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Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Food Samples by LC-MS/MS

MP 213

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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 and 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 forty PFAS compounds in foods with two SPE cartridges.

2. Methods and Results

2-1. Material, sample, and equipment

Standard compounds were purchased from Wellington Laboratories. Carrot was purchased from a grocery store and homogenized using a freeze grinder. Quantification was performed using fully polypropylene low-binding vials TORAST™-H Bio Vial (Shimadzu GLC Ltd.) with a triple quadrupole mass spectrometer LCMS-8050 equipped with Nexera™ X3 UHPLC (Shimadzu). 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 2.0 mm I.D., 1.9 µm)		
Delay column	: Shim-pack GIST C18 (3.0 mm x 50 mm I.D., 5 µm, HSS)		
Mobile phase A	: Acetonitrile/water=5:95(v/v) containing 2 mmol/L Ammonium acetate		
Mobile phase B	: Acetonitrile		
Rinse	: Methanol/water=50:50(v/v)		
Flow rate	: 0.4 mL/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 0.1 to 9.6 min using a flow switching valve.		
Column temp.	: 40 °C	Injection vol.	: 1 µL

<LCMS-8050>

Ionization	: ESI, Negative mode	DL temp.	: 200 °C
Interface temp.	: 200 °C	Heat block temp.	: 300 °C
Nebulizer gas	: 3 L/min	Heating gas	: 10 L/min
Drying gas	: 10 L/min	Probe position	: +2 mm

2-2. Extraction

Extraction was performed using a pre-processing method, taking reference from the QuEChERS method. It is a simple protocol that does not require glassware, and centrifugation is required only once. The procedure is shown in figure 1.

2-3. Development of purification process

Initially, purification was performed refer to 2nd draft method 1633 of EPA. However, due to significant losses in purification step with the SPE cartridge, detailed investigation was conducted focusing on the washing and elution steps. Two types of weak anion-exchanged SPE cartridges were evaluated: InertSep MA-2 (GL Sciences Inc.) or EVOLUTE® EXPRESS WAX (Biotage). Eleven aqueous solutions for washing and elution were prepared with methanol concentrations ranging from 0 to 100% in 10% increments. Three types of eluents were prepared: one containing formic acid (formic acid/methanol solution=1:1000(v/v)), one containing ammonium (28% ammonia solution/methanol solution=1:100(v/v/v)), and one containing nothing, and one set of eluent was sequentially supplied to each SPE cartridge. After loading the carrot extract containing PFAS into the SPE cartridge, elution was performed starting from a 5 mL portion with 0% methanol ratio, then gradually increasing (figure 2). Biotage® PRESSURE+ 48 was used for this process.
The compounds present in each fraction were quantified (figure 3). Since there was a compound that eluted up to 53.5% (3:3 FTCA) at methanol ratio of 50%, formic acid/methanol/water=1:400:600(v/v/v) was chosen as the washing solution. Only a small number of compounds required methanol ratio of 100% for elution, so 28% ammonia solution/methanol/water=1:90/10(v/v/v) was used as the eluent.

2-4. Evaluation of concentration

The feasibility of concentrating the eluate using nitrogen gas blow-down was investigated. After adding standard compounds to 5 mL of purified solution of carrot, it was concentrated to less than 1 mL and filled up to 1 mL with methanol. Upon quantification with LC-MS, there were loss ranging from 67.6 to 98.7% for eight compounds, PFOSA, NMeFOSA, NEtFOSA, NMeFOSE, NEtFOSE, 3:3 FTCA, 5:3 FTCA, and 7:3 FTCA. The losses for the other compounds were 27.8% or less. Therefore, the concentration step using nitrogen gas blow-down was avoided in this pre-processing method.

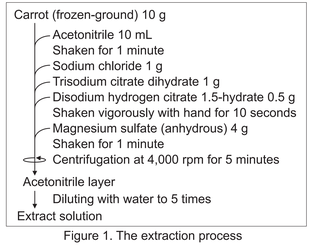

Figure 1. The extraction process

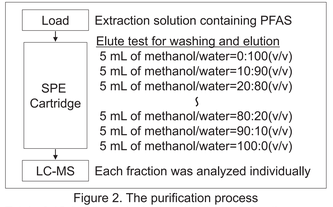

Figure 2. The purification process

Total of 12 fractions, including the through fraction, were collected and individually analyzed to quantify PFAS concentration present in each fraction.

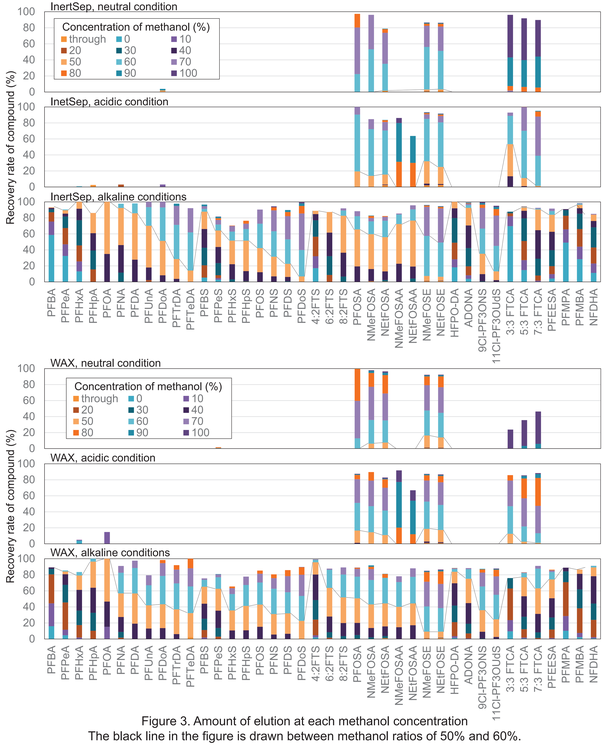

Figure 3. Amount of elution at each methanol concentration

The black line in the figure is drawn between methanol ratios of 50% and 60%.

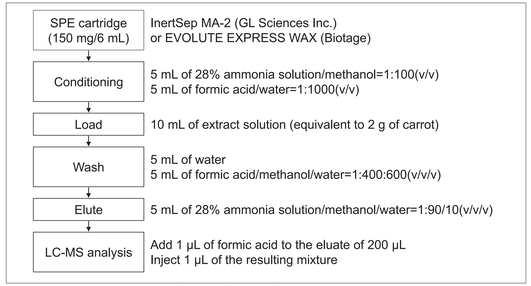

Figure 4. The purification process

2-5. Recovery rate test

To confirm the matrix effects, the eluate obtained carrot extract purification was analyzed after adding standard compounds (1 ng of PFOS in 5 mL of eluate). Additionally, to evaluate purification efficiency, standard compounds were added to the solution obtained from carrot extraction (1 ng of PFOS in 10 mL extraction 5-fold diluted with water), and the purified eluent was analyzed. Furthermore, to quantify the total loss in extraction step and purification step, ground carrot with added standard compounds (5 ng of PFOS in 10 g of carrot) were extracted, and the eluent was analyzed with LC-MS.
The recovery rates were calculated by comparing the peak areas of standard solutions, without correction by surrogate compounds. The results of recovery rate and Final concentration in vial before addition of formic acid are shown in table 1, and MS chromatograms are shown in figure 5. We obtained good recovery rate of within 70% to 120% for both cartridges. When injecting 5 µL, 6:2FTS exhibited higher concentration than that of theoretically expected due to matrix effect. However, reducing the injection volume suppressed matrix effect, suggesting a good recovery rates for all compounds.

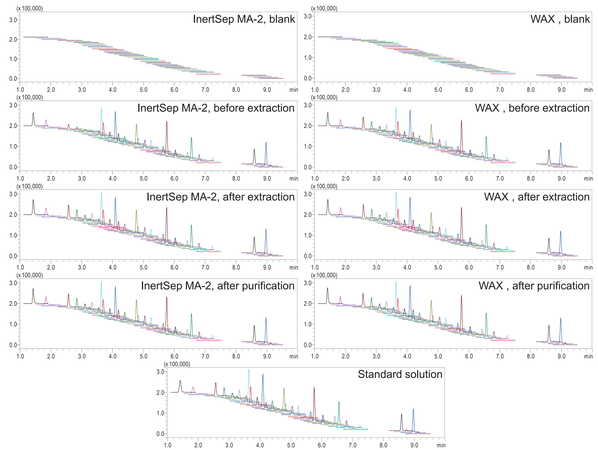

Figure 5. MS chromatograms of PFAS 40 compounds

Table 1. Recovery rate of PFAS using InertSep MA-2 or WAX

Compound	Retention time (min)	Conc. in carrot (ng/g)	Final conc. (ng/mL)	Recovery rate (%) InertSep MA-2 After purification	InertSep MA-2 After extraction	InertSep MA-2 Before extraction	WAX After purification	WAX After extraction	WAX Before extraction
PFBA	1.38	2.0	0.8	93.3	105.1	100.2	89.7	106.5	106.3
PFPeA	2.55	1.0	0.4	90.5	100.9	96.2	86.8	100.0	100.7
PFHxA	3.33	0.5	0.2	97.7	102.7	100.8	97.7	101.3	109.4
PFHpA	3.92	0.5	0.2	101.0	107.8	96.9	93.4	105.0	106.4
PFOA	4.40	0.5	0.2	86.0	99.7	88.6	84.2	103.1	88.5
PFNA	4.84	0.5	0.2	93.7	103.3	102.5	97.6	108.7	104.6
PFDA	5.25	0.5	0.2	97.5	94.4	98.4	87.2	95.6	99.8
PFUnA	5.65	0.5	0.2	87.4	95.0	92.1	81.5	91.3	92.2
PFDoA	6.05	0.5	0.2	95.9	101.1	102.1	89.1	96.3	99.0
PFTrDA	6.43	0.5	0.2	82.3	83.5	89.4	79.2	82.5	87.3
PFTeDA	6.81	0.5	0.2	88.2	89.8	91.9	85.9	95.7	94.9
PFBS	3.39	0.5	0.2	100.5	94.0	103.7	104.5	105.6	105.9
PFPeS	4.04	0.5	0.2	98.1	98.1	104.7	96.3	96.9	91.7
PFHxS	4.56	0.5	0.2	100.7	88.7	92.1	85.4	98.5	86.4
PFHpS	5.03	0.5	0.2	92.3	104.6	100.9	97.1	105.9	102.8
PFOS	5.45	0.5	0.2	89.8	86.2	88.9	75.9	80.4	98.8
PFNS	5.87	0.5	0.2	95.6	97.7	95.6	94.9	96.2	98.7
PFDS	6.27	0.5	0.2	87.4	101.0	95.0	83.1	92.1	97.6
PFDoS	7.04	0.5	0.2	86.6	94.1	92.8	92.9	91.6	94.8
4:2FTS	3.11	2.0	0.8	78.0	93.5	92.9	81.6	99.6	99.1
6:2FTS	4.20	2.0	0.8	86.5	103.3	93.9	93.8	101.9	92.3
8:2FTS	5.05	2.0	0.8	85.7	92.9	91.9	84.3	90.0	97.7
PFOSA	7.23	0.5	0.2	95.0	92.5	98.5	91.3	93.5	100.0
NMeFOSA	8.76	0.5	0.2	91.2	99.8	92.1	86.3	94.6	104.6
NEtFOSA	9.13	0.5	0.2	92.0	102.8	100.3	90.9	92.1	106.3
NMeFOSAA	5.27	0.5	0.2	84.6	109.3	101.8	97.9	93.8	98.4
NEtFOSAA	5.45	0.5	0.2	104.5	111.4	79.0	90.6	99.9	114.0
NMeFOSE	8.60	5.0	2.0	91.4	94.2	95.9	85.2	88.7	99.3
NEtFOSE	8.98	5.0	2.0	93.8	94.8	99.0	87.7	89.2	98.9
HFPO-DA	3.56	2.0	0.8	87.8	99.3	98.0	85.1	99.6	103.5
ADONA	4.10	2.0	0.8	90.9	100.1	98.9	93.2	99.9	95.5
9Cl-PF3ONS	5.76	2.0	0.8	95.0	100.2	97.8	95.7	100.1	101.1
11Cl-PF3OUdS	6.57	2.0	0.8	92.8	97.4	96.0	93.3	96.1	100.1
3:3 FTCA	1.87	2.5	1.0	84.7	86.4	84.6	85.2	80.8	89.9
5:3 FTCA	3.63	12.5	5.0	91.2	100.1	93.6	87.9	98.0	98.7
7:3 FTCA	4.76	12.5	5.0	85.0	97.3	96.8	85.9	98.0	99.7
PFEESA	3.70	1.0	0.4	91.5	97.7	95.1	91.2	96.8	101.0
PFMPA	1.79	1.0	0.4	88.2	100.5	96.4	86.2	102.2	101.2
PFMBA	2.84	1.0	0.4	86.3	98.6	92.5	88.9	97.5	99.0
NFDHA	3.26	1.0	0.4	82.7	93.6	93.8	85.2	100.3	92.9
<70%				0	0	0	0	0	0
70-120				40	40	40	40	40	40
>120%				0	0	0	0	0	0

3. Discussion

Purification conditions using SPE cartridges were studied and the optimized eluents was selected. Purification was performed using the optimized process and good recoveries were obtained. Two SPE cartridges of InertSep NA-2 and WAX were compared, but no significant difference was observed.

4. Conclusions

- An LC-MS method for forty PFAS within fifteen minutes analysis were created.
- The development of the pre-processing step, and a recovery test were conducted under low PFAS concentration conditions, resulting in favorable results.

References: Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Aqueous, Solid, Biosolids, and Tissue Samples by LC-MS/MS, 2nd Dragt Method 1633, EPA (June 2022).