RESTEK Pure Chromatography

The Analysis of PFAS in Milk by LC-MS/MS

Tina Brandscher[1)], Jamie York[2)], 1) Restek GmbH, Bad Homburg, DE 2) Restek Corporation, Bellefonte, PA, USA

Abstract & Introduction

Per- and polyfluoroalkyl substances (PFAS) are a class of manufactured organic compounds that are used for a wide array of applications and products. The environmental prevalence and bioaccumulation of these compounds can lead to contamination of produce and other commodities meant for human consumption. The Guidance Document on Analytical Parameters for Determination of Per- and Polyfluoroalkyl Substances (PFAS) in Food and Feed released by the European Union Reference Laboratory (EURL) for Halogenated POPs in Feed and Food was used as guidance.[1] The required limits of quantification (LOQ) are outlined for four compounds in milk; PFOS (0.02 µg/kg) PFOA (0.01 µg/kg), PFNA (0.05 µg/kg), and PFHxS (0.06 µg/kg). The lowest LOQ of the four compounds, 0.01 µg/kg, was used as the target LOQ for the complete list of 28 PFAS compounds in milk including perfluoroalkyl carboxylic acids (PFCA), perfluoroalkyl sulfonic acids (PFSA), FOSA, and other substitutes. The analytical method developed herein can resolve bile acid interferences from isobaric target PFAS compounds and can be applied to other sample matrices of animal origin.

Sample preparation

- Weigh 10 grams milk (+/- 0.1 g) in 50 mL centrifuge tube
- Fortify sample at 0.01 µg/kg
- Spike IS (50 µL of 20 ppb)
- Add 10 mL ACN
- Add 150 µL formic acid
- Add Q-sep QuEChERS Salt packet (original) (cat.#25847)
- Vortex ~30 seconds, shaker table 10 min.
- Centrifuge 5 min. 4200 rpm
- Aliquot into 15 mL dSPE, 300 mg PSA, 150 mg GCB (cat.#26126)
- Vortex ~ 30 seconds, centrifuge 5 mins 4200 rpm
- Aliquot 6 mL to clean 15 mL centrifuge vial
- Dry down for 90 minutes at 35 ºC
- Reconstitute using 400 µL methanol: water 60:40, vortex
- Centrifuge 5 min. 4200 rpm
- Aliquot supernatant to polypropylene vial and cap
- Inject 5 µL

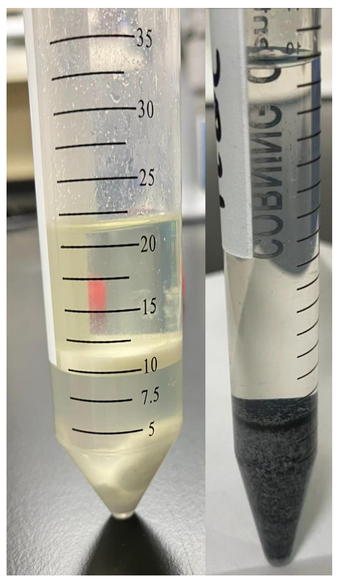

Figure 1: (Left) Milk prepared by QuEChERS, (Right) Milk after dSPE.

Analytical Method

Table 1: Analytical Conditions.

Column:	Force C18 50 x 2.1 mm, 3µm (cat.#9634352)
Guard:	Force C18 EXP Guard Cartridge 5 x 2.1 mm (cat.#963450252)
Delay:	PFAS Delay Column 50 x 2.1 mm, 5µm (cat.#27854)
MPA:	Water, 5 mM Ammonium Acetate
MPB:	Methanol: Acetonitrile 50:50
Sample Diluent:	Methanol: Water 60:40
Column Temp:	50 °C
Injection Volume:	5 µL
Flow Rate:	0.6 mL/min

Time (min)	%B
0.00	5
3.00	40
9.00	70
9.50	95
10.50	100
11.00	100
11.01	5
13.00	5

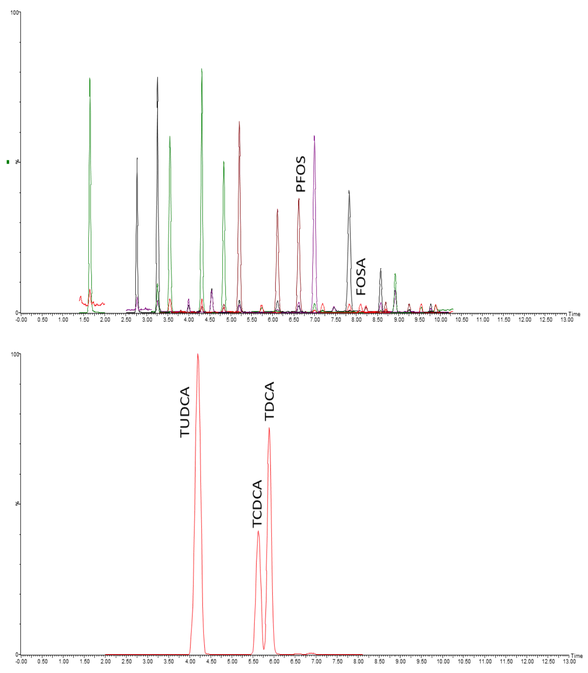

Figure 2: Analytes and bile acids analyzed by final method conditions.

#	Analyte	Retention Time (mins)	#	Analyte	Retention Time (mins)	#	Analyte	Retention Time (mins)
1	PFBA	1.64	15	M3PFHxS	4.84	29	PFUnDA	7.82
2	M4PFBA	1.64	16	PFOA	5.21	30	M7-PFUnDA	7.82
3	PFPeA	2.77	17	M8PFOA	5.21	31	FOSA	8.10
4	M5PFPeA	2.77	18	PFHpS	5.74	32	PFDS	8.23
5	PFBS	3.25	19	Capstone B	5.98	33	PFDoDA	8.57
6	M3PFBS	3.25	20	PFNA	6.11	34	M2-PFDoDA	8.57
7	PFHxA	3.55	21	M9PFNA	6.11	35	11Cl-PF3OUdS	8.69
8	M5PFHxA	3.55	22	Capstone A	6.23	36	PFUnDS	8.92
9	HFPO-Da	3.81	23	PFOS	6.62	37	PFTrDA	9.25
10	PFPeS	3.99	24	M8PFOS	6.62	38	PFDoDS	9.54
11	PFHpA	4.31	25	PFDA	6.99	39	PFTeDA	9.77
12	M4PFHpA	4.31	26	M6PFDA	6.99	40	M2-PFTeDA	9.77
13	DONA	4.55	27	9Cl-PF3ONS	7.19	41	PFTrDS	9.88
14	PFHxS	4.84	28	PFNS	7.46			

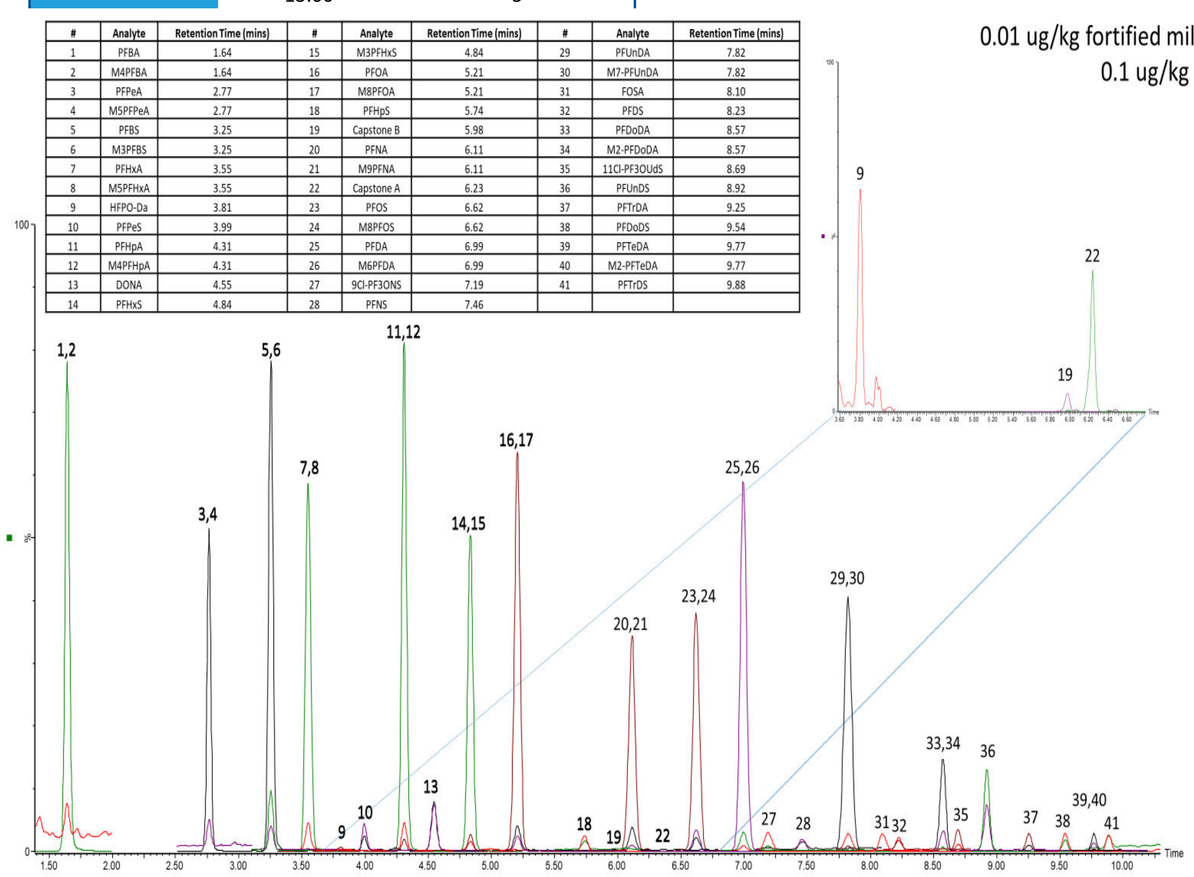

Figure 3: Chromatogram of milk fortified at 0.01 µg/kg and prepared by outlined sample preparation conditions. Isotopically labeled internal standards spiked at a concentration of 0.1 µg/kg.

Results and Discussion

Chromatographic Performance: Bile acids can often be of concern when analyzing tissue samples for PFAS. TDCA is an endogenous compound that is formed in the liver and can be detected at high concentrations in samples of animal origin. This compound, along with TUDCA and TCDCA all share the same MRM (m/z 499→80) as PFOS. A method was developed here to ensure the resolution of bile acids from isobaric compounds with excellent resolution (Figure 2).

Linearity: The method was assessed using an 8-point calibrations curve and a 1/x weighted linear regression with solvent calibrators using isotope dilution. Calibration levels were analyzed from 0.03/0.1 – 50 ppb and all analytes had an R^2 of >0.992.

Accuracy and Precision: Accuracy and precisions were assessed using the target LOQ of 0.01 µg/kg. Each sample was spiked in triplicate and analyzed in triplicate. Blank matrix samples were extracted to determine endogenous levels of PFAS. The accuracy ranged from 101 – 113% and precision 2.56 – 16.6% for the four compounds referenced in the EURL document are outlined in Table 2. The majority of other analytes monitored in these experiments returned acceptable results for accuracy and precision (Figures 4,5).

Table 2: Precision and Accuracy. Compounds noted with * had positive blanks that were subtracted from fortified levels.

Compound	Percent Recovery (Percent RSD)					
	Sample 1		Sample 2		Sample 3	
	0.01 µg/kg	0.05 µg/kg	0.01 µg/kg	0.05 µg/kg	0.01 µg/kg	0.05 µg/kg
PFBA*	90(4.8)	105(7.1)	103(8.6)	116(2.9)	115(11.5)	116(4.9)
PFPeA	105(7.9)	110(5.5)	102(6.7)	111(2.5)	107(10.8)	114(2.8)
PFHxA	114(10.6)	113(5.1)	104(8.1)	112(5.5)	118(11.3)	113(6.1)
PFHpA	117(5.1)	112(7.3)	108(7.1)	115(2.7)	111(16.2)	118(4.2)
PFOA*	108(5.1)	109(4.6)	102(6.2)	113(2.6)	105(7.7)	113(3.3)
PFNA	112(8)	109(2.6)	106(8.2)	110(3.8)	101(16.6)	101(10.4)
PFDA	111(9)	113(5.6)	107(6.1)	112(4.1)	112(7.4)	115(3.3)
PFUnDA	104(7.5)	109(11.6)	103(10.9)	107(7.6)	109(8.9)	110(4.5)
PFDoDA	110(7.6)	109(4.3)	102(6.7)	112(2.8)	108(9.8)	114(2.4)
PFTrDA	76(12.5)	66(7.7)	87(8.9)	73(8.6)	72(11)	69(12.2)
PFTeDA	83(14.5)	84(12.4)	103(6.9)	109(9)	74(17.2)	78(11.3)
PFBS*	106(11.9)	109(11.4)	110(11.2)	112(5.2)	103(7.2)	109(5.3)
PFPeS	113(6.3)	119(5.8)	105(6.2)	113(4.9)	114(8)	115(5.1)
PFHxS	112(5.9)	114(5.3)	106(7.3)	111(3.6)	106(12.8)	113(3.9)
PFHpS	117(5.1)	121(9.1)	112(5.6)	116(3.8)	121(5)	119(5.3)
PFOS*	109(7.3)	110(7.2)	109(3.8)	113(4)	102(6.7)	107(4.6)
PFNS	114(17.1)	89(18.1)	110(11.5)	90(15.4)	103(15.5)	84(19.9)
PFDS	93(11.7)	96(6)	101(7.3)	100(9.3)	99(12.3)	102(11.8)
PFUnDS	74(9.5)	96(9.1)	100(9.9)	113(9.3)	81(20.7)	104(12.7)
PFTrDS	68(15.6)	46(9.5)	88(14.1)	95(10.6)	43(15.1)	55(6)
PFDoDS	56(9.8)	60(8.2)	96(10)	103(9.3)	62(11.5)	74(7.3)
FOSA	61(12.6)	59(10.2)	93(8.2)	95(9.4)	87(15.3)	84(12.5)
DONA	111(7.2)	109(7.9)	94(11.3)	99(4.5)	94(9.5)	104(6)
HFPO-Da	106(13.7)	110(7.7)	111(15.4)	114(12.7)	125(9)	124(4.3)
9Cl-PF3ONS	95(3.4)	99(8.1)	97(10.3)	99(7.6)	102(12.8)	106(6.1)
11Cl-PF3OUdS	94(6.7)	98(6.8)	103(12.2)	114(10)	98(11.2)	102(13.1)
Capstone A	120(12.1)	126(9.3)	102(16.5)	108(7.3)	119(6.4)	114(5.1)
Capstone B	92(11.6)	68(11)	85(15.2)	72(8.2)	85(15)	71(10.6)

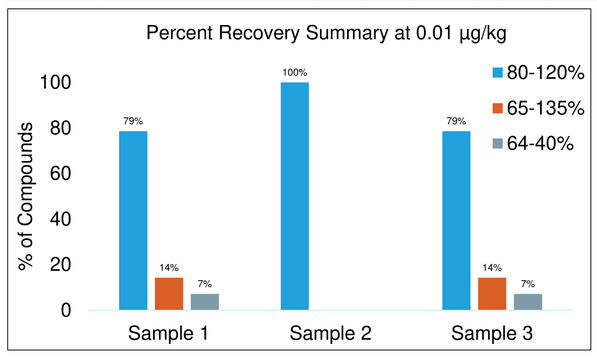

Figure 4: Percentage of compounds that meet each range for percent recovery.

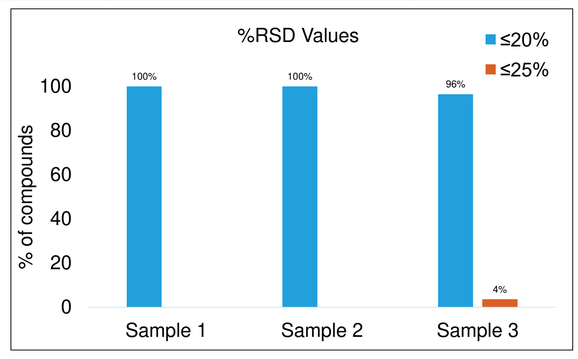

Figure 5: Percentage of compounds that meet each range for %RSD.

Conclusion

- An LC method was established in milk to meet the requirements outlined in the EURL document for detecting PFAS in food commodities.
- The method utilized a Force C18 50 x 2.1 mm, 3 µm column, with guard, and PFAS Delay column and can resolve bile acid interferences from analytes that share the same mass transition.
- Sample preparation was optimized for milk and included a QuEChERS + dSPE approach. This workflow returned exceptional results for the four PFAS compounds required for monitoring, and acceptable results for the majority of other analytes.
- Long-chain sulfonic acid (C11-C13) showed low recovery values possibly due to strong interactions with the milk fat layer.
- The chromatographic method developed herein is suitable to apply to other matrix samples of animal origin for the detection of PFAS compounds where high levels of bile acids may be present.

References

1. European Union Reference Laboratory for halogenated POPs in Feed and Food. Guidance Document on Analytical Parameters for the Determination of Per- and Polyfluoroalkyl Substances (PFAS) in Food and Feed. Version 1.2. May 2022 [cited 2024 May 10]. Available from: https://eurl-pops.eu/news/guidance-document-pfas/guidance-document-pfas

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For more information contact Tina Brandscher
Tina.Brandscher@restek.com - LC Specialist • Restek GmbH