A Simple, Rapid, and Highly Sensitive Multicomponent Simultaneous Analysis Method for PFAS Combining Liquid-Liquid Extraction and Solid-Phase Extraction in Food and Serum

SPL-W100
for SPE-LC system

Introduction

Analysis of PFAS in food and serum involves numerous interfering components, requiring significant time and effort for sample preparation. Furthermore, as the number of target analytes increases, the complexity of sample preparation continues to grow. In this application, we first reduced the burden on the solid phase by removing highly polar ionic interferents at high concentrations via liquid-liquid extraction. Next, sensitivity and purity were improved by retaining and concentrating PFAS via anion exchange solid-phase extraction while removing non-ionic contaminants. Furthermore, by employing an automated online SPE-LC/MS system integrating solid-phase extraction with LC/MS measurement, we introduce a rapid, simple, and highly sensitive multi-component simultaneous analysis method.

Experimental Methods

【In the case of horse mackerel】

Frozen crushed sample 5 g
- Water 7 mL
  ※10 - Moisture content in sample (mL)
- ACN 20 mL

Shake (10 min.)

Centrifugal (3500rpm, 3min)

【For Milk / Serum】

Sample 100 µL
- ACN-Water(4/1) 500µL

Stirring (Touch Mixer)

Centrifugal (14000rpm, 1分)

Supernatant Take 300 µL — liquid-liquid partitioning
- 0.5N HCl-Water 10 µL
- TBME 800 µL

Stirring (Touch mixer, 10 sec.)

Centrifugal (14000rpm, 1min.)

Organic phase (extract)

※To the online system

Online SPE-LC/MS System

SPL-W100 — Automatic preprocessing time: 12 min.

Flash-SPE WAXs : Hold
- Conditioning: 0.1% Ammonia_MeOH-Water MeOH TBME
- Load [Hold] Extraction Solution 40 µL
- Washing TBME
- Dry nitrogen gas: 10 sec.
- Elution: 0.1% ammonia MeOH-water (95/5) 60 µL

Eluted solution
- Mixture of 0.1% formic acid and water 120 µL
  ※Mixing within MiVS

The entire volume of the eluate mixed water is introduced into the LC/MS.

■ Removal of Highly Polar Ionic Impurities by Liquid-Liquid Distribution

【Key Points for Liquid-Liquid Separation①】

Supernatant : 300 µL
➡ACN 200 µL
Water 100 µL
Addition
TBME 800 µL
HCl Water 10 µL

Organic phase : 1 mL
ACN 200 µL
TBME 800 µL

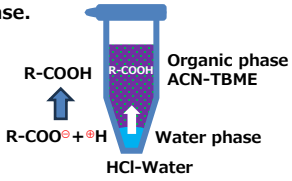
Water phase : 110 µL
Water 100 µL
HCl Water 10 µL

PFAS is to the organic phase

Highly polar anionic impurities are transferred to the aqueous phase.

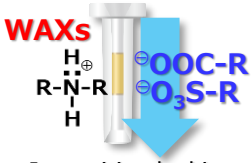
【Key Points for Liquid-Liquid Separation②】

The addition of HCl suppresses the dissociation of carboxyl groups, preventing dissociation and enabling hydrophilic PFAS to migrate into the organic phase.



■ Solid-phase extraction by ion exchange

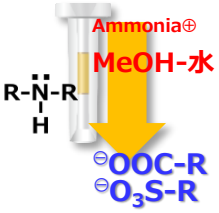
Load [Hold] & Cleaning



Impurities lacking anions are removed.

Retention of anionic PFAS in ACN-TBME by ion exchange onto cationic solid-phase WAXs.

Elution



The alkaline environment deionizes the solid-phase waxes, eluting the target substances.

Effect of liquid-liquid partitioning:
While adsorption via anion exchange may be affected by anionic impurities such as nitrate ions, potentially reducing the retention of target substances, prior removal through liquid-liquid partitioning enabled recovery without compromising retention capacity.

Sample

Information

The 33rd Environmental Chemistry Symposium

"Development of a Rapid, Simple, and Highly Sensitive Multicomponent Simultaneous Analysis Method Combining Liquid-Liquid Extraction and Solid-Phase Extraction for PFAS in Food and Serum"

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Key Word

PFAS
Solid-phase extraction
Online SPE-LC/MS/MS

AiSTI SCIENCE

Product

SPL-W100
Flash-SPE WAXs

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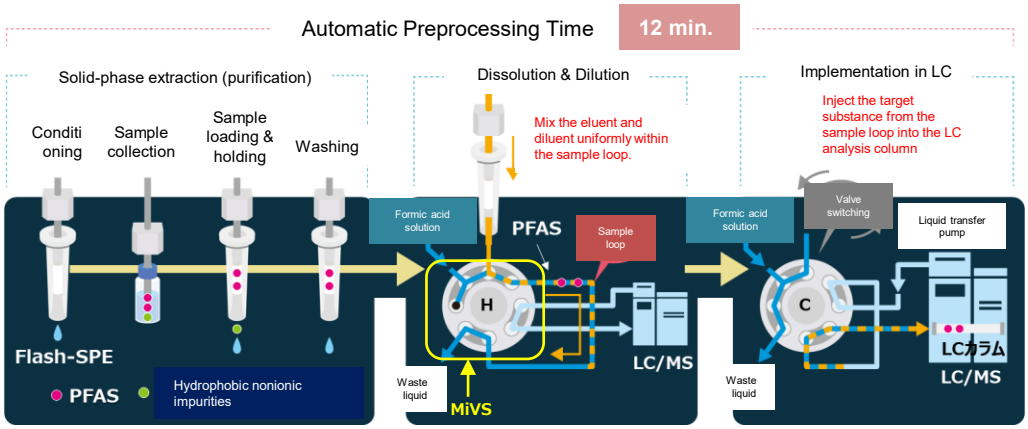
Online SPE-LC/MS System

This system achieves “reduced sample volume,” “shortened pretreatment time,” and “online operation” through the use of the dedicated solid-phase cartridge Flash-SPE and the mixed injection valve system [MiVS].
Furthermore, by overlapping the pretreatment (solid-phase extraction) and instrument measurement steps, analysis is performed more efficiently, enabling high throughput.



Measurement conditions

【Equipment】
SPL-W100(AiSTI SCIENCE)、 LCMS-8045(Shimadzu)

【LC conditions】
Delay Column: Inertsil ODS-3, 3 μm, 3.0 mm ID × 33 mm (UP)
Analytical Column: Inertsil ODS-3, 3 μm, 2.1 mm ID × 75 mm
Mobile Phase A: 2 mM ammonium acetate-water
B: 2 mM ammonium acetate-MeOH-acetonitrile (1/1)
Flow rate: 0.3 mL/min
Gradient: B.Conc. 30% (0-0.25 min) → 40% (0.5-2.5 min)
→ 100% (9-12 min) → 30% (12.5-15 min)
Column temperature: 40 °C

【MS condition】
Ionization mode : ESI Negative
Measurement Mode : MRM

SPL-W100(AiSTI SCIENCE)
LC-MS-8045(Shimdzu)

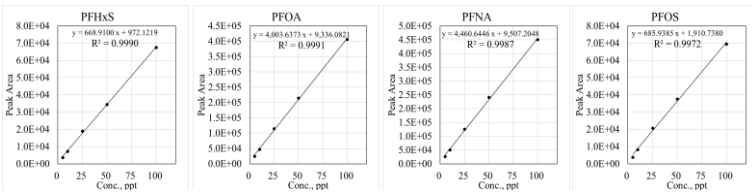
Table: Recovery Rates and Reproducibility of PFAS in Each Sample (n=5)

No.	Ingredient Name	Mackerel 2 ppb Addition recovery rate %	Mackerel 2 ppb Addition *RSD n=5, %*	Milk 1 ppb Addition recovery rate %	Milk 1 ppb Addition *RSD n=5, %*	Serum 1 ppb Addition recovery rate %	Serum 1 ppb Addition *RSD n=5, %*
1	PFBA	71	*7.1*	82	*3.4*	106	*5.1*
2	PFMPA	69	*5.9*	88	*4.1*	91	*1.8*
3	PFPeA	90	*3.2*	89	*6.3*	102	*9.4*
4	PFMBA	73	*8.4*	88	*3.8*	91	*6.3*
5	PFBS	107	*2.4*	79	*6.5*	108	*11.1*
6	4:2 FTS	94	*8.1*	60	*6.0*	121	*8.0*
7	NFDHA	147	*4.3*	94	*4.9*	185	*7.3*
8	PFHxA	93	*3.5*	84	*4.2*	94	*6.4*
9	PFEESA	90	*4.0*	89	*2.1*	105	*5.7*
10	HFPO-DA	87	*4.5*	85	*6.3*	91	*8.8*
11	PFPeS	107	*4.1*	84	*7.2*	106	*9.2*
12	PFHpA	94	*3.8*	85	*3.6*	95	*5.7*
13	PFHxS	106	*4.3*	92	*8.6*	107	*6.1*
14	6:2FTSA	130	*4.7*	81	*6.3*	192	*5.1*
15	PFOA	108	*2.1*	85	*3.0*	102	*3.9*
16	PFHpS	107	*4.3*	88	*2.9*	102	*10.7*
17	8:2 FTUCA	123	*1.6*	86	*4.2*	115	*7.1*
18	PFNA	101	*1.6*	88	*4.3*	102	*4.7*
19	*FOSA*[1)]	-	-	-	-	-	-
20	PFOS	96	*3.7*	80	*3.8*	82	*5.1*
21	8:2FTSA	142	*8.1*	80	*8.1*	133	*10.7*
22	PFDA	99	*4.4*	88	*2.9*	106	*6.6*
23	NMeFOSAA	113	*5.1*	97	*4.1*	135	*8.2*
24	PFNS	80	*5.3*	88	*3.6*	96	*9.7*
25	*N-MeFOSA*[1)]	-	-	-	-	-	-
26	NEtFOSAA	51	*4.9*	87	*7.5*	93	*7.4*
27	PFUnA	-[2)]	*3.2*	85	*2.6*	84	*4.7*
28	PFDS	93	*5.6*	93	*4.4*	98	*2.6*
29	*N-EtFOSA*[1)]	-	-	-	-	-	-
30	PFDoDA	63	*3.9*	83	*3.6*	77	*5.2*
31	PFTrDA	74	*2.6*	80	*5.0*	87	*7.5*
32	PFTeDA	89	*4.2*	80	*3.7*	98	*5.8*
33	PFHxDA	74	*6.0*	86	*3.3*	98	*5.8*
34	8:2 diPAP	89	*2.0*	87	*6.7*	79	*8.9*
35	PFOcDA	113	*2.6*	94	*3.4*	138	*5.3*

1) Components for which recovery could not be obtained
2) Recovery rate could not be evaluated due to peaks from unknown samples
*Calculations are based on absolute area values without corrections using stable isotopes or similar methods.

Result

Absolute calibration curve using this system



Additive Recovery Test : Additive recovery tests were conducted using horse mackerel, milk, and serum. For the three components FOSA, NMePFOSA, and NEtPFOSA, recovery was not achieved. This is likely because they lack anionic properties, resulting in weak retention on the WAX, and because loading with hydrophobic organic solvents prevents retention on the WAX. For the other major components, good recovery rates and reproducibility were obtained in all samples.

Blank Countermeasures : The solvent used in solid-phase extraction was incorporated into the pretreatment apparatus to pass through a filter (adsorbent) beforehand, thereby reducing contamination.

Summary

Using this system, combining liquid-liquid partitioning after protein removal with solid-phase extraction enabled rapid, simple, and highly sensitive simultaneous multi-component analysis of PFAS even in samples rich in impurities, such as food and serum.

References

Sasano et al., 3rd Joint Conference on Environmental Chemical Substances: “Development of an Analytical Method for PFOA in River Water Using Online SPE-LC/MS”