean-up step using dispersive solid phase extraction (dSPE), followed by LC-MS/MS analysis.[4]

For FDA method C-010.02, an extraction salt mixture containing 6.0 g $MgSO_4$ as well as 1.5 g NaCl, and a dSPE clean-up mix containing 900 mg $MgSO_4$, 300 mg PSA and 150 mg graphitized carbon, are specified. The Supel™ QuE non-buffered extraction salt mix and the specifically designed Supel™ QuE PSA/ENVI-Carb™ Tube 3 for clean-up have been used to meet the method requirements.

This application note describes the analysis of 16 PFAS compounds in milk and was performed in accordance with FDA method C-010.02.

Experimental

Solutions and Standards Preparation

Native and isotopically labeled PFAS standards were used as methanolic 50 µg/mL stock solutions. These standards were then diluted following the dilution scheme of the method C-010.02 to obtain calibration standards in the required concentrations (external calibration: 0.01, 0.05, 0.10, 0.50, 1.0, 5.0, 10, and 25 ng/mL).

Sample Preparation

Evaluation of Background Contamination

In accordance with U.S. FDA method C-010.02, analysis was performed for water and milk samples. UHPLC-MS grade water was used to test PFAS background contamination and found to be free of the 16 analytes covered by the FDA method. The water samples (5 mL) were fortified with the isotopically labeled internal standards and were further mixed with 5 mL water, 150 µL formic acid, and 10 mL acetonitrile. After addition of the Supel™ QuE extraction salt package (**55295-U**), the mixture was placed on a shaker (1500 rpm for 10 minutes) and the PFAS analytes were extracted from the water phase into the organic phase. For further clean-up of complex samples like food matrices, dSPE is required. The organic layer was therefore transferred into a second tube, containing Supel™ QuE PSA/ENVI-Carb™ (**55479-U**) and shaken for 10 minutes at 1500 rpm. After centrifugation (4000 x g for 10 minutes), the sample was filtered (Millex® filters, **SLGNX13**) and used for LC-MS/MS analysis.

MERCK

Method Performance Assessment

Following the background assessment of the method using the Supel™ QuE materials, method performance was investigated using milk as an exemplary sample matrix for quantitation of PFAS in processed foods. For that purpose, 5 mL of UHT, reduced-fat (1.5%) milk were spiked at 0.5 or 2.0 ng/mL with 16 native PFAS and 8 isotopically labeled surrogate standards. The samples were analyzed using the same methodology for the presence of PFAS analytes. Extraction and purification were performed as described in FDA method C-010.02.

LC-MS/MS analysis

An Agilent 1290 Infinity II instrument coupled to an Agilent 6495C triple quadrupole mass spectrometer was used for the LC-MS/MS analysis. Analyte separation was achieved using Ascentis® Express PFAS 90 Å (15 cm x 2.1 mm, 2.7 µm, **53560-U**) as analytical column. In addition, a delay column (Ascentis® Express 90 Å PFAS Delay Column, 5 cm x 3.0 mm, 2.7 µm, **53572-U**), was installed after the mixing valve and before the autosampler to offset potential PFAS contamination potentially originating from the LC system (e.g., pump, tubings, fittings, filters). Polypropylene snap cap vials were used instead of standard glass vials to avoid possible PFAS adherence to the glass surface. The LC conditions used are shown in **Table 1**.

Table 1. LC Conditions used for analysis of 16 PFAS compounds

LC Conditions	
Instrument:	Agilent 1290 Infinity II instrument coupled to an Agilent 6495C triple quadrupole mass spectrometer
Columns:	Ascentis® Express 90 Å PFAS HPLC Column, 2.7 µm, 15 cm x 2.1 mm (**53560-U**) Delay column: Ascentis® Express 90 Å PFAS Delay Column, 2.7 µm, 5 cm x 3.0 mm (**53572-U**)
Mobile phase:	[A] 5 mM Ammonium acetate*; [B] methanol
Gradient:	**Time (min) / A (%) / B (%)** Initial 90.0 10.0 3.0 90.0 10.0 3.1 60.0 40.0 26.0 10.0 90.0 26.1 90.0 10.0 28.0 90.0 10.0
Flow rate:	0.30 mL/min
Pressure:	320 bar
Column temp.:	40 °C
Detector:	MS/MS, ESI (-), MRM (see Table 2 for details)
Injection:	10 µL
Sample(s):	See text

* mobile phase A was modified compared to FDA method C-010.02 and used without addition of 1-methyl piperidine

Table 2. MRM, chromatographic and linearity (R^2) data for 16 PFAS analytes

Peak	Acronym	Compound	MRM	Collision Energy (eV)	RT (min)	R^2
1	PFBA	Perfluorobutanoic acid	213.0->169.0	4	6.3	0.9965
2	PFPeA	Perfluoropentanoic acid	263.0->219.0	4	8.8	0.9967
3	PFBS	Perfluorobutanesulfonic acid	298.9->80.0	40	9.3	0.9975
4	PFHxA	Perfluorohexanoic acid	313.0->269.0	4	11.7	0.9964
5	PFPeS	Perfluoropentanesulfonic acid	348.9->99.0	37	12.1	0.9951
6	HFPO-DA	Hexafluoropropylene oxide dimer acid	285.0->169.0	4	12.5	0.9965
7	PFHpA	Perfluoroheptanoic acid	363.0->319.0	4	14.4	0.9960
8	PFHxS	Perfluorohexanesulfonic acid	398.9->99.0	41	14.6	0.9949
9	NaDONA	Sodium dodecafluoro-3H-4,8-dioxanonanoate	377.0->251.0	8	14.7	0.9956
10	PFOA	Perfluorooctanoic acid	413.0->369.0	8	16.7	0.9964
11	PFHpS	Perfluoroheptanesulfonic acid	448.9->99.0	45	16.8	0.9961
12	PFNA	Perfluorononanoic acid	463.0->419.0	8	18.7	0.9974
13	PFOS	Perfluorooctanesulfonic acid	498.9->80.0	76	18.7	0.9976
14	9Cl-PF3ONS	9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	530.9->351.0	28	19.7	0.9961
15	PFDA	Perfluorodecanoic acid	513.0->469.0	8	20.4	0.9961
16	11Cl-PF3OUdS	11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	630.9->451.0	32	22.4	0.9953

Results and Discussion

A chromatogram of a solvent calibration standard containing the 16 native compounds is shown in **Figure 1**. All 16 compounds demonstrated a lower limit of quantitation (LLOQ) of 0.01 ng/mL for the HPLC method and an LLOQ of 0.02 ng/mL in the context of the milk sample. Linear calibration curves (0.01-25 ng/mL) with R^2 ≥0.99 were obtained for all PFAS analytes (**Table 2**).

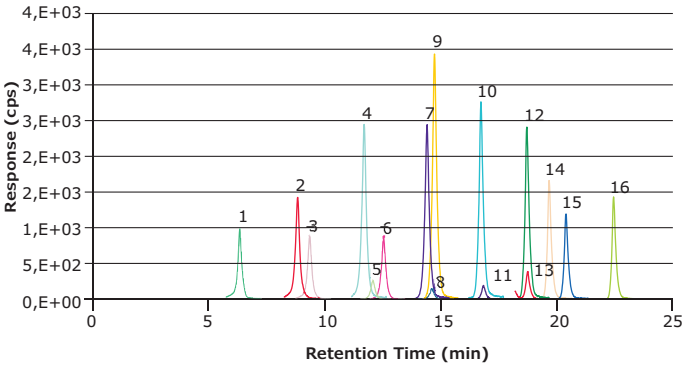

Figure 1. 16 PFAS compounds at 1 ng/mL in methanol (Peak IDs see **Table 2**)

The background evaluation of the FDA method C-010.02 using the recommended salt package and dSPE material showed negligible background levels for all the studied PFAS compounds (**Table 3**), as shown by values below the respective lower limits of quantification (LLOQ) of the LC-MS/MS method of 0.01 ng/mL (0.02 ng/mL in relation to the milk sample). Furthermore, an upfront screening of PFAS compounds in the UHPLC-MS solvents revealed concentrations below 0.01 ng/mL.

Table 3. Results of method background testing for the evaluation (LLOQ of 0.01 ng/mL)

Analyte	Method Background using UHPLC-MS water as sample
PFBA	Below LLOQ
PFPeA	Below LLOQ
PFBS	Below LLOQ
PFHxA	Below LLOQ
PFPeS	Below LLOQ
HFPO-DA	Below LLOQ
PFHpA	Below LLOQ
PFHxS	Below LLOQ
NaDONA	Below LLOQ
PFOA	Below LLOQ
PFHpS	Below LLOQ
PFNA	Below LLOQ
PFOS	Below LLOQ
9Cl-PF3ONS	Below LLOQ
PFDA	Below LLOQ
11Cl-PF3OUdS	Below LLOQ

The acceptable recovery range for the investigated PFAS analytes based on the FDA guidelines for the validation of chemical methods is 40-120% (including RSD ≤ 22%) for concentrations at lower levels (i.e. 1 ng/mL). **Table 4** displays the recoveries and %RSD from the experimental study where 16 compounds were spiked in quintuplicate in milk samples. All recoveries and %RSD met the requirements of the FDA method and were thus in the recommended range.

Table 4. Precision and recovery (n = 5) of PFAS in milk samples at 2 fortification/spike levels (0.5 ng/mL and 2.0 ng/mL)

Analyte	Fortified conc. (ng/mL)	Mean recovery (%)	% RSD	Fortified conc. (ng/mL)	Mean recovery (%)	% RSD
PFBA	0.5	94	9.3	2.0	86	4.7
PFPeA		91	5.0		84	3.7
PFBS		86	4.1		84	3.2
PFHxA		85	2.3		84	3.2
PFPeS		80	7.1		84	3.7
HFPO-DA		87	8.2		86	1.9
PFHpA		94	10.9		96	4.3
PFHxS		88	5.1		83	3.0
NaDONA		81	2.6		82	3.3
PFOA		101	3.6		89	2.3
PFHpS		89	5.8		81	5.7
PFNA		100	4.5		90	3.0
PFOS		81	1.8		83	2.7
9Cl-PF3ONS		95	4.8		84	2.9
PFDA		95	5.0		82	4.7
11Cl-PF3OUdS		95	6.1		85	2.8

Conclusions

In this application note, the workflow for FDA method C-010.02 to analyze 16 PFAS in processed food using the QuEChERS method was investigated for milk samples. The background values of all used consumables and the LC-MS system resulted in levels below the LLOQs given in the method, thus ensuring an appropriate analysis of low levels of PFAS analytes. At both 0.5 ng/mL and 2.0 ng/mL fortified concentration levels, recoveries for all 16 compounds were well within the FDA method acceptable range of 40-120%. The calculated %RSDs were less than 11%, indicating satisfactory precision. Hence, the Supel™ QuE PSA/ENVI-Carb™ clean-up mix 3, Supel™ QuE extraction salt mix (non-buffered), Ascentis® Express PFAS columns, and Millex® syringe filters proved to be suitable tools for this PFAS analysis in milk samples.

Featured & Related Products

Description	Cat. No.
Sample Preparation	
Supel™ QuE PSA/ENVI-Carb™ Tube 3, volume 15 mL, Pk. 50	**55479-U**
Supel™ QuE Non-Buffered Tube 2, pk. 50	**55295-U**
Brand® PP graduated centrifuge tube, screw cap volume 50 mL, without base, non-sterile, Pk. 300	**BR114820**
Millex® Syringe Filter, Nylon, Non-sterile, 0.20 µm pore size, 13 mm diameter	**SLGNX13**
HPLC Analysis	
Ascentis® Express 90 Å PFAS HPLC Column, 2.7 µm, 15 cm x 2.1 mm	**53560-U**
Ascentis® Express 90 Å PFAS Delay Column, 2.7 µm, 5 cm x 3.0 mm	**53572-U**
Solvents & Reagents	
Water for UHPLC-MS LiChrosolv®	**1.03728**
Methanol hypergrade for LC-MS LiChrosolv®	**1.06035**
Acetonitrile hypergrade for LC-MS LiChrosolv®	**1.00029**
Ammonium acetate LiChropur™, eluent additive for LC-MS	**73594**
Formic acid for analysis EMSURE® ACS,Reag. Ph Eur	**1.00264**
Standardization and Calibration	
Perfluorobutanoic acid, analytical standard, 25 mg	**68808**
Perfluoropentanoic acid, analytical standard, 25 mg	**68542**
Perfluorohexanoic acid, analytical standard, 25 mg	**43809**
Perfluoroheptanoic acid, analytical standard, 25 mg	**43996**
Perfluorooctanoic acid, analytical standard, 100 mg	**33824**
Perfluorodecanoic acid, analytical standard, 25 mg	**43929**
Pentadecafluorooctanoic acid, 100 µg/mL in methanol, analytical standard, 1 mL	**33603**
Heptadecafluorooctanesulfonic acid, 100 µg/mL in methanol, analytical standard, 1 mL	**33607**

References

1. 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, Washington, DC, 2020. **https://cfpub.epa.gov/si/si_public_record_Report.cfm?dirEntryId=343042&Lab=NERL**
2. 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. United States Environmental Protection Agency, Office of Water, December 2019. **https://www.epa.gov/dwanalyticalmethods/method-533-determination-and-polyfluoroalkyl-substances-drinking-water-isotope**
3. Draft Method 1633 Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/ MS. United States Environmental Protection Agency, Office of Water, August 2021. **https://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=P101345B.txt**
4. Method C-010.02 Determination of 16 Per and Polyfluoroalkyl Substances (PFAS) in Processed Food using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS). United States Food and Drug Administration, December 2021. **https://www.fda.gov/media/131510/download**.

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