

Direct Injection Analysis of Organofluorine Compounds (PFAS) by Triple-Quadrupole LC/MS/MS

Organofluorine compounds such as perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) (Per/polyfluoroalkyl compound: PFAS) have superior functionality of water and oil repellency and are widely used as coating agents for daily necessities. However, PFAS is known to remain in the blood and is reported to be a toxic compound. Moreover, PFAS is chemically stable and highly persistent. PFOS, a typical organofluorine compound, is listed in Annex B of the Stockholm Convention on Persistent Organic Pollutants (Restriction), and its manufacture and use are internationally restricted. It is designated as a Class I Specified Chemical Substance under the Chemical Substances Control Law in Japan, and its manufacture and use are basically prohibited, with some exceptions.

Analyses of many types of organofluorine compounds have generally been reported after solid-phase extraction/concentration pretreatment, while simplification of pretreatment is required. In this news, PFOA, PFOS, and PFAS including related substances were analyzed by triple-quadrupole LC/MS/MS without the concentration procedure.

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Analysis of Reference Standards

PFOA and PFOS Reference Standards were dissolved and mixed in the solvent, water/methanol = 7/3 (v/v), to prepare a standard sample for the calibration curve. The analytical conditions are shown in Table 1.

In addition, PFOA from the on-line degassing unit and/or flow channel may be concentrated in the analytical column and eluted at the same retention time as the PFOA injected as the analytical sample. To separate background PFOA in the HPLC system from PFOA in the injected sample, a delay column was mounted between the gradient mixer and the autosampler (Figure 1).

This method of delaying the elution of impurities in the system from the target components in the sample is called the Impurity Delay method. By installing a delay column, the elution of PFOA in the system is delayed, and it is possible to analyze PFOA in the sample, as it is isolated.

Table 1 Analytical conditions

[HPLC conditions] (Nexera™ Series)		
Column	: Shim-pack Velox™ SP-C18 (150 mm L × 2.1 mm I.D., 2.7 μm, PN: 227-32003-04)	
Delay column	: Shim-pack™ XR-ODS II (75 mm × 2.0 mm I.D., 3 μm, PN: 228-41623-91)	
Mobile phases	: A) 20 mmol/L Ammonium Acetate in H ₂ O B) Methanol	
Gradient program	: B 60% (0.00 min) - B85% (25.00 - 30.00 min) - 60% (30.01 - 34.00 min)	
Flow rate	: 0.25 mL/min	
Column temp.	: 40 °C	
Injection volume	: 50 μL	
[MS conditions] (LCMS™-8060)		
Ionization	: ESI (Negative mode)	
Probe voltage	: -1 kV	
Mode	: MRM	
Nebulizing gas flow	: 3 L/min	
Drying gas flow	: 5 L/min	
Heating gas flow	: 15 L/min	
DL temp.	: 200 °C	
Heat block temp.	: 300 °C	
Interface temp.	: 300 °C	
[MS/MS parameters]		
Compound	MRM transition (m/z)	Collision energy (V)
PFOA	412.90>369.05	10.0
	412.90>169.10	18.0
PFOS	498.90>79.95	55.0
	498.90>98.95	40.0

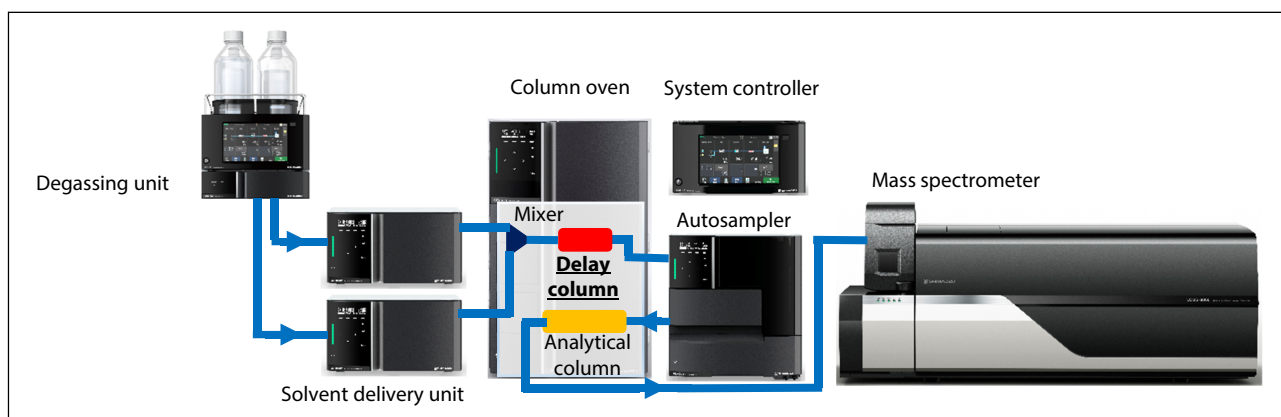


Figure 1 Impurity delay method

Results of the Standard Analysis

Calibration curves from 1 to 100 ng/L are shown in Figure 2. These calibration curves for both PFOA and PFOS indicate good linearity with $R^2 > 0.999$.

Table 2 shows the accuracy (%) at each calibration point. The accuracy of the calibration curve was 91.4 to 101.5%, which is a good result. Figure 3 shows each chromatogram of the 1 ng/L standard sample for PFOA and PFOS, respectively. The repeatability ($n = 3$) of the standard sample at this concentration was 5.8% for PFOA and 4.9% for PFOS, indicating good reproducibility.

Table 2 Accuracy at the calibration points (%)

	PFOA	PFOS
1 ng/L	93.5	92.7
2 ng/L	101.4	91.4
5 ng/L	94.6	95.4
10 ng/L	101.4	101.4
20 ng/L	102.2	101.5
50 ng/L	99.3	100.3
100 ng/L	100.1	99.9

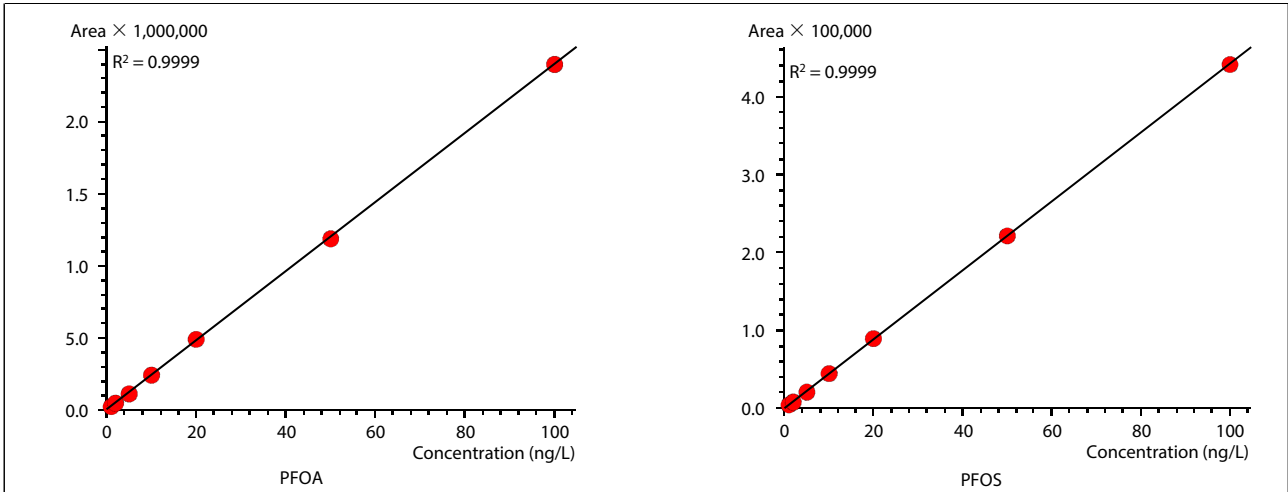


Figure 2 Calibration curves for PFOA and PFOS

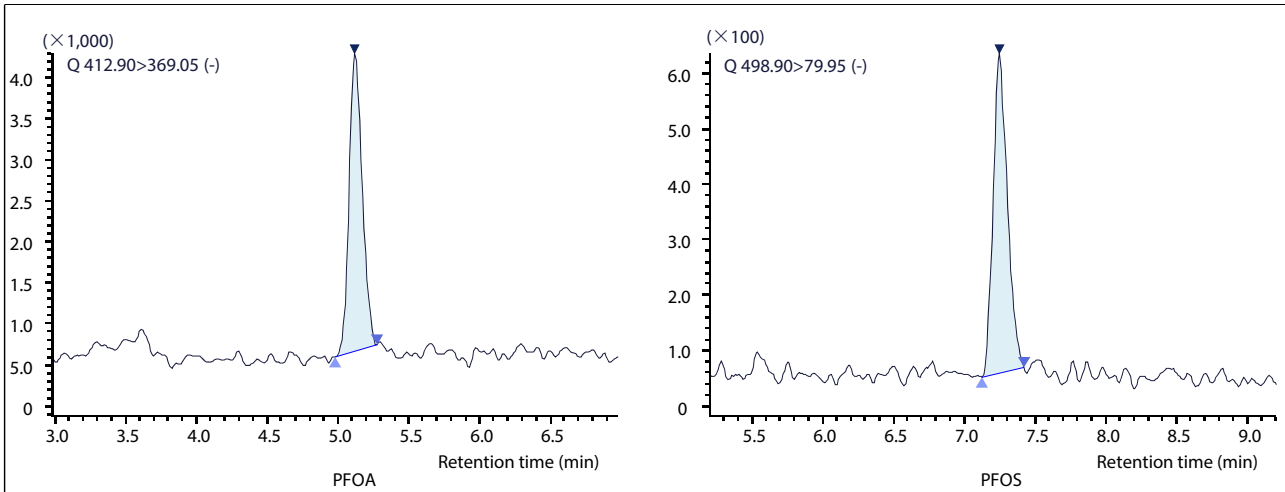


Figure 3 Chromatogram of PFOA and PFOS at 1 ng/L standard solution

■ Analysis of PFOA / PFOS Analogues

The reinforcement of PFAS regulations is advancing worldwide, and, at the same time, Method 537 of the U.S. Environmental Protection Agency (EPA) and ASTM D 7979 define analytical methods for PFOA and PFOS analogues.

Twenty-nine components (Table 3) including PFOA and PFOS were analyzed by the direct injection method using the Nexera MX system shown in Figure 4. This analysis was conducted under the conditions shown in Table 4, and calibration curves were prepared based on the internal standard method.

Table 3 List of compounds to be analyzed

	Compounds	Composition formula	Monoisotopic mass
1	4:2 FTS	C ₆ H ₅ F ₉ SO ₃	327.9816
2	6:2 FTS	C ₈ H ₅ F ₁₃ SO ₃	427.9752
3	8:2 FTS	C ₁₀ H ₅ F ₁₇ SO ₃	527.9688
4	10:2 FTS	C ₁₂ H ₅ F ₂₁ SO ₃	627.9624
5	N-EtFOSA	C ₁₀ H ₆ F ₁₇ NSO ₂	526.9848
6	N-EtFOSAA	C ₁₂ H ₈ F ₁₇ NSO ₄	584.9903
7	N-EtFOSE	C ₁₂ H ₁₀ F ₁₇ NSO ₃	571.0110
8	FOSA	C ₈ H ₂ F ₁₇ NSO ₂	498.9535
9	N-MeFOSAA	C ₁₁ H ₆ F ₁₇ NSO ₄	570.9746
10	N-MeFOSE	C ₁₁ H ₈ F ₁₇ NSO ₃	556.9953
11	N-MeFOSA	C ₉ H ₄ F ₁₇ NSO ₂	512.9691
12	PFTrDA	C ₁₃ HF ₂₅ O ₂	663.9577
13	PFBA	C ₄ HF ₇ O ₂	213.9865
14	PFBS	C ₄ F ₉ SO ₃ H	299.9503
15	PFDA	C ₁₀ F ₁₉ O ₂ H	513.9673
16	PFDoDA	C ₁₂ F ₂₃ O ₂ H	613.9609
17	PFHpA	C ₇ F ₁₃ O ₂ H	363.9769
18	PFHxA	C ₆ F ₁₁ O ₂ H	313.9801
19	PFHxS	C ₆ F ₁₃ SO ₃ H	399.9439
20	PFPeS	C ₅ F ₁₁ SO ₃ H	349.9471
21	PFNA	C ₉ F ₁₇ O ₂ H	463.9705
22	PFOA	C ₈ F ₁₅ O ₂ H	413.9737
23	PFDS	C ₁₀ F ₂₁ SO ₃ H	599.9311
24	PFHpS	C ₇ F ₁₅ SO ₃ H	449.9407
25	PFOS	C ₈ F ₁₇ SO ₃ H	499.9375
26	PFPeA	C ₅ HF ₉ O ₂	263.9833
27	PFTeDA	C ₁₄ HF ₂₇ O ₂	713.9545
28	PFUnDA	C ₁₁ HF ₂₁ O ₂	563.9641
29	PFHxDA	C ₁₆ HF ₃₁ O ₂	813.9482

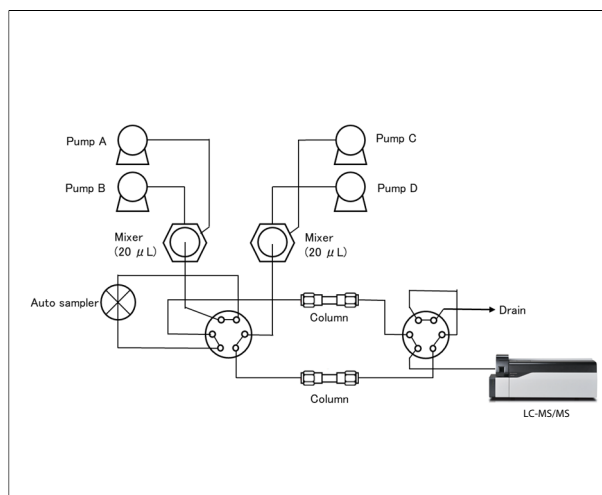


Figure 4 Flow diagram (High-throughput analysis)

Table 4 Analytical conditions

[HPLC conditions] (Nexera Series)

Column	: Shim-pack ODS III (50 mm x 2.0 mm I.D., 1.6 µm, P/N: 228-59922-91)
Delay column	: Shim-pack XR-ODS (30 mm x 3.0 mm I.D., 2.2 µm, P/N: 228-41606-91)
Mobile phases	: A) 5mM Ammonium Acetate / 0.05% Acetic Acid in H ₂ O B) Methanol
Gradient program	: B 50%(0.00 min) – B100%(4.60-5.50 min)
Flow rate	: 0.4 mL/min
Column temp.	: 40 °C
Injection volume	: 40 µL

[MS conditions] (LCMS-8050)

Ionization	: ESI (Negative mode)
Probe voltage	: -3 kV
Mode	: MRM
Nebulizing gas flow	: 2.6 L/min
Drying gas flow	: 7 L/min
Heating gas flow	: 9 L/min
DL temp.	: 180 °C
Heat block temp.	: 250 °C
Interface temp.	: 350 °C

Results of the Analogue Analysis

The TIC chromatogram obtained by measuring the standard mixture is shown in Figure 5. An analytical cycle is 5.5 minutes. The ranges of calibration curve and contribution ratios for each compound are shown in Table 5.

A good calibration curve with a contribution ratio (R^2) > 0.99 was obtained for each compound, and it was shown that the simultaneous analysis of analogues including PFOA and PFOS can be performed with high throughput.

Conclusions

- Samples of 1 ng/L could be analyzed for PFOA and PFOS without concentration.
- A total of 29 components including PFOA, PFOS, and their analogues could be simultaneously analyzed in just 5.5 minutes.
- The major PFAS can be determined without the solid-phase extraction or concentration steps described in EPA METHOD 537.1.

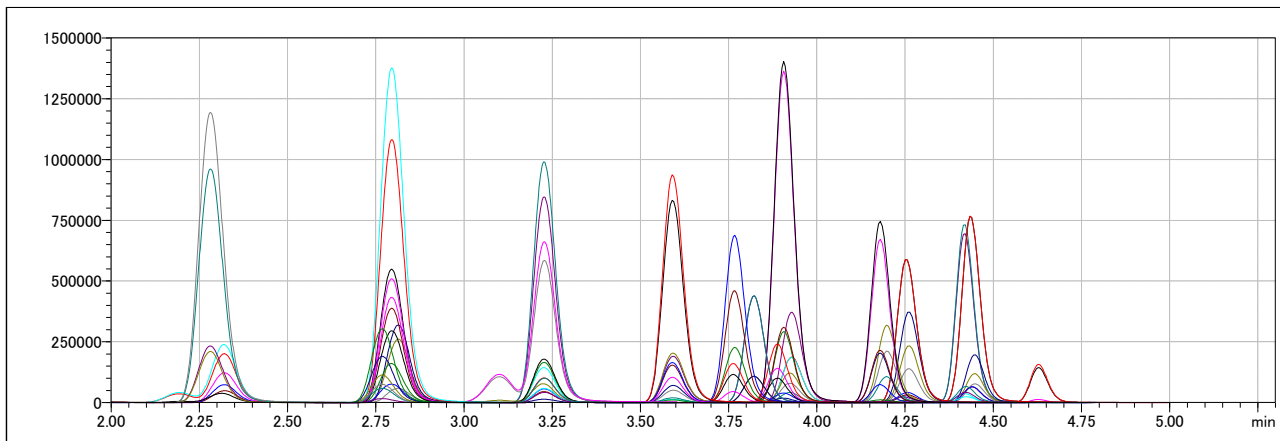


Figure 5 TIC Chromatogram of the standard mixture (each concentration; 1 - 25 µg/L)

Table 5 Calibration range and contribution ratio (R^2) for each target compound

	Compounds	Calibration range (µg/L)	Contribution ratio (R^2)
1	4:2 FTS	0.0934 – 2.3350	0.9936
2	6:2 FTS	0.0095 – 2.3700	0.9995
3	8:2 FTS	0.0096 – 2.3950	0.9948
4	10:2 FTS	0.0096 – 4.8200	0.9969
5	N-EtFOSA	0.0250 – 12.5000	0.9984
6	N-EtFOSAA	0.0400 – 5.0000	0.9976
7	N-EtFOSE	0.0250 – 12.5000	0.9997
8	FOSA	0.0100 – 5.0000	0.9998
9	N-MeFOSAA	0.0400 – 5.0000	0.9991
10	N-MeFOSE	0.0250 – 12.5000	0.9986
11	N-MeFOSA	0.0250 – 12.5000	0.9972
12	PFTrDA	0.0100 – 5.0000	0.9960
13	PFBA	0.0500 – 25.0000	0.9991
14	PFBS	0.0088 – 4.4200	0.9990
15	PFDA	0.0100 – 5.0000	0.9994

	Compounds	Calibration range (µg/L)	Contribution ratio (R^2)
16	PFDODA	0.0100 – 5.0000	0.9990
17	PFHpA	0.0100 – 5.0000	0.9999
18	PFHxA	0.0100 – 5.0000	0.9999
19	PFHxS	0.0095 – 4.7300	0.9992
20	PFPeS	0.0094 – 4.6900	0.9992
21	PFNA	0.0100 – 5.0000	0.9999
22	PFOA	0.0100 – 5.0000	0.9999
23	PFDS	0.0096 – 0.9640	0.9991
24	PFHpS	0.0095 – 2.3800	0.9990
25	PFOS	0.0093 – 9.2800	0.9996
26	PFPeA	0.0100 – 5.0000	0.9999
27	PFTeDA	0.0250 – 12.5000	0.9999
28	PFUnDA	0.0100 – 5.0000	0.9991
29	PFHxDA	0.0100 – 2.5000	0.9990

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