SHIMADZU

T024

Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Fish Fillet with LC-MS/MS

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1. Introduction

Per- and Polyfluoroalkyl Substances (PFAS) is the collective name for a chemical group of organic fluorinated compounds, perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) are representative compounds of PFAS. They have been used water repellents, surface treatment agents, fire extinguishers, and coatings. PFAS are persistent and bioaccumulative in the environment because of their stable structure, it is known that they are present in a wide range of environmental water and wildlife. Due to concerns about human exposure through diet, studies on the status of food contamination by PFAS are being conducted in various countries. We have examined a quantitative analysis method for thirty PFAS compounds in fish fillet samples.

2. Methods

2-1. Sample and equipment

Standard compounds were purchased from Wellington Laboratories. Fish fillet for sample was purchased from a local grocery store and homogenized using a freeze grinder FST-4000 (AiSTI SCIENCE). Quantification was performed with a triple quadrupole mass spectrometer LCMS-8060NX equipped with Nexera™ X3 UHPLC (Shimadzu Corporation, figure 1). The system configuration is shown below. To prevent contamination from an equipment, a delay column was added between a mixer and an autosampler.

Nexera X3 system
Column : Shim-pack Scepter™ C18-120 (100 mm x 2.1 mm I.D., 3 µm)
Delay column : Shim-pack Scepter C18-120 (50 mm x 2.1 mm I.D., 3 µm)
Mobile phase A : Acetonitrile/water = 5:95(v/v) with 2 mmol/L Ammonium acetate
Mobile phase B : Acetonitrile
Rinse : Methanol/water = 50:50(v/v)
Flow rate : 0.3 mL/min (0.6 mL/min only between 10.01-12 min)
Time 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 ºC Injection vol. : 5 µL

LCMS-8060NX
Ionization : ESI, Negative mode
DL temp. : 200 ºC
Interface temp. : 250 ºC
Heat block temp. : 400 ºC
Nebulizer gas : 2 L/min
Drying gas : 10 L/min
Heating gas : 10 L/min
Probe position : +2 mm

Figure 1. Nexera X3 and LCMS-8060NX

2-2. Extraction

The extraction procedure was performed with reference to the QuEChERS method. The flow is shown in Figure 2. A frozen and ground sample of 10 g was weighed and added with 10 mL of acetonitrile, then vigorously shaken for 1 minute. One packet of Q-sep QuEChERS extraction salt (Restek, P/N: 25849) was added and immediately shaken vigorously by hand for 1 minute. The mixture was centrifuged at 4,000 rpm at room temperature for 5 minutes, and the acetonitrile layer was collected. This acetonitrile layer was diluted 5 times with water to obtain the extraction solution.

2-3. Purification

EVOLUTE PFAS (Biotage, 150 mg/6 mL) and PRESSURE+ pressurized manifold (Biotage) was used for solid-phase purification. The flow is shown in figure 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. After washing with 5 mL of water and 5 mL of formic acid/methanol/water (1:400:600, v/v/v), the elution was performed with 5 mL of 28% ammonium hydroxide/methanol/water (1:90:10, v/v/v). The eluted solution was taken 500 µL and mixed with 2 µL of formic acid, then analyzed by LC-MS/MS.

3. Results

3-1. MS chromatogram and calibration curve

Figure 4 shows the MS chromatograms for simultaneous analysis of thirty PFAS compounds, and Figure 5 shows the calibration curves for representative compounds.

All compounds eluted within 8 minutes, indicating good separation. Additionally, although not shown in the figures, it has been confirmed that taurodeoxycholic acid (TDCA), taurolithocholic acid (TCDCA), and tauroursodeoxycholic acid (TUDCA) are sufficiently separated from PFOS using these conditions.

Good calibration curves can be obtained for all compounds in the range of 0.05 to 5 µg/kg, and the coefficient of determination R^2 was 0.98 for 10:2 FTS, and 0.99 or more for all other compounds, indicating good linearity.

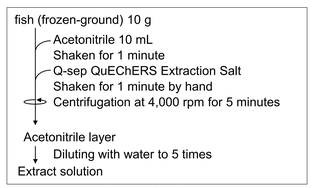

Figure 2. The extraction process

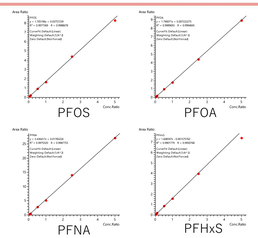

Figure 5. Calibration curves ranging from 0.05 - 5 µg/kg (spiked conc.)

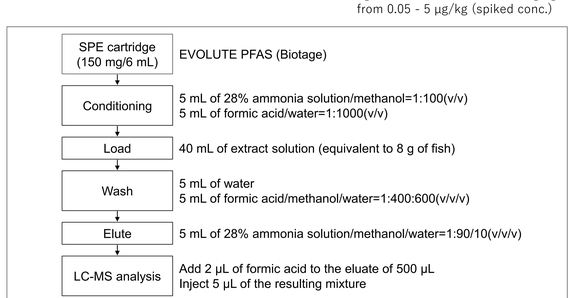

Figure 3. The purification process

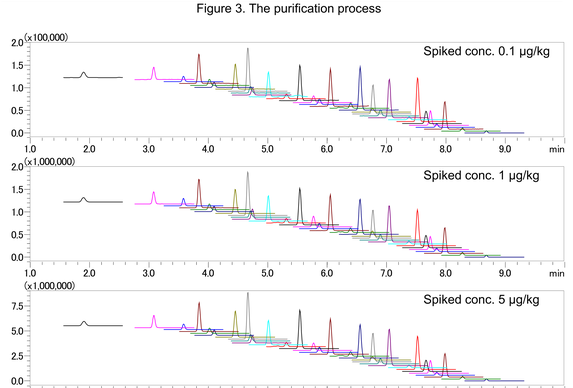

Figure 4. MS chromatograms of samples spiked with thirty PFAS compounds

3-2. Recovery rate test

Recovery tests were conducted at concentrations of 0.1, 1, and 5 µg/kg to verify the recovery rates and repeatability. Preprocessing was performed in triplicate, and matrix-matched calibration curves were used for quantification. According to the requirements of the AOAC SMPR, PFOS, PFOA, PFNA, and PFHxS have a LOQ of 0.1 µg/kg, a recovery rate of 80-120%, and a repeatability of less than 20%. Other PFAS compounds have a LOQ of 1.0 µg/kg, a recovery rate of 65-135%, and a repeatability of 25%. For all compounds, the recovery rates were within 80-120% and the repeatability was below 20% at the spiked concentrations of 0.1, 1, and 5 µg/kg.

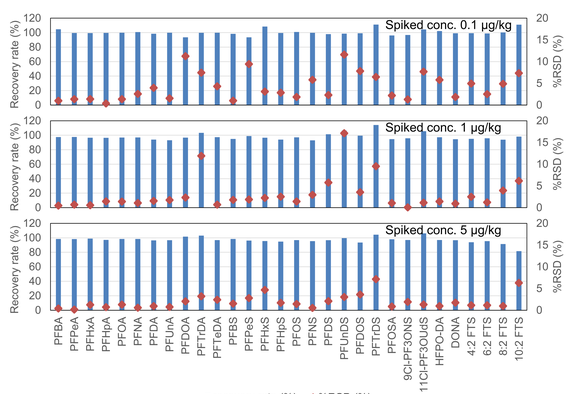

Figure 6. Recovery rate and repeatability (n=3)

3. Conclusions

- An LC-MS method for thirty PFAS within fifteen minutes analysis were created.
- The development of the pre-processing step, and a recovery test were conducted at 0.1, 1, and 5 µg/kg, resulting in favorable results. Recovery rates within 80-120% and repeatability below 20% were achieved for all compounds.

References
1) AOAC SMPR®2023.003