

Determination of various PFAS in egg matrix using stacked injection on-line SPE coupled to LC-MS/MS

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User Benefits

- ◆ Single vendor solution for UHPLC and MS system
- ◆ Quantification of 27 PFAS in ng/mL range using an on-line SPE approach
- ◆ Increased sensitivity due to the stacked injection combined with on-line SPE

Introduction

Perfluoroalkyl and polyfluoroalkyl substances (PFAS) refer to a class of more than 4000 individual chemicals that have been widely used since the 1950s, e.g. as fire retardants, food packaging materials or non-stick coatings. These compounds offer heat-resistant, and oil- and water-repellant properties as well as chemical and thermal stability, resistance to UV light and weathering. Due to their anthropogenic origin, PFAS cannot be degraded, and hence they accumulate and can now be detected ubiquitously in the environment. Due to this PFAS also found their way into the food chain and accordingly into our food. Concerns about human exposure through diet, studies on the status of food contamination are being conducted in various countries.

Here we describe the determination of various PFAS in egg matrix in a relevant concentration range. The analysis is based on a simple QuEChERS extraction coupled to an on-line SPE approach. This omits additional sample preparation steps like dSPE.

Materials and Methods

Fast, sensitive and robust LC-MS/MS systems provide the basis for routine analysis in food testing laboratories. For the described application, a Shimadzu LCMS-8060NX triple-quadrupole mass spectrometer coupled with a Nexera X3 UHPLC system was used (Figure 3).

27 PFAS standards and one IS-mixture (ISO 21675-LSS) were purchased (Wellington Laboratories / neochema). Stock solutions of these PFAS were diluted with methanol and combined to a single standard mixture with a final a concentration of 1ng/μL for each compound. Further dilutions of this mixture were produced to spike either the egg matrix before extraction or in case of calibrators, extracted egg matrix. Calibration samples in egg matrix were determined in the concentration range from 0.001 -0.025 ng/mL to 1 ng/mL. All samples (except blanks) were spiked with IS to a final concentration of 0.04 ng/mL.

Samples were extracted on the basis of QuEChERS AOAC method (Figure 1, RESTEK Q-Sep QuEChERS Extraction Packets AOAC Method). 50μL of sample was injected directly on a SPE-trap column using the stacked injection function offered by the Nexera SIL-40 autosampler. This results in 5x10 μL injections, where each injection is followed by aqueous sample loading phase removing the organic solvent from the sample extraction. This leads to improved trapping capability. With this approach higher volumes of the pure QuEChERS extract can be injected.

Analysis was performed within 15 minutes using MRM acquisition with at least two transitions for each compound (except PFBA, where only one transition is available).

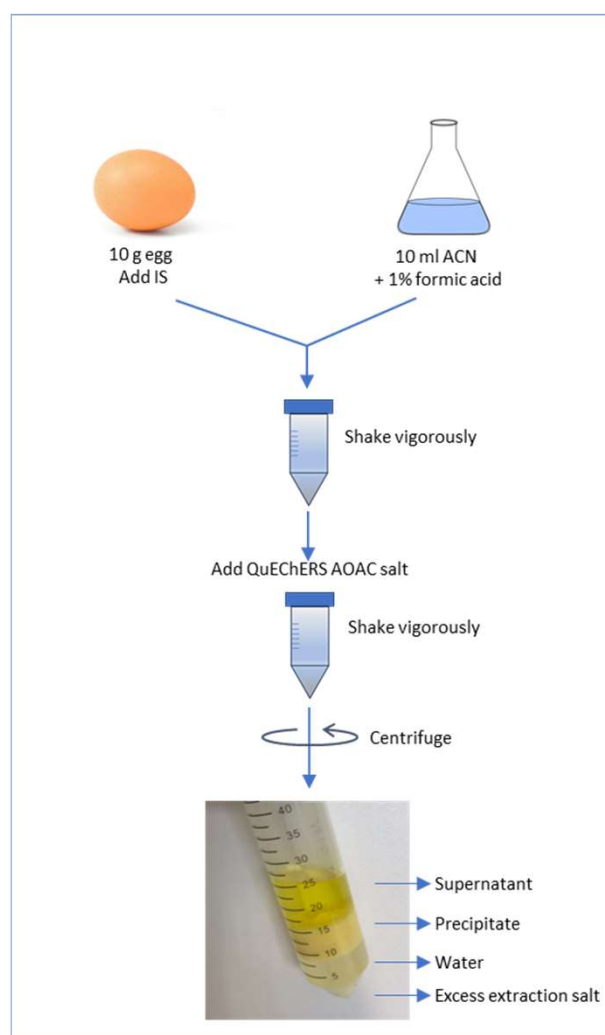


Figure 1 Extraction process

Analytical conditions are listed in Table 1. The optimized MRM transitions are summarized in Table 2.

Since PFAS can be present in reagents, glassware, pipettes, tubing, degassers and other parts from the LC-MS/MS instrument, the use of a solvent delay column is necessary. Small C18 columns are placed between mixer and autosampler respectively between mixer and valve to delay possible PFAS contaminations and separate them from sample-derived PFAS.

Table 1 Analytical conditions

Mass Spectrometer	: LCMS-8060NX
Ionization	: Electrospray Ionization (ESI), negative
Interface Voltage	: -1 kV
Focus Voltage	: -2.5 kV
Heating Gas	: 15 L/min
DL Temp.	: 150 °C
Interface Temp.	: 300 °C
Nebulizing Gas	: 3 L/min
Drying Gas	: 3 L/min
Heat Block	: 400 °C
Dwell-/Pause-time	: 4 (3 for IS) / 1 msec
CID	: 270 kPa

UHPLC	: Nexera X3
Pump A (Analytical)	: 2 mM ammonium acetate in H ₂ O
Pump B (Analytical)	: 2 mM ammonium acetate in Methanol
Pump C (Trap)	: H ₂ O + modifier (sample loading)
Pump D (Trap)	: Methanol (washing of SPE and delay column)
Analytical column	: Shim-pack™ Scepter 1.9 μm, C18-120, 2.1 x 50 mm
Delay column	: Shim-pack™ GIST HP 3 μm, C18-AQ, 3 x 30 mm
Trap column	: EVOLUTE® Express ABN on-line SPE cartridge
Injection Volume	: 5 x 10 μL
Cooler temperature	: 8 °C
Column Oven	: 50 °C
UHPLC	: Nexera X3

Results

Matrix matched calibration curves were calculated using weighted (1/conc) linear regression with an R² of >0.98 for all PFAS. Exemplary calibration curves and respective MRM-chromatograms at 0.1 ng/mL are shown in Figure 2.

All tested eggs already contained certain PFAS. These PFAS were marked with an asterisk. Lowest calibration point was adapted accordingly. Depending on availability of an appropriate ISTD either internal or external standard method was used for quantification.

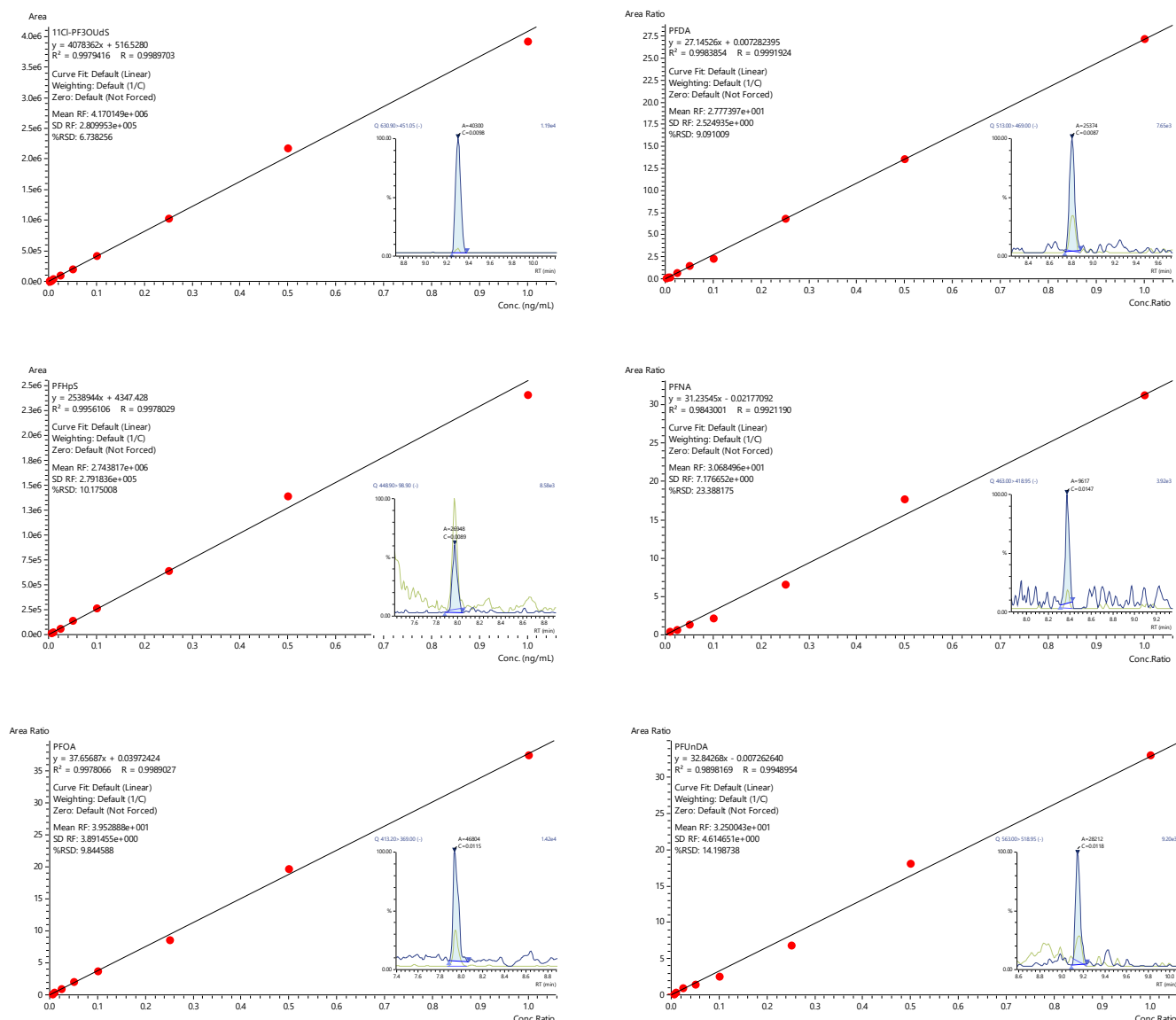


Figure 2 Exemplary calibration curves and a typical chromatogram at 0.01 ng/mL level

Five eggs from different origins were purchased locally and analysed together with the calibration samples. Results are shown in Table 4. In addition, these eggs were spiked with PFAS before extraction at concentrations of 0.01 ng/mL and 0.1 ng/mL.

The percentage relative standard deviation was typically lower than 20% (for 95% of the determined compounds resp. QCs) from these spiked samples (Table 3). Eggs where some PFAS could be detected at a relatively high level were not taken into account for the respective calculations.

Table 2 MRM transitions and calibration information

Acronym	RT	Type	ISTD used	Quantifier	Qualifier	Calibration range	Unit	R ²
11CI-PF3OUdS	9.309	Target	----	630.90>451.05	630.90>82.95	0.001-1	ng_mL	0.9979
9CI-PF3ONS	8.648	Target	PFOS-IS	530.90>351.10	530.90>82.90	0.001-1	ng_mL	0.9989
DONA	7.479	Target	PFHpA-IS	377.10>251.00	377.10>84.95	0.001-1	ng_mL	0.9957
FOSA	9.313	Target	FOSA-IS	497.90>77.90	497.90>478.15	0.01-1	ng_mL	0.9959
FOSA-IS	9.312	ISTD	----	505.90>78.00	505.90>172.00	----	ng_mL	----
HFPO-DA*	6.946	Target	HFPO-DA-IS	284.95>169.05	284.95>185.05	0.01-1	ng_mL	0.9945
HFPO-DA-IS	6.946	ISTD	----	286.85>168.90	286.85>118.85	----	ng_mL	----
PFDoS	9.674	Target	PFDoDA-IS	699.00>79.90	699.00>98.90	0.0025-1	ng_mL	0.9912
PFTTrDS	9.878	Target	PFDoDA-IS	749.00>99.10	749.00>79.90	0.0025-1	ng_mL	0.9867
PEESA	6.538	Target	----	315.00>135.00	315.00>82.90	0.001-1	ng_mL	0.9989
PFBA**	4.547	Target	PFBA-IS	213.00>169.00	----	0.01 -1	ng_mL	0.9846
PFBA-IS	4.541	ISTD	----	216.90>172.00	----	----	ng_mL	----
PFBS**	5.982	Target	PFBS-IS	299.00>79.90	299.00>98.90	0.01 -1	ng_mL	0.9997
PFBS-IS	6.139	ISTD	----	301.90>98.80	301.90>79.80	----	ng_mL	----
PFDA	8.802	Target	PFDA-IS	513.00>469.00	513.00>219.05	0.0025-1	ng_mL	0.9984
PFDA-IS	8.814	ISTD	----	519.00>473.90	519.00>219.00	----	ng_mL	----
PFDoDA	9.454	Target	PFDoDA-IS	613.00>568.95	613.00>169.10	0.01-1	ng_mL	0.9979
PFDoDA-IS	9.451	ISTD	----	614.90>570.10	614.90>269.10	----	ng_mL	----
PFDS	9.155	Target	PFOS-IS	598.80>79.95	598.80>98.85	0.0001-1	ng_mL	0.9971
PFHpA	7.389	Target	PFHpA-IS	363.10>319.00	363.10>169.00	0.0025-1	ng_mL	0.9905
PFHpA-IS	7.381	ISTD	----	367.00>322.10	367.00>169.00	----	ng_mL	----
PFHpS	7.974	Target	----	448.90>98.90	448.90>79.90	0.005-1	ng_mL	0.9956
PFHxA	6.693	Target	PFHxA-IS	313.10>269.00	313.10>119.00	0.01-1	ng_mL	0.9994
PFHxA-IS	6.692	ISTD	----	317.90>273.00	317.90>120.10	----	ng_mL	----
PFHxDA-IS	10.208	ISTD	----	814.90>769.90	814.90>369.00	----	ng_mL	----
PFHxS**	7.468	Target	PFHxS-IS	398.90>79.95	398.90>98.90	0.005-1	ng_mL	0.9988
PFHxS-IS	7.636	ISTD	----	402.00>79.90	402.00>98.80	----	ng_mL	----
PFNA	8.392	Target	PFNA-IS	463.00>418.95	463.00>219.00	0.01-1	ng_mL	0.9843
PFNA-IS	8.375	ISTD	----	471.90>427.00	471.90>223.00	----	ng_mL	----
PFNS	8.809	Target	----	549.10>79.90	549.10>98.90	0.005-1	ng_mL	0.9965
PFOA**	7.943	Target	PFOA-IS	413.20>369.00	413.20>169.05	0.005-1	ng_mL	0.9978
PFOA-IS	7.951	ISTD	----	421.00>376.10	421.00>172.00	----	ng_mL	----
PFOS	8.387	Target	PFOS-IS	498.90>98.90	498.90>169.05	0.025-1	ng_mL	0.9858
PFOS-IS	8.368	ISTD	----	506.90>79.90	506.90>98.80	----	ng_mL	----
PFPeA	5.771	Target	PFPeA-IS	263.10>219.00	263.10>69.10	0.01-1	ng_mL	0.9989
PFPeA-IS	5.861	ISTD	----	267.90>223.00	267.90>69.10	----	ng_mL	----
PFPeS / PFPS	6.992	Target	----	349.20>79.95	349.20>98.95	0.005-1	ng_mL	0.9972
PFTeDA	9.896	Target	PFTeDA-IS	713.00>669.05	713.00>169.05	0.005-1	ng_mL	0.9804
PFTeDA-IS	9.892	ISTD	----	714.90>670.00	714.90>368.90	----	ng_mL	----
PFTTrDA	9.698	Target	PFDoDA-IS	663.00>619.00	663.00>169.00	0.005-1	ng_mL	0.9877
PFUnDA	9.143	Target	PFUnDA-IS	563.00>518.95	563.00>269.05	0.005-1	ng_mL	0.9898
PFUnDA-IS	9.15	ISTD	----	570.00>524.90	570.00>268.90	----	ng_mL	----
PFUnDS	9.601	Target	----	649.00>79.95	649.00>98.95	0.0025-1	ng_mL	0.9917

* Contamination from ISTD

** Contamination from egg matrix

Table 3 Reproducibility of spiked samples

	11CI-PF3OUdS		9CI-PF3ONS		DONA		FOSA		HFPO-DA		L-PFDoS		L-PFTrDS		PEESA		PFBA	
	0.01 ng/mL		0.01 ng/mL		0.01 ng/mL		0.01 ng/mL		0.01 ng/mL		0.01 ng/mL		0.01 ng/mL		0.01 ng/mL		0.01 ng/mL	
	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy
Egg A QC 0.01	0.0101	100.64	0.0097	96.95	0.0102	102.08	0.0117	116.99	0.0088	88.39	0.0104	104.38	0.0067	67.41	0.0102	101.8	below LOQ	
Egg B QC 0.01	0.0107	106.84	0.0093	93.10	0.0096	95.63	0.0103	102.72	0.0062	61.63	0.0149	149.13	0.0117	117.46	0.0101	101.04	below LOQ	
Egg C QC 0.01	0.0092	91.70	0.0104	104.12	0.0100	100.08	0.0115	114.69	0.0112	111.54	0.0102	101.98	0.0108	108.27	0.0104	104.31	below LOQ	
Egg D QC 0.01	0.0113	113.08	0.0093	93.36	0.0087	87.41	0.0116	115.99	0.0099	98.96	0.0100	100.01	0.0061	61.48	0.0105	104.70	below LOQ	
Egg E QC 0.01	0.0109	108.64	0.0096	96.32	0.0098	98.12	0.0101	101.33	0.0073	72.54	0.0113	112.64	0.0117	117.26	0.0102	102.18	below LOQ	
Mean	104.18		96.77		96.66		110.34		86.61		113.63		94.38		102.81			
SD	8.28		4.45		5.70		7.65		19.99		20.42		27.65		1.61			
%RSD	7.95		4.60		5.89		6.94		23.09		17.97		29.30		1.57			

	0.1 ng/mL		0.1 ng/mL		0.1 ng/mL		0.1 ng/mL		0.1 ng/mL		0.1 ng/mL		0.1 ng/mL		0.1 ng/mL		0.1 ng/mL	
	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy
	Egg A QC 0.1	0.0992	99.25	0.0922	92.24	0.1027	102.72	0.0965	96.47	0.1102	110.25	0.1105	110.53	0.0984	98.35	0.1062	106.16	0.1551
Egg B QC 0.1	0.0816	81.65	0.0877	87.75	0.0971	97.10	0.1115	111.48	0.1055	105.46	0.1281	128.08	0.1112	111.16	0.1052	105.17	0.3562	*356.16
Egg C QC 0.1	0.0894	89.42	0.0916	91.58	0.0945	94.55	0.0985	98.47	0.1010	100.99	0.1028	102.79	0.0956	95.60	0.1018	101.79	0.1057	*105.71
Egg D QC 0.1	0.0923	92.31	0.0831	83.09	0.0963	96.33	0.1136	113.57	0.0993	99.32	0.1037	103.71	0.1143	114.29	0.1020	101.96	0.1197	*119.69
Egg E QC 0.1	0.1060	105.99	0.0919	91.92	0.0934	93.35	0.0906	90.64	0.1064	106.37	0.1022	102.22	0.0992	99.21	0.1061	106.13	0.2795	*279.55
Mean	93.72		89.32		96.81		102.13		104.48		109.47		103.72		104.24			
SD	9.32		3.93		3.62		9.95		4.38		10.93		8.40		2.20			
%RSD	9.94		4.40		3.74		9.74		4.19		9.98		8.10		2.11			

	PFBS		PFDA		PFDODA		PFDS		PFHpA		PFHpS		PFHxA		PFHxS		PFNA	
	0.01 ng/mL		0.01 ng/mL		0.01 ng/mL		0.01 ng/mL		0.01 ng/mL		0.01 ng/mL		0.01 ng/mL		0.01 ng/mL		0.01 ng/mL	
	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy
Egg A QC 0.01	0.0100	99.89	0.0124	123.67	0.0081	80.88	0.0097	97.40	0.0097	97.33	0.0099	99.04	0.0107	106.80	0.0101	101.16	0.0093	92.70
Egg B QC 0.01	0.0117	116.9	0.0347	*346.61	0.0314	*314.12	0.0093	92.53	0.0125	*124.63	0.0108	107.58	0.0111	111.20	0.0205	*204.82	0.0253	*252.65
Egg C QC 0.01	0.0119	119.42	0.0107	106.55	0.0106	105.54	0.0088	87.75	0.0096	96.29	0.0103	102.74	0.0103	103.00	0.0089	88.72	0.0153	153.41
Egg D QC 0.01	0.0095	95.05	0.0105	104.55	0.0090	90.09	0.0072	71.81	0.0091	90.63	0.0098	97.96	0.0115	114.63	0.0111	111.11	0.0115	115.49
Egg E QC 0.01	0.0092	92.33	0.0297	*297.08	0.0315	*314.53	0.0085	85.27	0.0148	*147.64	0.0116	116.28	0.0121	121.23	0.0370	*370.26	0.0348	*347.85
Mean	104.72		111.59		92.17		86.95		94.75		104.72		111.37		100.33		120.53	
SD	12.60		10.51		12.46		9.66		3.61		7.48		7.05		11.22		30.67	
%RSD	12.03		9.42		13.52		11.11		3.81		7.14		6.33		11.18		25.44	

	0.1 ng/mL		0.1 ng/mL		0.1 ng/mL		0.1 ng/mL		0.1 ng/mL		0.1 ng/mL		0.1 ng/mL		0.1 ng/mL		0.1 ng/mL	
	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy
	Egg A QC 0.1	0.0975	97.52	0.1148	114.81	0.0974	97.43	0.0901	90.11	0.0973	97.26	0.1074	107.45	0.1039	103.89	0.1038	103.78	0.0906
Egg B QC 0.1	0.0945	94.50	0.1168	116.80	0.1076	107.56	0.0926	92.62	0.1013	101.27	0.1107	110.70	0.0986	98.58	0.1082	108.25	0.0925	92.55
Egg C QC 0.1	0.1001	100.07	0.0832	83.20	0.0863	86.28	0.0979	97.90	0.1051	105.10	0.1127	112.74	0.0986	98.62	0.0816	81.56	0.1043	104.30
Egg D QC 0.1	0.0965	96.50	0.1006	100.57	0.0921	92.13	0.0991	99.13	0.0980	98.05	0.1087	108.71	0.0964	96.41	0.0956	95.64	0.1056	105.57
Egg E QC 0.1	0.0937	93.66	0.1312	131.21	0.1040	104.03	0.0854	85.37	0.0938	93.79	0.1153	115.34	0.1040	103.99	0.1111	111.13	0.1194	119.44
Mean	96.45		109.32		97.49		93.03		99.09		110.99		100.30		100.07		102.49	
SD	2.54		18.19		8.64		5.66		4.28		3.15		3.44		11.89		11.62	
%RSD	2.64		16.64		8.86		6.09		4.32		2.84		3.43		11.88		11.34	

	PFNS		PFOA		PFOS		PFPeA		PFPS		PFTeDA		PFTrDA		PFUnDA		PFUnDS	
	0.01 ng/mL		0.01 ng/mL		0.01 ng/mL		0.01 ng/mL		0.01 ng/mL		0.01 ng/mL		0.01 ng/mL		0.01 ng/mL		0.01 ng/mL	
	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy
Egg A QC 0.01	0.0109	109.26	0.0098	97.74	below LOQ		0.0070	69.92	0.0102	102.39	0.0110	109.83	0.0085	85.08	0.0110	109.54	0.0086	86.25
Egg B QC 0.01	0.0108	108.41	0.0510	*509.67	below LOQ		0.0084	84.20	0.0104	103.83	0.0183	*183.07	0.0265	*265.47	0.0248	*247.73	0.0123	122.98
Egg C QC 0.01	0.0100	99.86	0.0115	114.81	below LOQ		0.0118	117.66	0.0104	103.71	0.0091	90.60	0.0074	73.54	0.0111	111.06	0.0090	90.32
Egg D QC 0.01	0.0095	95.28	0.0105	104.76	below LOQ		0.0104	103.82	0.0097	97.22	0.0115	115.26	0.0085	85.21	0.0111	111.27	0.0082	82.14
Egg E QC 0.01	0.0106	106.16	0.0923	*922.92	below LOQ		0.0091	90.57	0.0113	113.23	0.0196	*196.07	0.0330	*329.81	0.0230	*229.98	0.0111	111.09
Mean	103.79		105.77				93.23		104.08		105.23		81.28		110.62		98.56	
SD	6.02		8.58				18.31		5.78		12.96		6.70		0.94		17.62	
%RSD	5.80		8.11				19.64		5.56		12.31		8.24		0.85		17.88	

	0.1 ng/mL		0.1 ng/mL		0.1 ng/mL		0.1 ng/mL		0.1 ng/mL		0.1 ng/mL		0.1 ng/mL		0.1 ng/mL		0.1 ng/mL	
	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy	Conc.	Accuracy
	Egg A QC 0.1	0.0971	97.10	0.1009	100.88	0.0856	85.60	0.0986	98.56	0.1131	113.11	0.1189	118.90	0.1067	106.69	0.1024	102.41	0.0900
Egg B QC 0.1	0.0855	85.53	0.1410	*140.97	0.3793	*379.3	0.1044	104.40	0.1036	103.57	0.1480	*148.04	0.1265	126.50	0.1198	119.76	0.0796	79.64
Egg C QC 0.1	0.0881	88.14	0.1042	104.22	0.0953	95.29	0.0976	97.58	0.1015	101.55	0.1143	114.29	0.0943	94.32	0.0992	99.23	0.0894	89.45
Egg D QC 0.1	0.1008	100.85	0.0908	90.83	0.0999	99.93	0.0949	94.93	0.1006	100.64	0.1053	105.34	0.1060	105.96	0.0976	97.57	0.0794	79.39
Egg E QC 0.1	0.1035	103.45	0.1707	*170.69	0.43	*429.96	0.0955	95.49	0.1087	108.69	0.1514	*151.42	0.1179	117.94	0.1181	118.07	0.0956	95.59
Mean	95.01		98.64		93.61		98.19		105.51		112.84		110.28		107.41		86.81	
SD	7.85		6.97		7.31		3.77		5.27		6.89		12.33		10.66		7.08	
%RSD	8.27		7.07		7.81		3.84		4.99		6.11		11.18		9.93		8.16	

*compound already found in sample

Table 4 Sample results (positive results only)

	PFBA	PFBS	PFDA	PFDODA	PFHpA	PFHpS	PFHxS	PFNA	PFOA	PFOS	PFPeA	PFTeDA	PFTrDA	PFUnDA	PFUnDS
	Conc.	Conc.	Conc.	Conc.	Conc.	Conc.	Conc.	Conc.	Conc.	Conc.	Conc.	Conc.	Conc.	Conc.	Conc.
Egg A	<LOQ	<LOQ	----	<LOQ	----	----	----	----	----	----	----	----	----	----	----
Egg B	0.2662	<LOQ	0.0221	0.0167	0.0032	----	0.0125	0.0273	0.0411	0.3121	----	----	0.0125	0.0182	0.0144



Figure 3 Scheme of the Nexera on-line SPE LCMS-8060NX system

■ The Package

□ Main Unit

LCMS-8060NX:	TQ Mass spectrometer
Nexera X3:	Liquid chromatograph
	CBM-40
	DGU-405
	2x LC-40D X3
	LC-40B X3
	SIL-40C X3
	CTO-40S
	2x Reservoir Tray

□ Accessory

Valve:	FCV-0206H3
Mixer:	2x Mir20 μ L
Loop:	50 μ L

□ Main Consumables:

Shim-pack Scepter C18 (50 mm x 2.1 mm I.D., 1.9 μ m; P/N 227-31012-03)
Shim-pack GIST HP C18-AQ (2x) (30 mm x 3.0 mm I.D., 3 μ m; P/N 227-30766-01)
EVOLUTE® Express ABN on-line SPE cartridge (Biotage) (30 mm x 2.1 mm I.D.; P/N OSPE-620-32150)
Shimadzu LabTotal Vial for LC/LCMS (P/N 227-34001-01)
RESTEK® Q-Sep QuEChERS Extraction Packets / AOAC Method (P/N 25851)

□ Software and Libraries

LabSolutions LCMS
LabSolutions Insight

■ Conclusions

This application note describes an on-line SPE LC-MS/MS method to monitor 27 PFAS and internal standards in egg matrix. This proof of concept study using the LCMS-8060NX coupled with a Nexera UHPLC system equipped for on-line SPE demonstrates a sensitive method for PFAS analysis in egg matrix with minimal sample preparation steps.

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