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Application News

High Performance Liquid chromatograph Mass Spectrometer LCMS-8060NX

Analysis of Per- and Polyfluoroalkyl Substances (PFAS) Using Triple Quadrupole Mass Spectrometer Part 2 -Milk-

Nozomi Maeshima

User Benefits

- The optimized procedure for pretreatment and LC-MS/MS analytical conditions enable accurate quantification of thirty major PFASs targeted by AOAC SMPR from 0.01 µg/kg in milk.
- The method allows the initiation of PFASs analysis in food.

■ Introduction

Per- and Polyfluoroalkyl Substances (PFASs) are a collective name for more than four thousand organofluorine compounds. Perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) are representative compounds of PFAS. They are used in a wide range of applications, such as fire retardants, food packaging materials, and non-stick coatings, due to their water-repellent, oil-repellent, heat-resistant, and chemical-resistant properties. Due to their structural stability, PFAS widely remains in the environment.

Recent studies have revealed that PFAS can transfer into dairy products when dairy cows ingest feed or water contaminated with PFAS[1]. There are concerns about the potential health risks caused by human ingestion of PFAS-contaminated dairy products. Therefore, quantitative assessment of PFAS levels in dairy products should be important. To monitor PFAS concentrations in dairy products, a highly accurate and sensitive quantification method is essential.

This application news introduces a quantitative analysis of PFAS in dairy milk with LC-MS/MS. Thirty PFASs targeted by AOAC INTERNATIONAL[2] were analyzed and evaluated by spike recovery test. By optimizing the analytical conditions, good recoveries were obtained for all compounds.

■ Sample and pretreatment

As standards and internal standards (ISTDs), L-PFUdS, L-PFTrDS, L-PFDoS, and 10:2 FTS and two standard mixtures of PFAC30PAR and MPFAC-HIF-ES were purchased from Wellington Laboratories. Whole milk was purchased at a local grocery store and stored at 4˚C until pretreatment.

Extraction and purification were performed using the QuEChERS method based on a method published by the FDA[3]. The procedure is shown in Fig. 2 and 3.

10 g of milk sample was weighed into a 50 mL tube, followed by the addition of 10 µL of ISTDs, 10 mL of acetonitrile, and 150 µL of formic acid, and shaken vigorously by hand for 10 seconds. One packet of QuEChERS extraction salt (Supel QuE, Sigma-Aldrich, P/N: 55295-U) was added and immediately shaken vigorously by hand for 10 seconds, then shaken on a shaker for another 5 minutes. The sample was centrifuged at 4,000 rpm at room temperature for 5 minutes, and 8 mL of the acetonitrile layer was transferred to a new 50 mL tube.

Supel QuE PSA/ENVI-Carb Tube 3 (Sigma-Aldrich, P/N: 55479-U) was added as a dispersive SPE (d-SPE) sorbent, and the mixture was immediately shaken vigorously by hand for 10 seconds, then shaken on a shaker for another 5 minutes. The sample was centrifuged again at 4,000 rpm at room temperature for 5 minutes, and 6 mL of the supernatant was collected into a new tube.

The sample was concentrated to less than 1 mL using TurboVap LV blowdown evaporator (Biotage) with nitrogen gas and then adjusted to a final volume of 1 mL with 90% methanol aqueous solution. Based on preliminary experiments, complete drying of the sample solution resulted in significantly reduced recoveries of PFOSA, NMeFOSA, NEtFOSA, NMeFOSE, and NEtFOSE. Therefore, care was taken to avoid reducing the sample solution volume to less than 0.5 mL during nitrogen blow-down. Following vortexing for 10 seconds, the solution was transferred to 1.5 mL tubes and centrifuged at 15,000 rpm 4˚C for 10 minutes. The supernatant was then transferred to vial for LC-MS/MS analysis.

■ Analytical conditions

Analysis was performed using a triple quadrupole mass spectrometer LCMS-8060NX equipped with an ultra-high performance liquid chromatograph Nexera™ X3 UHPLC (Fig. 1). The analytical conditions are shown in Table 1. To prevent interference caused by PFAS contamination from solvents, a delay column was installed between mixer and autosampler using SUS piping (300 mm x 0.3 mm I.D., P/N: 228-69955-41). The delay column increased the elution time of PFAS derived from solvents, allowing it to be separated from the PFAS in the sample. PP vials (Shimadzu GLC, P/N: GLC-IVS-100) confirmed to have no detectable PFAS were observed.

Fig. 1 Nexera™ X3 and LCMS-8060NX

Table 1 Analytical conditions of LC-MS/MS

[HPLC conditions] Nexera X3

Column	: Shim-pack Scepter™ C18-120 (100 mm x 2.1 mm I.D., 3 µm)*1
Delay column	: Shim-pack Scepter C18-120 (50 mm x 2.1 mm I.D., 3 µm)*2
Mobile phase A	: 2 mmol/L Ammonium Acetate in Acetonitrile/Water (5:95, v/v)
Mobile phase B	: Acetonitrile
Flow rate	: 0.3 mL/min (0.6 mL/min only between 10.01-12 min)
Gradient program	: B conc. 20% (0 min) → 100% (10-12 min) → 20% (12.01-15 min) The flow was introduced into the mass spectrometer between 1 to 9.6 min using a flow switching valve.
Column temp.	: 40˚C
Injection volume	: 5 µL (Co-injection of 10 µL of water before and after injection.)

[MS conditions] LCMS-8060NX

Ionization	: ESI, Negative mode
Nebulizing gas	: 2 L/min
Heating gas	: 10 L/min
Drying gas	: 10 L/min
DL temp.	: 200˚C
Interface temp.	: 250˚C
Heat block temp.	: 400˚C
Probe position	: +2 mm

*1 227-31014-05
*2 227-31014-03

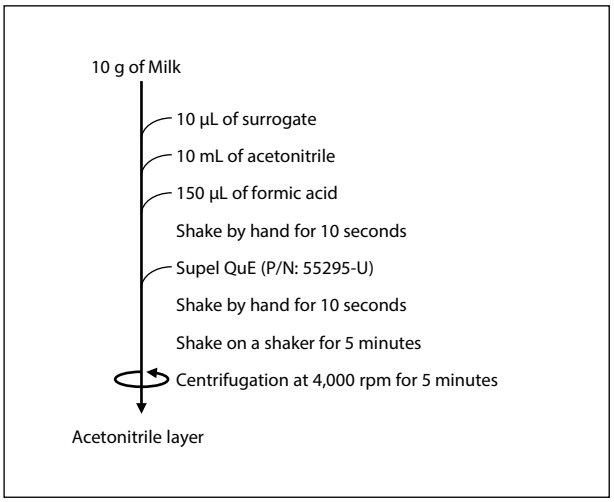

Fig. 2 Extraction procedure

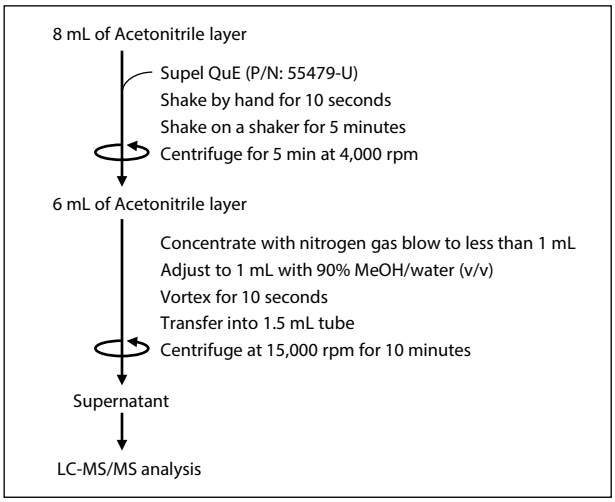

Fig. 3 Purification procedure

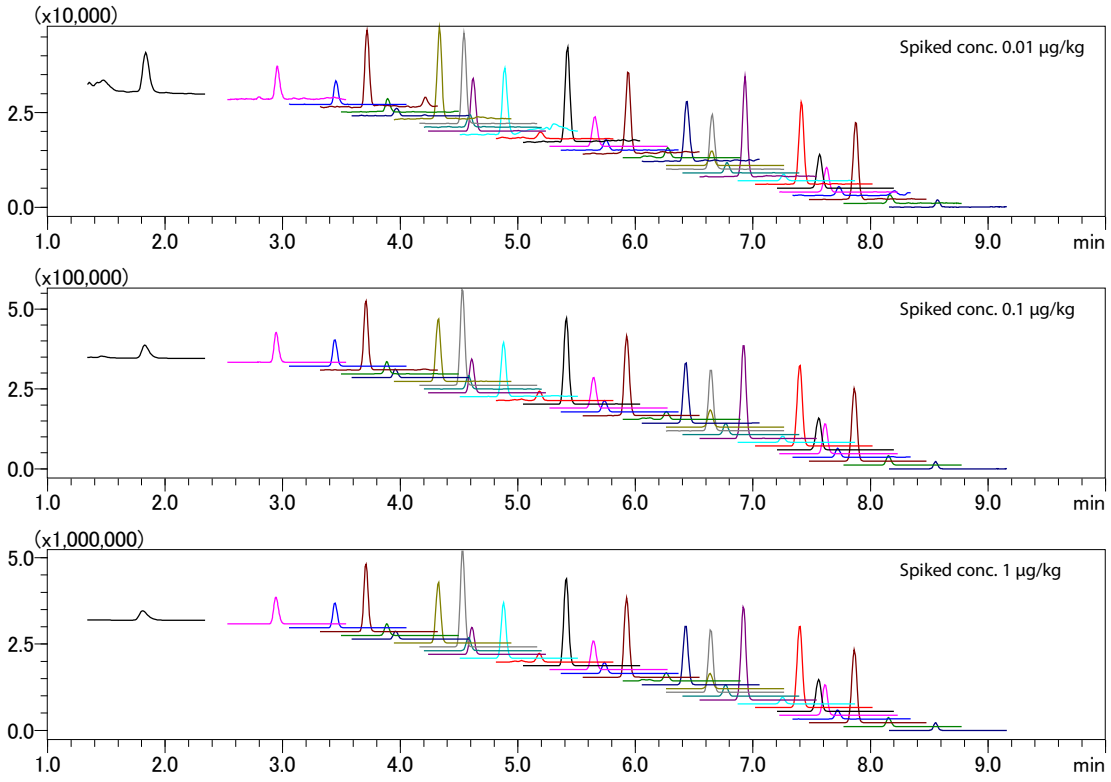

Fig. 4 MRM chromatograms of spiked PFAS in milk samples

■ MRM chromatograms and calibration curves

MRM chromatograms of simultaneous analysis for thirty PFAS are shown in Fig. 4. All compounds were eluted within 8 min in great separation. Taurodeoxycholic acid (TDCA), taurochenodeoxycholic acid (TCDCA), and tauroursodeoxycholic acid (TUDCA), which were concerned to affect the MRM transitions of PFOS, were confirmed to be sufficiently separated from PFOS (data not shown).

Calibration curves for representative compounds are shown in Fig. 5. The standard compounds spiked into milk sample along with ISTD were subjected to the pretreatment procedure, and a calibration curve was created in the range from 0.005 to 1 µg/kg for all target compounds. Internal standards shown in Table 2 were used for the correction of peak area values. The coefficients of determination (R^2) were higher than 0.99 for all compounds, indicating good linearity was achieved. The concentration of the solution in the vial ranged from 0.03 to 6 ng/mL.

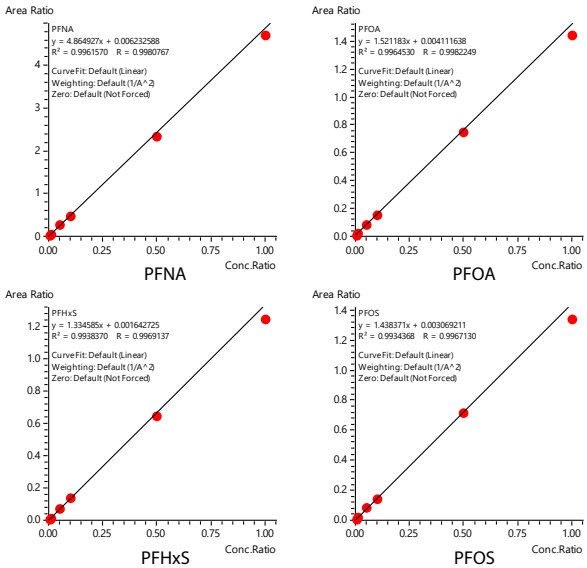

Fig. 5 Calibration curve at spiked concentrations ranging from 0.005 to 1 µg/kg

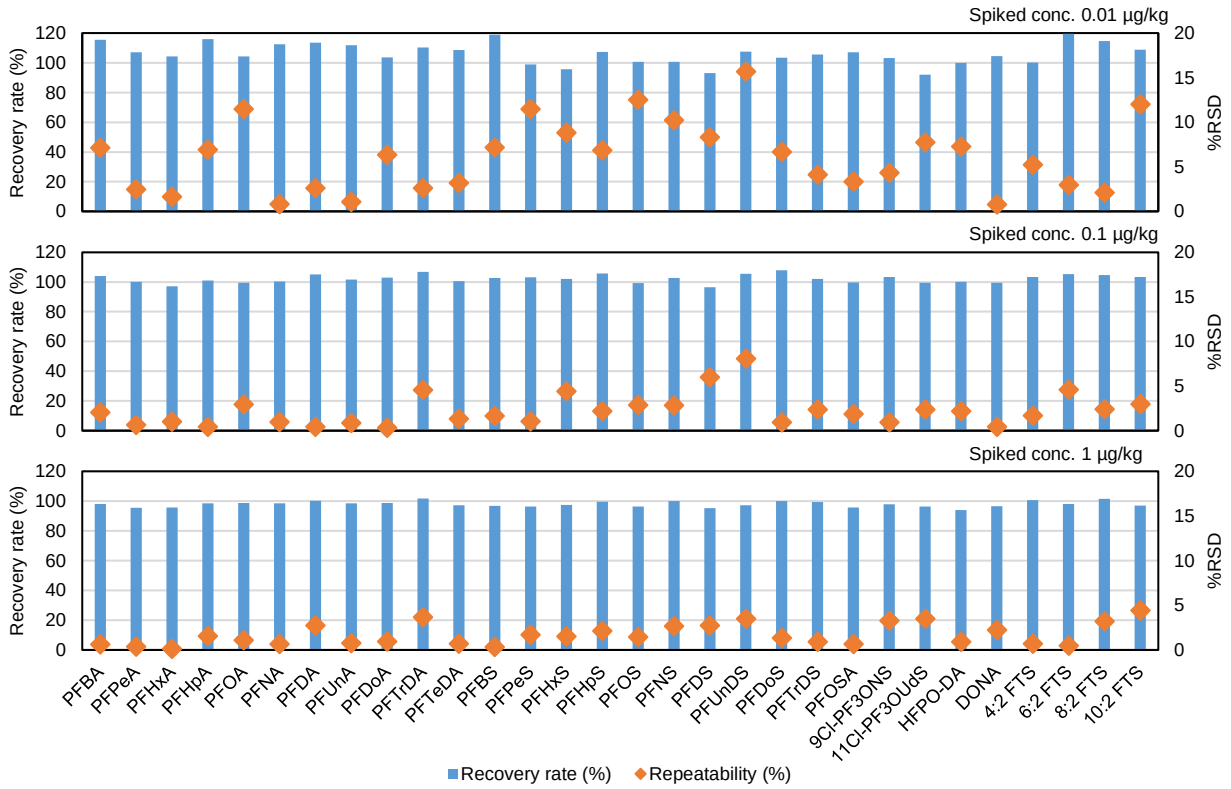

Fig. 6 Recovery rate and repeatability of QC samples (n = 3)

■ Recovery test

Recovery tests were conducted at 0.01, 0.1, and 1 µg/kg, and the recovery rates and repeatability were evaluated. The results are shown in Fig. 6. The procedure was performed from pretreatment in triplicate, and quantification was carried out using a matrix-matched calibration curve. According to the requirements of AOAC SMPR, PFOS, PFOA, PFNA, and PFHxS are specified to have limit of quantification (LOQ) of 0.01 µg/kg or less, a recovery rate within 65-135%, and repeatability of 25% or less. The LOQs for other PFAS are 1.0 µg/kg for PFBA and PFPeA, and 0.1 µg/kg for the others (Table 3).

In this validation, recovery rates for all compounds at a spiked concentration of 0.01 µg/kg were 92.0-119.6% with repeatability of 15.7% or less, 96.6-107.8% with repeatability of 8.1% or less at an added concentration of 0.1 µg/kg, and 93.9-101.7% with repeatability of 4.4% or less at an added concentration of 1 µg/kg.

Table 2 Combination of compound and ISTD

Compound	ISTD	Compound	ISTD
PFBA	$^{13}C_4$-PFBA	PFOS	$^{13}C_8$-PFOS
PFPeA	$^{13}C_5$-PFPeA	PFNS	$^{13}C_7$-PFUnA
PFHxA	$^{13}C_5$-PFHxA	PFDS	$^{13}C_2$-PFDoA
PFHpA	$^{13}C_4$-PFHpA	PFUnDS	$^{13}C_8$-PFOSA
PFOA	$^{13}C_8$-PFOA	PFDOS	$^{13}C_2$-PFTeDA
PFNA	$^{13}C_9$-PFNA	PFTrDS	$^{13}C_2$-PFTeDA
PFDA	$^{13}C_6$-PFDA	PFOSA	$^{13}C_8$-PFOSA
PFUnA	$^{13}C_7$-PFUnA	9Cl-PF3ONS	$^{13}C_8$-PFOS
PFDOA	$^{13}C_7$-PFUnA	11Cl-PF3OUdS	$^{13}C_3$-HFPO-DA
PFTrDA	$^{13}C_8$-PFOSA	HFPO-DA	$^{13}C_3$-HFPO-DA
PFTeDA	$^{13}C_2$-PFTeDA	DONA	$^{13}C_4$-PFHpA
PFBS	$^{13}C_3$-PFBS	4:2 FTS	$^{13}C_2$-4:2 FTS
PFPeS	$^{13}C_4$-PFHpA	6:2 FTS	$^{13}C_2$-6:2 FTS
PFHxS	$^{13}C_3$-PFHxS	8:2 FTS	$^{13}C_2$-8:2 FTS
PFHpS	$^{13}C_6$-PFDA	10:2 FTS	$^{13}C_2$-8:2 FTS

Table 3 Criteria from AOAC SMPR

Compound	LOQ (µg/kg)	Recovery (%)	Repeatability (%)
PFOA	≦0.01	65-135	≦25
PFNA	≦0.01	65-135	≦25
PFHxS	≦0.01	65-135	≦25
PFOS	≦0.01	65-135	≦25
PFBA and PFPeA	≦1.0	-	-
Other PFAS	≦0.1	-	-

■ Conclusion

This application news describes the analysis of thirty PFAS targeted by AOAC INTERNATIONAL in dairy milk samples. The analysis was performed using LCMS-8060NX coupled with a Nexera X3 UHPLC system. A Shim-pack Scepter column was employed to achieve good separation and peak shape. Recovery tests demonstrated satisfactory results, with recovery rates within 92.0-119.6% and repeatability below 15.7% for all compounds at spiking concentrations of 0.01, 0.1, and 1 µg/kg. Notably, PFOA, PFNA, PFHxS, and PFOS showed recovery rates within 95.7-112.5% at all spiking concentrations.

Reference

1) E.D. Van Asselt, et al. Transfer of perfluorooctane sulfonic acid (PFOS) from contaminated feed to dairy milk, Food Chem. 141 (2013) 1489–1495
2) AOAC SMPR®2023.003
3) Determination of 30 Per and Polyfluoroalkyl Substances (PFAS) in Food and Feed using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS), FDA, C-010.03

01-00770-EN First Edition: Apr. 2025

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