Application News

Liquid Chromatograph Mass Spectrometer LCMS-8060NX

Simultaneous Quantitation of 32 PFAS in Baby Food by LC-MS/MS

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User Benefits

◆ A simple QuEChERS extraction procedure has been employed for quantifying the PFAS with normal dSPE cleanup.

◆ This method complies with all acceptance criteria (as per AOAC SMPR 2023.003) without using ISTDs.

◆ Method with shorter run time can contribute to achieving high throughputs in testing laboratories.

■ Introduction

PFAS (Per- and polyfluoroalkyl substances) are a class of highly persistent chemicals widely known as "forever chemicals" due to their strong resistance to environmental degradation (Figure 1). Found in products like firefighting foams, non-stick cookware, and water-resistant fabrics, PFAS are now recognized as major environmental and health concerns. These substances accumulate in soil, water, and living organisms, including humans, where they may cause serious health impacts over time.

Given their potential for long-term contamination, the detection and quantification of PFAS are critical. Advanced analytical methods, particularly LC-MS/MS with Multiple Reaction Monitoring (MRM), offer the specificity and sensitivity needed to accurately measure PFAS concentrations. However, one of the primary challenges in PFAS analysis is minimizing background contamination from PFAS residues within the LC system and during sample collection and preparation. Effective strategies to reduce these sources of contamination are essential for reliable results in PFAS quantification.
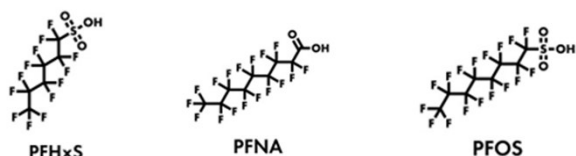

Figure 1: Structure of PFHxS, PFNA and PFOS

In this application news, we describe a single laboratory validation study with a rapid extraction of trace level concentrations of 32 PFAS (Table 1) in infant formula (baby food) involving the use of the QuEChERS as an extraction

technique followed by mass spectrometric analysis using a Shimadzu LCMS-8060NX triple quadrupole mass spectrometer coupled to a Shimadzu Nexera™ X3 liquid chromatograph (Figure 2).

■ Material and Method

The reference standards were procured from Dr. Ehrenstorfer with below catalogue numbers:
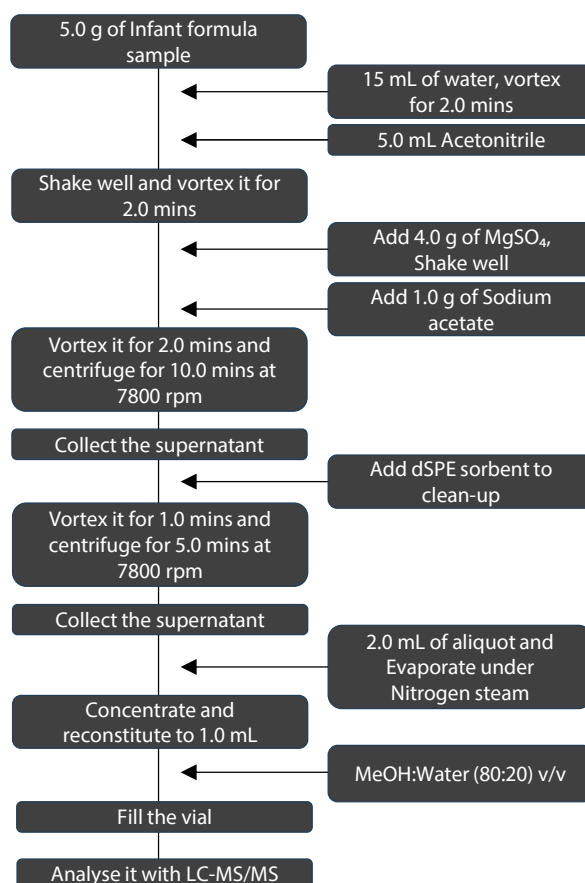EPA Method 533 PFAS Mixture 151 100 µg/mL (DRE-A50000151MW).
The calibration standards were analyzed in the range of 0.005 to 10 µg/L (for some compounds 0.05-10 µg/L and for PFBA was 0.25-10 µg/L). Calibration curves were generated by external standard method with weighted regression of $1/C^2$. Fortified samples were prepared in six replicates of each 0.01, 0.05 and 0.25 µg/kg.

Compared the recovery and precision against the requirements of AOAC SMPR 2023.003. In addition, we determined the Limit of Quantitation (LOQ) as the lowest concentration meeting recovery accuracy and precision. All recovery, precision, and LOQ's met the acceptance criteria. We optimized the chromatography and instrument operating parameters to achieve excellent peak shape, separation, and sensitivity (Figure 3).

Figure 2: Shimadzu LCMS-8060NX triple quadrupole mass spectrometer coupled to a Nexera™ X3 UHPLC

■ Sample Preparation

- 5.0 g of Infant formula sample
  - 15 mL of water, vortex for 2.0 mins
  - 5.0 mL Acetonitrile
- Shake well and vortex it for 2.0 mins
  - Add 4.0 g of $MgSO_4$, Shake well
  - Add 1.0 g of Sodium acetate
- Vortex it for 2.0 mins and centrifuge for 10.0 mins at 7800 rpm
- Collect the supernatant
  - Add dSPE sorbent to clean-up
- Vortex it for 1.0 mins and centrifuge for 5.0 mins at 7800 rpm
- Collect the supernatant
  - 2.0 mL of aliquot and Evaporate under Nitrogen steam
- Concentrate and reconstitute to 1.0 mL
  - MeOH:Water (80:20) v/v
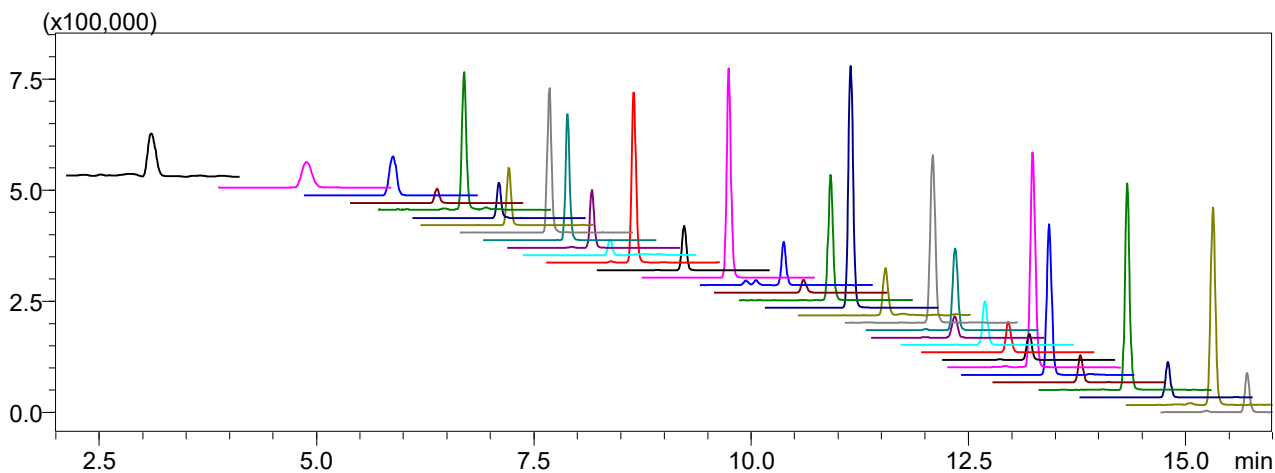- Fill the vial
- Analyse it with LC-MS/MS

Figure 3: Representative Total ion Chromatograph of 0.1 μg/kg PFAS in Infant formula (baby food) matrix with separation of all peaks in twenty minutes

Table 1: PFAS Analytes, Acronyms, CAS No. and SMPR LOQ

Native PFAS analyte	Acronym	CAS No.	LOQ as per SMPR (ppb)
Perfluorobutanoic acid	PFBA	375-22-4	1.0
Perfluoropentanoic acid	PFPeA	2706-90-3	1.0
Perfluorobutanesulfonic acid	PFBS	375-73-5	0.1
1H,1H, 2H, 2H-Perfluorohexane sulfonic acid	4-2 FTS	757124-72-4	0.1
Perfluorohexanoic acid	PFHxA	307-24-4	0.1
Hexafluoropropylene oxide dimer acid	HFPO-DA	13252-13-6	0.1
Perfluoropentansulfonic acid	PFPeS	2706-91-4	0.1
Perfluoroheptanoic acid	PFHpA	375-85-9	0.1
4,8-Dioxa-3H-perfluorononanoic acid	DONA	919005-14-4	0.1
Perfluorohexanesulfonic acid	PFHxS	355-46-4	0.01
1H,1H, 2H, 2H-Perfluorooctane sulfonic acid	6-2 FTS	27619-97-2	0.1
Perfluorooctanoic acid	PFOA	335-67-1	0.01
Perfluoroheptanesulfonic acid	PFHpS	375-92-8	0.1
Perfluorononanoic acid	PFNA	375-95-1	0.01
Perfluorooctanesulfonic acid	PFOS	1763-23-1	0.01
1H,1H, 2H, 2H-Perfluorodecane sulfonic acid	8-2 FTS	39108-34-4	0.1
Perfluorodecanoic acid	PFDA	335-76-2	0.1
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonic acid	9Cl-PF3ONS	756426-58-1	0.1
Perfluorononanesulfonic acid	PFNS	68259-12-1	0.1
Perfluoroundecanoic acid	PFUnA	2058-94-8	0.1
Perfluorooctanesulfonamide	PFOSA	754-91-6	0.1
N-methyl Perfluorooctanesulfonamidoacetic Acid	N-MeFOSAA	2355-31-9	0.1
Perfluorodecanesulfonic acid	PFDS	335-77-3	0.1
1H,1H, 2H, 2H-Perfluorododecane sulfonic acid	10-2 FTS	120226-60-0	0.1
N-ethyl Perfluorooctanesulfonamidoacetic Acid	N-MeFOSAA	2991-50-6	0.1
Perfluorododecanoic acid	PFDoA	307-55-1	0.1
11-Chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	11Cl-PF3OUdS	763051-92-9	0.1
Perfluoroundecanesulfonic acid	PFUnDS	749786-16-1	0.1
Perfluorotridecanoic acid	PFTriDA	72629-94-8	0.1
Perfluorododecanesulfonic acid	PFDoS	79780-39-5	0.1
Perfluorotetradecanoic acid	PFTeDA	376-06-7	0.1
Perfluorotridecanesulfonic acid	PFTrDS	791563-89-8	0.1

■ Results and Discussion

Validation parameters like specificity, linearity, recovery and precision were studied as per SANTE guidelines. Results obtained on LC-MS/MS are shown in Table 2.

❖ System precision and specificity

System precision was evaluated by calculating variation of the peak area and retention time of six replicates of 1 µg/kg PFAS mixture. The % RSD was found to be less than 20 for peak area and retention times were within tolerance limit of ±0.1 min. Specificity of the method was determined by comparing the response of blank sample (reagent and matrix) against reporting level. Response in reagent/matrix blank sample was well within (<30 %) of the reporting limit and met the acceptance criteria.

❖ Linearity study

For linearity study, matrix-matched calibration standards were used. Calibration curve ranges from 0.005 to 10 µg/L (for three compounds 0.05-10 µg/L and for PFBA was 0.25-10 µg/L). All calibration standards showed % accuracies within 80 to 120 % which is acceptable as per SANTE guidelines. The LOQ level chromatograms and linearity graphs of representative compounds are shown in Figure 4.

❖ Recovery study

Recovery was evaluated by analysing fortified samples at 0.01, 0.05 and 0.25 µg/kg concentration level with six replicates at each level against matrix-matched calibration curve. Mean recoveries for most of the compounds were found within 65-135 %. As per AOAC SMPR, all the compounds were found to be reproducible at their LOQ levels.

❖ Precision study

For precision, repeatability and within-laboratory reproducibility studies were carried out. Concentrations of fortified samples were back calculated against matrix matched linearity.

❖ Repeatability (RSD$_r$)/ Reproducibility (RSD$_R$)

The % RSD for repeatability of six injections at 0.01, 0.05 and 0.25 µg/kg concentration levels were found to be less than 25 %,
Reproducibility experiment for recoveries was performed on six different fortified samples at 0.01, 0.05 and 0.25 µg/kg concentration levels. The % RSD for recovery of six fortified samples at their respective LOQ levels were found to be less than 40 %.

Table 2: Summary results of LC-MS/MS analysis

Sr. No.	Compound Name	Ret. Time (min)	R^2	Target Ion	Ref. Ion	LOQ (ppb)	RSD$_r$ (%)	RSD$_R$ (%)	Recovery (%)
1	PFBA	3.107	0.9958	213.00>169.10	----	0.25	19.50	1.60	113.32
2	PFPeA	4.891	0.9927	263.20>219.15	----	0.01	16.70	5.73	83.17
3	PFBS	5.893	0.9673	299.10>80.00	299.10>98.95	0.01	11.49	8.43	110.05
4	4-2 FTS	6.394	0.9930	327.20>80.90	327.20>306.95	0.05	15.33	11.54	90.89
5	PFHxA	6.701	0.9964	312.90>269.10	312.90>119.10	0.01	6.45	6.94	96.07
6	HFPO-DA	7.100	0.9919	285.00>185.10	285.00>169.20	0.01	18.07	10.77	100.37
7	PFPeS	7.216	0.9924	349.10>99.00	349.10>79.95	0.01	13.16	8.53	110.9
8	PFHpA	7.683	0.9947	363.20>319.05	363.20>169.05	0.01	7.01	9.64	111.01
9	DONA	7.894	0.9960	377.10>251.00	377.10>84.90	0.01	4.70	6.20	119.90
10	PFHxS	8.172	0.9953	398.90>80.00	398.90>98.95	0.01	6.24	6.78	115.62
11	6-2 FTS	8.377	0.9958	426.90>406.95	426.90>386.90	0.05	4.12	6.42	113.28
12	PFOA	8.657	0.9951	412.90>368.95	412.90>219.10	0.01	9.04	6.26	117.1
13	PFHpS	9.231	0.9949	449.10>79.85	449.10>169.15	0.01	9.52	9.15	118.86
14	PFNA	9.750	0.9955	463.10>418.95	463.10>169.05	0.01	12.11	7.62	91.97
15	PFOS	10.384	0.9927	498.90>79.95	498.90>98.90	0.01	11.92	13.48	94.48
16	8-2 FTS	10.615	0.9932	526.80>506.95	526.80>81.00	0.01	10.45	12.11	116.55
17	PFDA	10.919	0.9973	513.10>468.95	513.10>169.10	0.01	6.02	7.14	115.24
18	9Cl-PF3ONS	11.153	0.9976	530.90>350.95	533.00>352.95	0.01	4.52	5.67	111.38
19	PFNS	11.559	0.9976	549.00>98.95	549.00>79.95	0.01	9.76	3.24	108.28
20	PFUnA	12.101	0.9965	563.10>519.00	563.10>169.30	0.01	16.96	10.83	115.76
21	PFOSA	12.360	0.9970	498.10>77.90	498.10>477.85	0.01	11.90	7.73	104.67
22	N-Me-FOSAA	12.346	0.9836	570.10>418.90	570.10>511.90	0.05	13.41	7.88	63.07
23	PFDS	12.703	0.9977	598.90>79.95	598.90>98.95	0.01	14.36	11.25	109.3
24	10-2 FTS	12.969	0.9942	626.90>606.90	626.90>80.90	0.01	13.99	7.48	72.03
25	N-Et-FOSAA	13.215	0.9836	584.10>419.00	584.10>525.95	0.01	13.26	8.81	70.50
26	PFDoA	13.248	0.9967	612.80>169.10	612.80>568.90	0.01	4.86	5.03	117.82
27	11ClPF3OUdS	13.443	0.9964	630.70>450.90	631.10>198.90	0.01	10.93	5.78	114.78
28	PFUnDS	13.802	0.9925	648.90>80.05	648.90>98.90	0.01	9.16	11.57	113.27
29	PFTriA	14.336	0.9929	662.90>618.90	662.90>269.00	0.01	8.59	14.34	105.5
30	PFDoS	14.800	0.9967	698.90>79.90	698.90>98.90	0.01	13.99	4.98	106.13
31	PFTeDA	15.321	0.9957	713.00>668.95	713.00>468.90	0.01	8.79	13.62	90.24
32	PFTrDS	15.711	0.9987	749.00>230.00	749.00>98.85	0.01	6.05	18.20	115.62
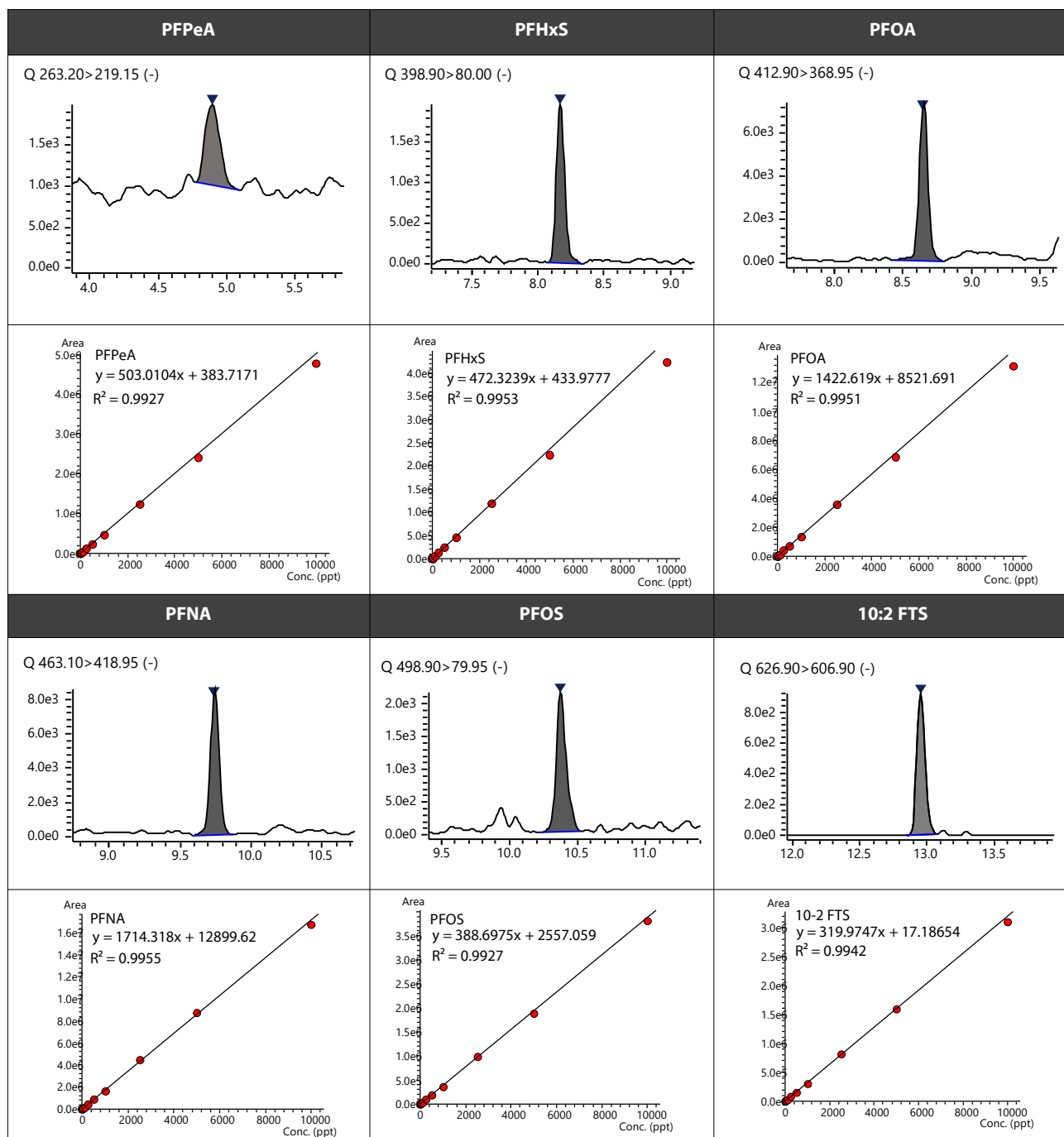
Figure 4: Representative linearity graphs and chromatograms at LOQ level

The charts show:

PFPeA — Q 263.20>219.15 (-); $y = 503.0104x + 383.7171$, $R^2 = 0.9927$

PFHxS — Q 398.90>80.00 (-); $y = 472.3239x + 433.9777$, $R^2 = 0.9953$

PFOA — Q 412.90>368.95 (-); $y = 1422.619x + 8521.691$, $R^2 = 0.9951$

PFNA — Q 463.10>418.95 (-); $y = 1714.318x + 12899.62$, $R^2 = 0.9955$

PFOS — Q 498.90>79.95 (-); $y = 388.6975x + 2557.059$, $R^2 = 0.9927$

10:2 FTS — Q 626.90>606.90 (-); $y = 319.9747x + 17.18654$, $R^2 = 0.9942$

■ Conclusion

✓ A simple, sensitive, and rapid LC-MS/MS method has been developed to quantify 32 PFAS compounds in infant formula.

✓ This method utilizes a QuEChERS-based extraction technique for efficient sample preparation.

✓ With Shimadzu's LC-MS/MS, the method demonstrated high sensitivity and reproducibility, achieving low relative standard deviations (RSD_r and RSD_R) in accordance with AOAC SMPR standards at trace levels.

✓ This approach offers a reliable solution for multi-PFAS analysis in infant formula, making it highly applicable for testing laboratories focused on food safety.

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