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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 1 -Fish Fillet-

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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 0.1 µg/kg.
- 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. There are concerns about health risks caused by human ingestion of fish that have ingested seawater, river water, or feed contaminated with PFAS. Therefore, quantitative assessment of PFAS levels in fish should be important.

This application news introduces a quantitative analysis of PFAS in tuna fillets with LC-MS/MS. Thirty principal PFASs targeted by AOAC INTERNATIONAL, a North American organization that standardizes food testing methods and verifies analytical methods, were analyzed using newly created method, which was investigated from the pretreatment procedure then validated by recovery test. By reducing losses during the pretreatment and optimizing the analytical conditions, good recovery rates 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. Tuna fillet was purchased at a local grocery store, and frozen and crushed with dry ice using a freeze grinder FST-4000 (AiSTI SCIENCE). The samples were left in a freezer overnight, and the dry ice was removed before the pretreatment.

Extraction was performed using the QuEChERS method as a reference. The procedure is shown in Fig. 2. 10 g of frozen crushed sample was weighed, added ISTDs and 10 mL of acetonitrile, and shaken vigorously for 1 min. One packet of QuEChERS extraction salt (Restek, P/N: 25849) was added and immediately shaken vigorously by hand for 1 min then centrifuged at 4,000 rpm for 5 min at room temperature. Acetonitrile layer was collected then diluted 5-fold with water and used as extract.

EVOLUTE PFAS (Biotage, P/N: 614-0015-CP, 150 mg/6 mL) and a positive pressure manifold PRESSURE+ (Biotage) were used for solid-phase extraction. The procedure is shown in Fig. 3. After conditioning with 5 mL of 28% ammonium hydroxide/methanol (1:100, v/v) and 5 mL of formic acid/methanol (1:1000, v/v), 40 mL of extraction solution (equivalent to 8 g of fish sample) was loaded. Following washing with 5 mL of water and 5 mL of formic acid/methanol/water (1:400:600, v/v/v), the analytes were eluted with 5 mL of 28% ammonium hydroxide/methanol/water (1:90:10, v/v/v). The eluate was taken 500 µL and mixed with 2 µL of formic acid, then analyzed by LC-MS/MS.

■ 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 analytical equipment, solvents and glassware, 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 the LC system, 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 loaded onto the mass spectrometer between 1 to 9.6 min using a flow switching valve.
Column temp.	: 40°C
Injection volume	: 5 µL
[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

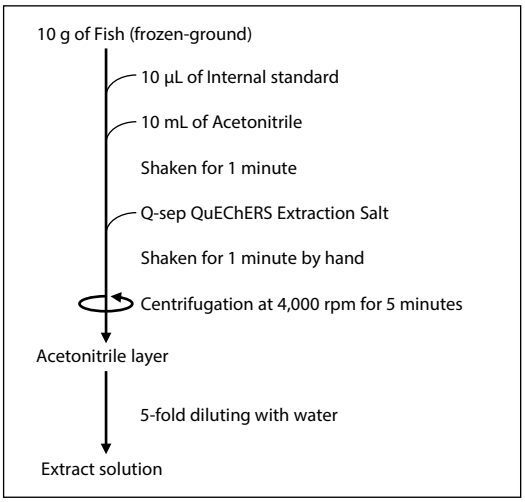

Fig. 2 Extraction procedure

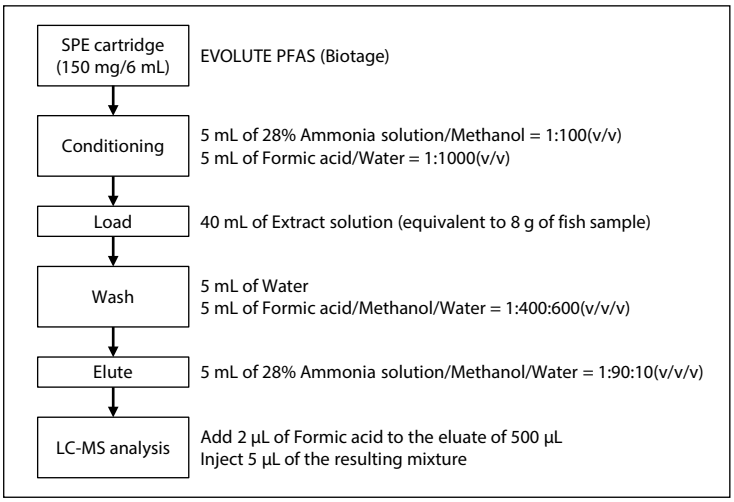

Fig. 3 Purification procedure

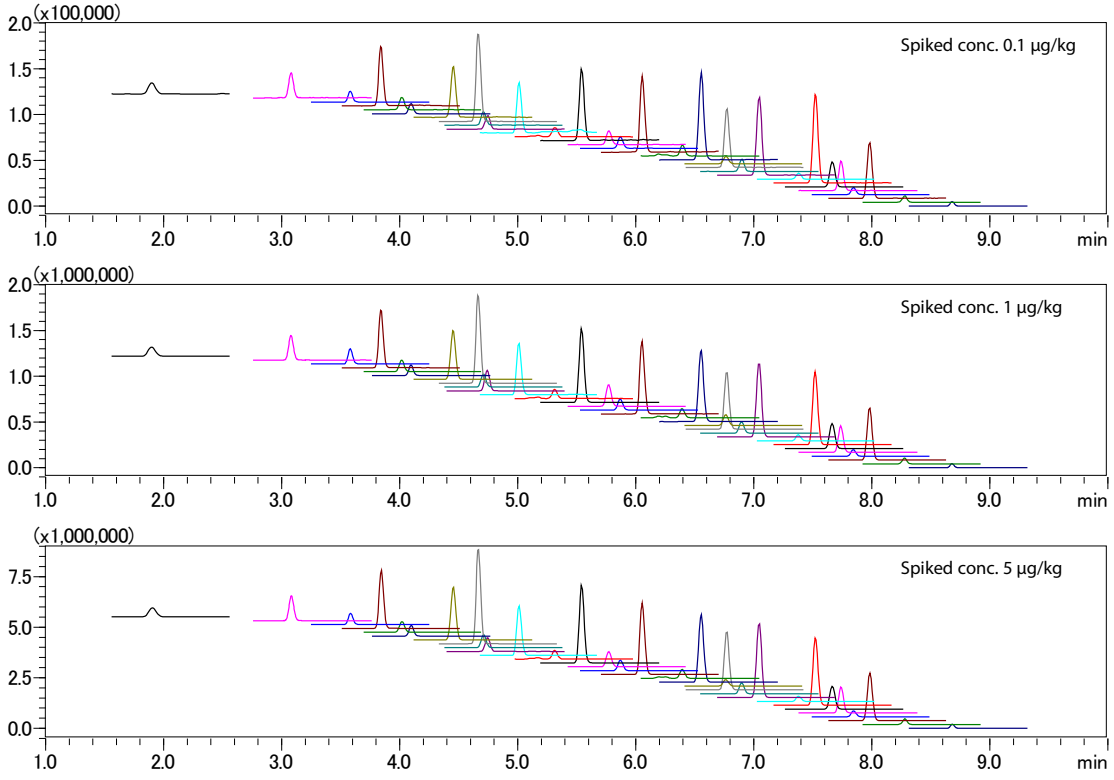

Fig. 4 MRM chromatograms of spiked PFAS in tuna fillet samples

■ 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 frozen crushed tuna fillets along with ISTD were subjected to the pretreatment procedure, and a calibration curve was created in the range from 0.05 to 5 µg/kg for all target compounds. The coefficients of determination (R^2) were 0.98 for 10:2 FTS, and higher than 0.99 for the other compounds, indicating good linearity was achieved. The concentration of the solution in the vial ranged from 0.07 to 7 ng/mL.

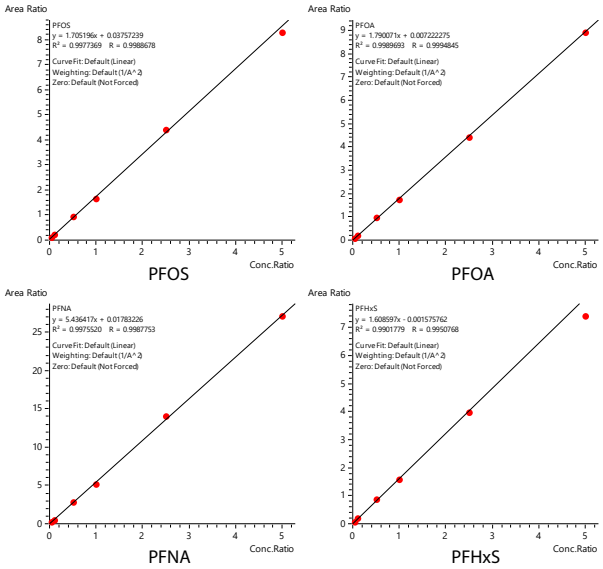

Fig. 5 Calibration curve at spiked concentrations ranging from 0.05 to 5 µg/kg

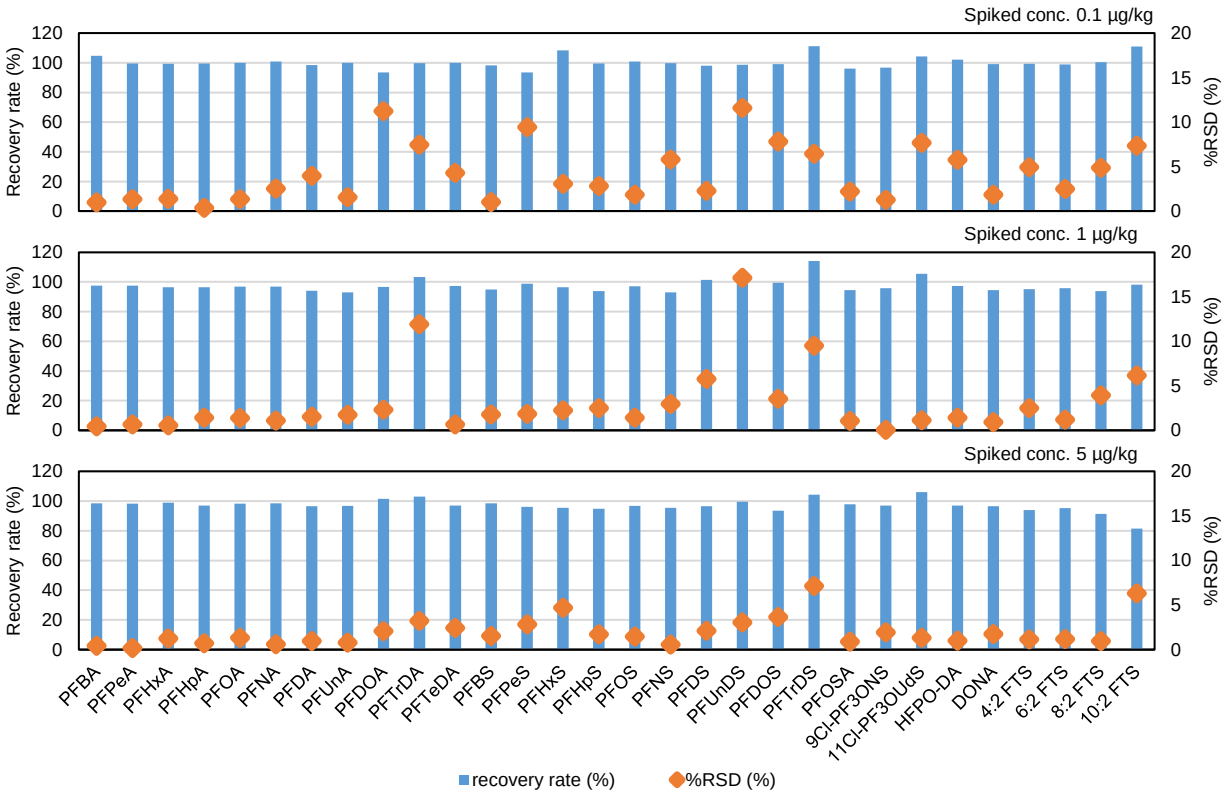

Fig. 6 Recovery rate and repeatability of QC samples (n = 3)

■ Recovery test

Recovery tests were conducted at 0.1, 1, and 5 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 the area values were corrected using the combination of the standard substances and ISTDs shown in the table 2, with quantification carried out using a matrix calibration curve. According to the requirements of AOAC SMPR, PFOS, PFOA, PFNA, and PFHxS are specified to have limit of quantification (LOQ) of 0.1 µg/kg or less, recovery rate within 80-120%, and repeatability 10% or less. For other compounds, LOQ of 1.0 µg/kg or less, recovery rate within 65-135%, and repeatability of 25% or less (Table 3). For all compounds, recovery rate within 81.6-114.1% and repeatability below 17.1% were achieved at spiked concentrations of 0.1, 1, and 5 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 (%)
PFOS	≦0.1	80-120	≦20
PFOA	≦0.1	80-120	≦20
PFNA	≦0.1	80-120	≦20
PFHxS	≦0.1	80-120	≦20
Other PFAS	≦1.0	65-135	≦25

■ Conclusion

This application news described a quantitative analysis of thirty PFAS targeted by AOAC INTERNATIONAL in tuna fillet. The analysis was performed using a quadrupole mass spectrometer LCMS-8060NX equipped with ultra-high performance liquid chromatograph Nexera X3 UHPLC system. Shim-pack Scepter, which provides good separation and peak shape, was used as the column. A spiked recovery test were conducted, and all compounds showed recovery rate within 80-120% and repeatability below 20% at spiked concentrations of 0.1, 1, 5 µg/kg. In particular, recovery rates of PFOS, PFOA, PFNA, and PFHxS were within 95.5-108.4% at all spiked concentrations. Using optimized pretreatment and analytical methods, accurate quantitation is possible from 0.1 µg/kg.

Reference

1) AOAC SMPR®2023.003

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