Application Note

Food

Quantitation of 18 Per and Polyfluoroalkyl Substances (PFAS) in Shrimp with Agilent LC/TQ

Routine analysis using the Agilent 6475 triple quadrupole LC/MS System and simplified QuEChERS

Authors

Vikrant Goel, Prasanth Joseph, and Ramprasad Ganni
Agilent Technologies, Inc.

Abstract

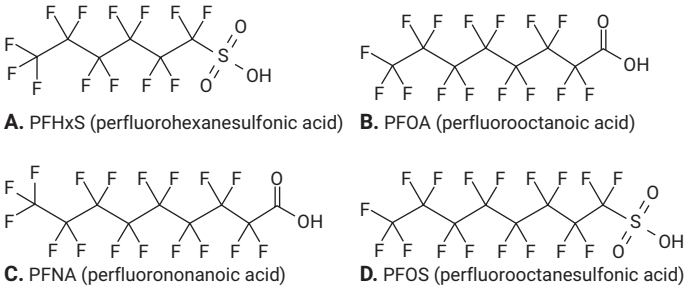
Per and polyfluoroalkyl substances (PFAS) are a group of chemicals that can be harmful to humans and are persistent in the environment, earning them the nickname "forever chemicals." An essential component of many manufacturing and industrial processes, these chemicals can make their way into human food products via environmental contamination. The European Union (EU) has set food contamination limits covering four PFAS compounds and has recommended monitoring for a broad range of PFAS compounds in commonly consumed foods.

This application note presents a highly selective "dynamic multiple reaction monitoring" (dMRM) liquid chromatography/triple quadrupole (LC/TQ) method for the quantitative assessment of 18 PFAS in shrimp, using the Agilent 6475 triple quadrupole LC/MS system. The developed method is found to be linear within the concentration range from 0.1 ng/mL to 2.5 ng/mL with a minimum R^2 value of 0.993. A quality control (QC) sample prepared at 0.2 µg/kg with six replicates showed recoveries from 80 to 120%. The relative standard deviation (RSD) of calculated concentrations at QC level in the matrix was found to be below 10%.

Introduction

PFAS are a group of anthropogenic chemicals used across various industrial applications and known to enter environmental matrices such as air, soil, and water. Their amphiphilic chemical structure makes them highly soluble in water, which leads to accumulation in animal and human tissues. EU Commission Regulation 2023/915 sets maximum levels for four PFAS in food, including seafood such as crustaceans (Figure 1).

In this application note, a highly selective dMRM-based LC/MS method was developed for analysis of residual PFAS analytes in a shrimp matrix using an Agilent 6475 triple quadrupole LC/MS at the required maximum residue limit, as per the EU directive.



A. PFHxS (perfluorohexanesulfonic acid) **B.** PFOA (perfluorooctanoic acid)

C. PFNA (perfluorononanoic acid) **D.** PFOS (perfluorooctanesulfonic acid)

Figure 1. Structures of four PFAS compounds in food.

Experimental conditions

Consumables

Acetonitrile (part number 34967), methanol (part number 34966), and formic acid (part number 56302) were purchased from Honeywell (Charlotte, NC, U.S.), and type-1 water was purchased from MilliporeSigma (Burlington, MA, U.S.). The PFAS standard mix (part number PFS-537-ADPS), PFAS Internal standard mix (part number PFS-537-IPDS), QuEChERS extraction salt (part number 5982-7550), QuEChERS dispersive kit (part number 5982-4921), polypropylene vial (part number 5182-0567), snap caps with polypropylene septa (part number 5182-0542), and ceramic homogenizer (part number 5982-5313) were from Agilent.

Instrumentation and methodology

The instruments used in this experiment included an Agilent 1290 Infinity II high-speed pump (G7120A), Agilent 1290 Infinity II multisampler (G7167B), Agilent 1290 Infinity II multicolumn thermostat (G7116B), Agilent 6475 triple quadrupole LC/MS system, and an Agilent PFC-free HPLC conversion kit (part number 5004-0006). Table 1 details the LC method, and source parameters are listed in Table 2. An intermediate stock of 2 ppm was prepared using MeOH:Water (1:1) as diluent. Further individual dilutions were prepared at 50, 25, 10, 5, and 2 ppb levels for spiking in a blank shrimp matrix to generate prep-spike calibration, as shown in Table 3.

Table 1. HPLC gradient method.

Parameter	Value		
Column	Agilent ZORBAX Extend-C18 column, 2.1 mm × 50 mm, 1.8 µm (part number 757700-902), kept at 50 °C		
Mobile Phase	(A) 5 mM ammonium acetate (B) Methanol Flow rate: 0.35 mL/min		
Multisampler	10 µL injection volume from sampler kept at 5 °C		
Diluent and Needle Wash	Methanol:water (1:1)		
Gradient	Time (min)	Mobile Phase A	Mobile Phase B
	0.0	90	10
	3.0	45	55
	10.5	30	70
	12.0	5	95
	15.0	5	95
	15.1	90	10
	20.0	90	10

Table 2. Source parameters in the Agilent 6475 triple quadrupole LC/MS system.

Parameter	Value
Drying Gas (Nitrogen)	11 L/min at 250 °C
Nebulizer Gas (Nitrogen)	25 psi
Sheath Gas (Nitrogen)	11 L/min at 350 °C

Parameter	Value
Capillary Voltage	2,500
Nozzle Voltage	0
Ionization mode	ESI Negative

Table 3. Dilution and spike preparation.

S. No.	Initial Conc.	Volume (µL)	Diluent (µL)	Obtained Solvent Conc. (ppb)	Final Concentration in Shrimp*
1	2 ppm	25	975	50	5 ppb (5 µg/kg)
2	50 ppb	500	500	25	2.5 ppb (2.5 µg/kg)
3	25 ppb	400	600	10	1 ppb (1 µg/kg)
4	10 ppb	500	500	5	0.5 ppb (0.5 µg/kg)
5	5 ppb	400	600	2	0.2 ppb (0.2 µg/kg)
6	2 ppb	500	500	1	0.1 ppb (0.1 µg/kg)
7	NA	NA	NA	Matrix Blank	0.2 mL diluent in 2g shrimp

*Spike 0.2 mL of obtained concentration in 2 g sample, before extraction.

Sample preparation

Shrimp samples were cleaned and washed with ultrapure water and homogenized in a mixer grinder. A 2 ± 0.05 g quantity of paste was weighed in a 50 mL polypropylene tube and spiked with 0.2 mL of individual PFAS standard mix at the respective concentrations, followed by mixing, as mentioned in Table 3. A ceramic homogenizer was used wherever aggregate formation occurred during the 1-minute vortex mixing step. Extraction was performed with 10 mL of acetonitrile and buffered QuEChERS salt. The removal of biological matrix components including hydrophobic substances was performed with a dispersive clean-up step. The final stages included adding ultrapure water to the supernatant and collecting it in polypropylene vials. The pre-spiked (matrix-based) sample was processed according to the developed LC/TQ method for 18 PFAS analytes. The extraction protocol is seen in Figure 2.

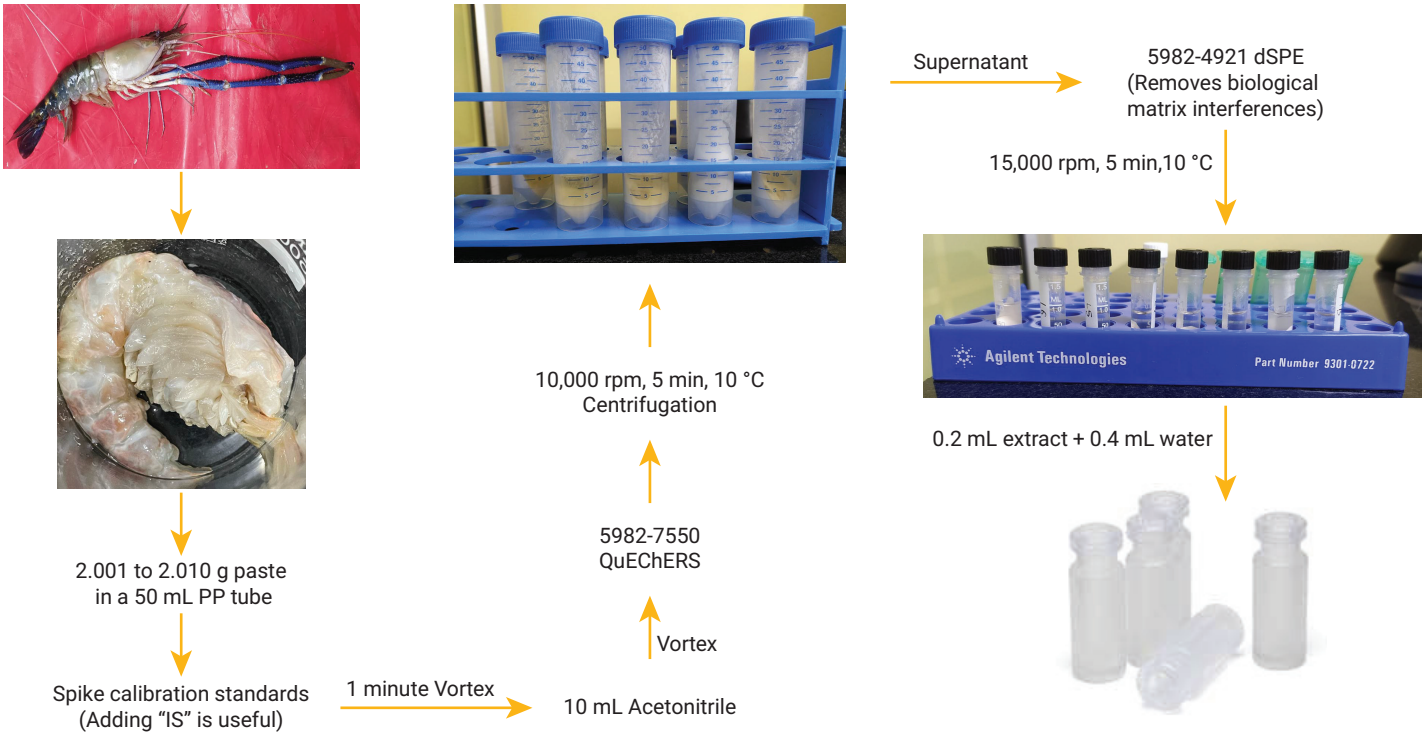


Figure 2. Sample preparation methodology.

Results and discussion

Matrix linearity and sensitivity data

The matrix-based (prespiked) shrimp extracts containing PFAS at various concentrations had good reproducible data while using LC/TQ in dMRM scan mode. Per EU directive 2023/915, the maximum PFAS level acceptable in wet weight for crustaceans is 0.2 µg/kg. The linearity was obtained from 0.1 to 2.5 µg/kg with R^2 values above 0.993 for all 18 analytes, with the obtained signal-to-noise values above 35:1 at 0.2 µg/kg (Figures 3 and 4), indicating the developed methodology's suitability for such difficult matrices. Incorporating deuterated internal standards was useful to mitigate the calibration and recovery issues. Further, the calibration tables for four major PFAS compounds with the internal standard are seen to have ± 20% accuracy, ± 30% qual/quant ion ratio, and QC samples with ± 20% accuracy at 0.2 µg/kg level.

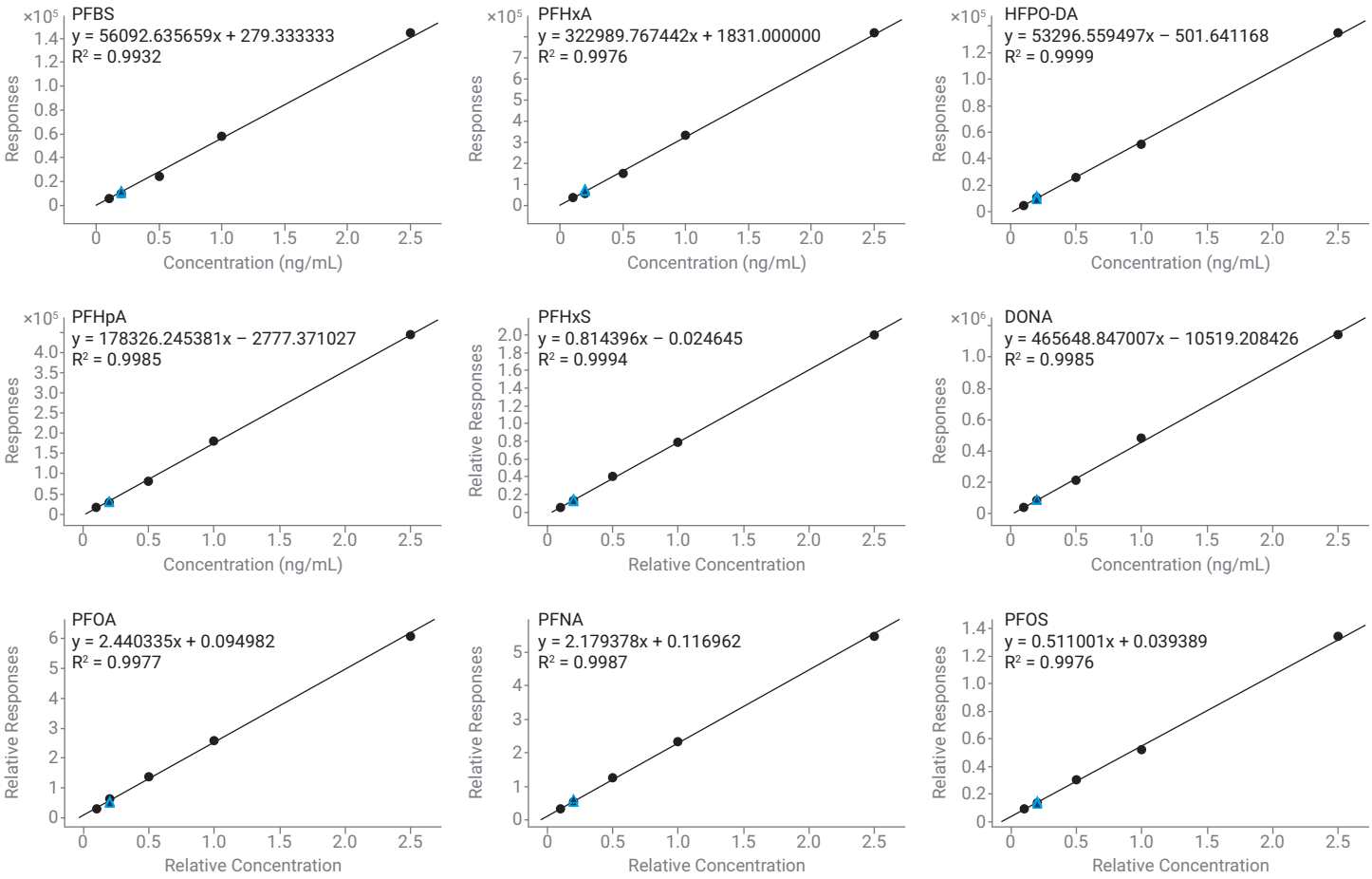


Figure 3. Linear calibration plot with R^2 values and 1/x weight for 18 PFAS compounds (continued on next page).

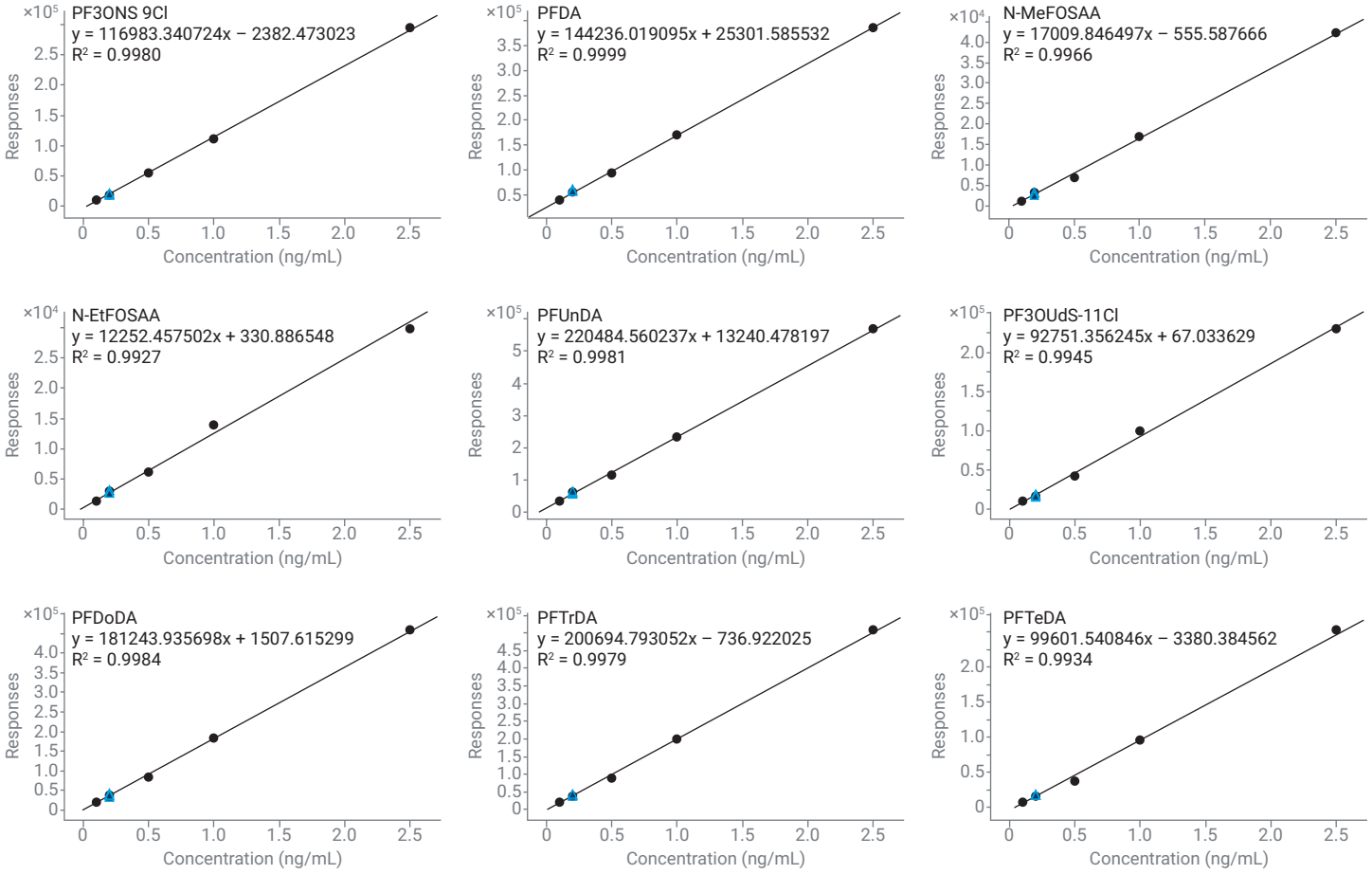


Figure 3. Linear calibration plot with R^2 values and 1/x weight for 18 PFAS compounds (continued).

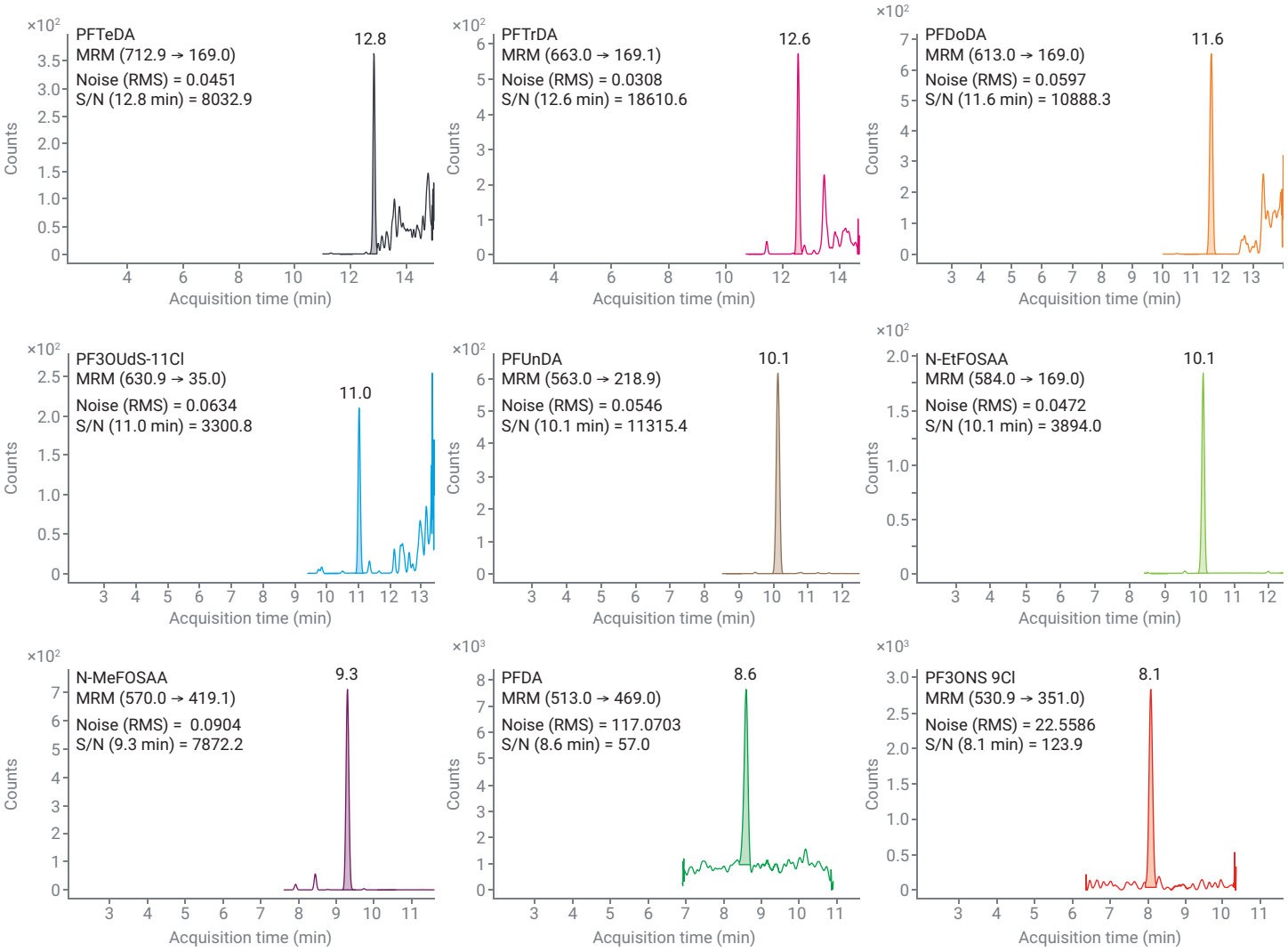


Figure 4. Signal-to-noise values for 18 PFAS compounds in a shrimp matrix at 0.2 µg/kg (continued on next page).

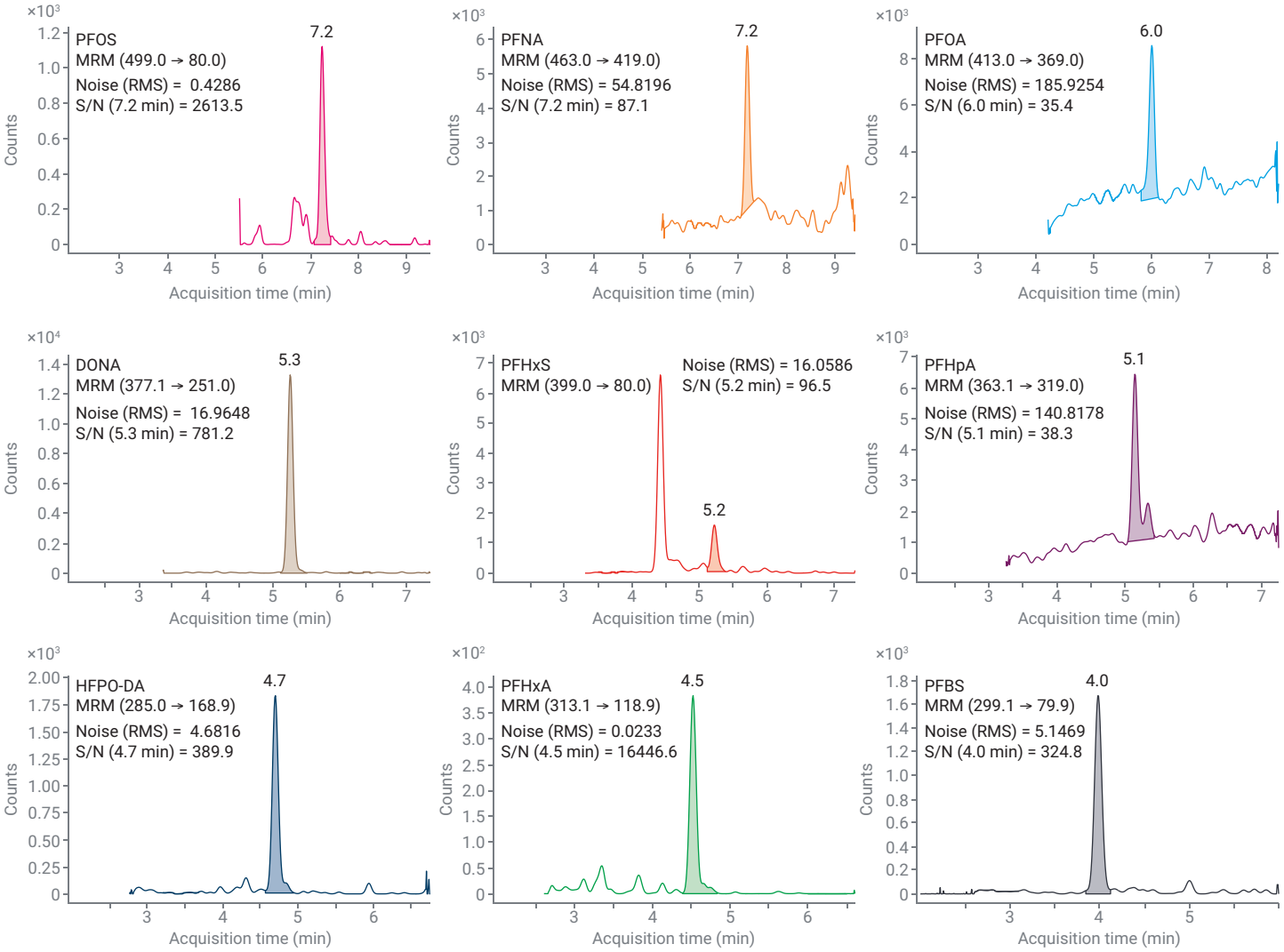


Figure 4. Signal-to-noise values for 18 PFAS compounds in a shrimp matrix at 0.2 µg/kg.

Sample			PFHxS Method	PFHxS Results				Quali ...	IS-PFOS (I...	
Data File	Type	Level	Exp. Conc.	RT	Resp.	Final Conc.	Accuracy	Ratio	RT	Resp.
MB_2.d	Blank			5.27	2025	0.070			7.25	62001
L1.d	Cal	1	0.1	5.22	3433	0.099	99.2	51.8	7.24	61114
L2.d	Cal	2	0.2	5.21	7529	0.193	96.4	41.8	7.24	56881
L3.d	Cal	3	0.5	5.22	23930	0.526	105.1	60.1	7.24	59308
L4.d	Cal	4	1.0	5.21	47944	0.999	99.9	49.6	7.24	60775
L5.d	Cal	5	2.5	5.21	119575	2.483	99.3	44.5	7.24	59854
MB_3.d	Blank			5.63	1584	0.059			7.23	68368
QC_1.d	QC	2	0.2	5.22	9107	0.180	90.0	42.2	7.23	74712
QC_2.d	QC	2	0.2	5.22	7983	0.181	90.3	49.1	7.23	65223
QC_3.d	QC	2	0.2	5.21	9688	0.223	111.4	45.5	7.22	61814
QC_4.d	QC	2	0.2	5.21	9159	0.202	101.0	50.3	7.22	65502
QC_5.d	QC	2	0.2	5.22	8961	0.200	100.2	41.8	7.23	64644
QC_6.d	QC	2	0.2	5.21	8069	0.187	93.7	46.0	7.22	63064
MB_4.d	Blank			5.61	714	0.044			7.22	63317
Dil_3.d	DoubleBlank			5.22	41	3.903			7.36	13

Sample			PFOA Method	PFOA Results				Quali ...	Quali ...	IS-PFOA (I...	
Data File	Type	Level	Exp. Conc.	RT	Resp.	Final Conc.	Accuracy	Ratio	Ratio	RT	Resp.
MB_2.d	Blank			6.02	4764	0.000		13.9	23.7	6.01	73284
L1.d	Cal	1	0.1	6.01	20842	0.085	85.2	15.3	32.6	6.01	68819
L2.d	Cal	2	0.2	6.00	39507	0.220	110.1	10.1	32.4	6.00	62482
L3.d	Cal	3	0.5	6.01	91802	0.523	104.6	12.5	32.4	6.00	66951
L4.d	Cal	4	1.0	6.00	177342	1.021	102.1	13.7	32.2	6.00	68552
L5.d	Cal	5	2.5	6.01	428352	2.451	98.0	12.2	36.6	6.00	70510
MB_3.d	Blank			6.00	3781	0.000		9.0	27.0	6.00	72080
QC_1.d	QC	2	0.2	6.00	36099	0.175	87.3	16.1	33.5	6.00	69290
QC_2.d	QC	2	0.2	6.00	36717	0.169	84.5	15.3	32.5	6.00	72338
QC_3.d	QC	2	0.2	6.00	40328	0.199	99.5	14.9	34.5	6.00	69485
QC_4.d	QC	2	0.2	6.00	35344	0.170	84.9	17.1	34.1	6.00	69392
QC_5.d	QC	2	0.2	6.00	36613	0.178	89.2	13.2	31.2	6.00	69048
QC_6.d	QC	2	0.2	6.00	35034	0.166	83.0	14.2	35.9	6.00	70038
MB_4.d	Blank			5.98	6848	0.000		9.0	19.6	6.00	75960
Dil_3.d	DoubleBlank			5.93	950	129.725		0.7	12.4	6.04	3

Sample			PFNA Method	PFNA Results				Quali ...	IS-PFOA (I...	
Data File	Type	Level	Exp. Conc.	RT	Resp.	Final Conc.	Accuracy	Ratio	RT	Resp.
MB_2.d	Blank			7.19	8118	0.000		6.3	6.01	73284
L1.d	Cal	1	0.1	7.17	23015	0.100	99.8	23.1	6.01	68819
L2.d	Cal	2	0.2	7.17	32812	0.187	93.6	30.9	6.00	62482
L3.d	Cal	3	0.5	7.18	84963	0.529	105.7	29.4	6.00	66951
L4.d	Cal	4	1.0	7.17	161087	1.025	102.5	24.4	6.00	68552
L5.d	Cal	5	2.5	7.17	386231	2.460	98.4	26.7	6.00	70510
MB_3.d	Blank			6.52	2487	0.000			6.00	72080
QC_1.d	QC	2	0.2	7.17	37930	0.198	98.8	27.2	6.00	69290
QC_2.d	QC	2	0.2	7.17	40010	0.200	100.1	25.9	6.00	72338
QC_3.d	QC	2	0.2	7.16	38273	0.199	99.5	25.5	6.00	69485
QC_4.d	QC	2	0.2	7.17	38650	0.202	101.0	24.0	6.00	69392
QC_5.d	QC	2	0.2	7.17	37660	0.197	98.3	26.7	6.00	69048
QC_6.d	QC	2	0.2	7.17	44538	0.238	119.1	24.7	6.00	70038
MB_4.d	Blank			7.17	12147	0.020		9.4	6.00	75960
Dil_3.d	DoubleBlank			7.27	15321	2343.276		0.2	6.04	3

Sample			PFOS Method	PFOS Results				Quali ...	IS-PFOS (I...	
Data File	Type	Level	Exp. Conc.	RT	Resp.	Final Conc.	Accuracy	Ratio	RT	Resp.
MB_2.d	Blank			7.24	1190	0.000		76.6	7.25	62001
L1.d	Cal	1	0.1	7.24	5778	0.108	107.9	60.3	7.24	61114
L2.d	Cal	2	0.2	7.24	7632	0.185	92.7	59.9	7.24	56881
L3.d	Cal	3	0.5	7.24	18010	0.517	103.4	56.4	7.24	59308
L4.d	Cal	4	1.0	7.24	31539	0.938	93.8	62.6	7.24	60775
L5.d	Cal	5	2.5	7.24	80379	2.551	102.0	58.8	7.24	59854
MB_3.d	Blank			7.24	1739	0.000		83.1	7.23	68368
QC_1.d	QC	2	0.2	7.23	11006	0.211	105.6	52.8	7.23	74712
QC_2.d	QC	2	0.2	7.22	9825	0.218	108.8	47.7	7.23	65223
QC_3.d	QC	2	0.2	7.23	9053	0.210	104.8	67.6	7.22	61814
QC_4.d	QC	2	0.2	7.23	8319	0.171	85.7	60.0	7.22	65502
QC_5.d	QC	2	0.2	7.22	8787	0.189	94.5	55.5	7.23	64644
QC_6.d	QC	2	0.2	7.22	8174	0.177	88.3	58.4	7.22	63064
MB_4.d	Blank			7.22	1558	0.000		52.0	7.22	63317
Dil_3.d	DoubleBlank			7.26	15	2.181		613.3	7.36	13

Figure 5. Calibration tables for for major PFAS compounds across 0.1 to 2.5 µg/kg and six QC samples at 0.2 µg/kg.

Reproducibility and recovery data

Further reproducibility of the developed method was established as a good chromatographic overlay across five calibration levels (Figure 6) and by generating RSD data for six replicates at maximum levels of 0.2 µg/kg. The final calculated concentration values were between 2.4 and 8.3 %RSD for six replicates of 18 PFAS compounds in the shrimp matrix (Table 4). The recovery values at 0.2 µg/kg levels were within limits of 70 to 120%.

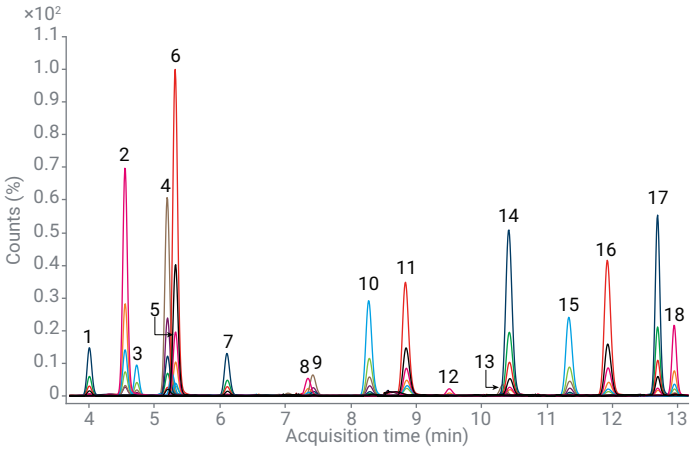


Figure 6. Chromatographic overlay of 18 PFAS compounds from 0.1 to 2.5 µg/kg in shrimp.

Table 4. MRM Parameters and RSD values for 18 PFAS compounds in shrimp.

Sr. No	Name and Precursor (*m/z*)	Product (*m/z*)	RT (min)	Fragmentor (V)	Collision Energy (V)	%RSD
1	PFTeDA (712.9)	668.9, 169	13	125	16, 35	2.43
2	PFTrDA (663.0)	619, 369	12.7	100	9, 15	4.50
3	PF3OUdS-11Cl (630.9)	451, 35	11.4	200	30, 65	5.41
4	PFDoDA (613.0)	569, 169	12	100	8, 30	7.26
5	N-EtFOSAA (584.0)	418.9, 169	10.4	150	24, 34	8.34
IS	IS_N-MeFOSAA (573.0)	419.1	9.6	150	24	NA
6	N-MeFOSAA (570.0)	482.8, 419.1, 169	9.6	150	15, 24, 34	5.44
7	PFUnDA (563.0	519, 269.2, 218.9	10.5	100	8, 15, 15	4.96
8	PF3ONS 9Cl (530.9)	351, 35	8.35	175	30, 50	3.46
9	PFDA (513.0)	469, 269.2, 219.1	8.9	85	4, 16, 16	4.06
IS	IS-PFOS (507.0)	80.0	7.5	120	65	NA
10	PFOS (499.0)	98.9, 80.0	7.5	120	50, 65	5.34
11	PFNA (463.0)	419.0	7.4	100	4, 17	7.07
IS	IS-PFOA (421.0)	376	6.2	90	4	NA
12	PFOA (413.0)	369, 219.1, 169	6.2	90	4, 16, 16	6.21
13	PFHxS (399.0)	119, 99, 80	5.3	120	50, 50, 65	8.03
14	DONA (377.1)	251, 85	5.3	90	10, 35	3.56
15	PFHpA (363.1)	319, 169	5.25	75	5, 15	3.57
16	PFHxA (313.1)	269.1, 118.9	4.6	75	5, 20	6.07
17	PFBS (299.1)	99, 9.9	4.0	130	30, 40	6.78
18	HFPO-DA (285.0)	185, 168.9	4.7	55	15, 5	6.82

Conclusion

This application note describes sensitivity values lower than targeted residue limits for PFAS under EU Commission Regulation 2023/915. The recovery values between 80 and 120% at 0.2 µg/kg for 18 PFAS analytes were demonstrated, while matrix calibration from 0.1 to 2.5 µg/kg with the use of a PFC-free HPLC conversion kit makes it more suitable for evaluating samples under future guidelines that cover more analytes in shrimp. Sample preparation using original extraction salt and dispersive solid phase extraction takes less than 1 hour, ensuring a greater number of samples per day. Further, using the Agilent 6475 triple quadrupole LC/MS system for this analysis makes it possible for a routine testing lab to cater to a wider range of prospective food analysis.

References

1. European Union. 2023. Commission Regulation (EU) 2023/915 of 25 April 2023 on Maximum Levels for Certain Contaminants in Food and Repealing Regulation (EC) No 1881/2006. https://eur-lex.europa.eu/eli/reg/2023/915/oj (accessed 2025-03-20).
2. Agilent. Containment Without Contamination: New Agilent PFC-Free Vials and Caps for LC/MS. *Agilent Technologies flyer*, publication number 5994-3089EN, **2025**.
3. Kamuf, M.; Wälz, M.; Borowiak, A. Reduce PFAS Background with the Agilent PFC-Free* HPLC Conversion Kit. *Agilent Technologies technical overview*, publication number 5994-2291EN, **2024**.
4. Agilent. Installation Video for the InfinityLab PFC-free HPLC Conversion Kit for PFAS Applications. https://www.agilent.com/en/video/pfc-free-hplc-conversion-kit (accessed 2025-03-20).
5. Agilent QuEChERS Selection Guide, *Agilent Technologies guide*, publication number 5990-8590EN, **2011**.

www.agilent.com

DE-007437

Printed in the USA, June 27, 2025
5994-8443EN

Agilent
Trusted Answers