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Application News

High Performance Liquid Chromatograph Mass Spectrometer

Analysis of PFAS in Wastewater Based on ISO 21675 Using Triple Quadrupole LC/MS/MS

Nami Iwasa

User Benefits

- Based on ISO 21675, 30 PFAS can be analyzed in various water samples, including short-chain PFAS with 4 carbon atoms and long-chain PFAS with 16 and 18 carbon atoms.
- Accurate measurements are possible even in low concentration spiking recovery tests using ultrapure water and wastewater.

■ Introduction

Perfluoroalkyl and polyfluoroalkyl substances (PFAS) are a large group of over 10,000 chemical compounds that are mainly composed of a carbon chain and fluorine atoms. The characteristics of PFAS depend on the carbon chain length and attached functional groups, though excellent water and oil-repelling properties and heat and chemical stability of some PFAS have led to them being used in a wide range of consumer products and industrial applications. However, their exceptional stability also makes them resistant to degradation. Concerns over the persistence of PFAS in the environment and their toxicity to organisms have led to stricter regulations globally and the development of various analytical methods to determine PFAS levels.

ISO 21675[1)] is an international standard for determining PFAS in water samples that was drafted by the National Institute of Advanced Industrial Science and Technology (AIST) and then developed by the International Organization for Standardization (ISO). The U.S. Environmental Protection Agency (EPA) Method 1633 targets PFAS with 4 to 14 carbons in water samples, while ISO 21675 includes additional long-chain PFAS with 16 and 18 carbons, which are not covered by EPA Method 1633. ISO 21675 enables the simultaneous analysis of 30 short- to long-chain PFAS (Table 1) using liquid chromatography–tandem mass spectrometry (LC/MS/MS).

This article describes the results of PFAS analysis in industrial wastewater conducted using the LCMS-8060RX (Fig. 1) based on ISO 21675.

Table 1 Analyte List

Target	ISTD
PFBS	$^{13}C_3$-PFBS
PFHxS	$^{13}C_3$-PFHxS
PFHpS	$^{13}C_8$-PFOS
PFOA	$^{13}C_8$-FOSA
PFDS	d_3-N-MeFOSA
FOSA	d_5-N-EtFOSA
N-MeFOSA	d_3-N-MeFOSAA
N-EtFOSA	d_5-N-EtFOSAA
N-MeFOSAA	$^{13}C_2$-6:2FTSA
N-EtFOSAA	$^{13}C_2$-8:2FTSA
6:2FTSA	$^{13}C_4$-PFBA
8:2FTSA	$^{13}C_5$-PFPeA
9Cl-PF3ONS	$^{13}C_5$-PFHxA
PFBA	$^{13}C_4$-PFHpA
PFPeA	$^{13}C_8$-PFOA
PFHxA	$^{13}C_9$-PFNA
PFHpA	$^{13}C_6$-PFDA
PFOA	$^{13}C_7$-PFUnA
PFNA	$^{13}C_2$-PFDoA
PFDA	$^{13}C_2$-PFTeDA
PFUnA	$^{13}C_2$-PFHxDA
PFDoA	$^{13}C_2$-8:2 FTUCA
PFTrDA	$^{13}C_4$-8:2 diPAP
PFTeDA	$^{13}C_3$-HFPO-DA
PFHxDA	
PFOcDA	
8:2 FTUCA	
8:2 diPAP	
HFPO-DA	
DONA	

Fig. 1 LCMS- 8060RX

■ Sample Pretreatment

Sample pretreatment was performed according to ISO 21675. An internal standard was added to a 100 mL water sample, and solid-phase extraction was performed with a mixed-mode reversed-phase/anion-exchange column. After washing and drying the solid-phase column, neutral PFAS were eluted first (first elution), followed by the elution of ionic PFAS (second elution). The eluate was dried down and reconstituted to 1 mL, and then the 100-fold concentrated sample was analyzed by LC/MS/MS. The sample preparation workflow is shown in Fig. 2.

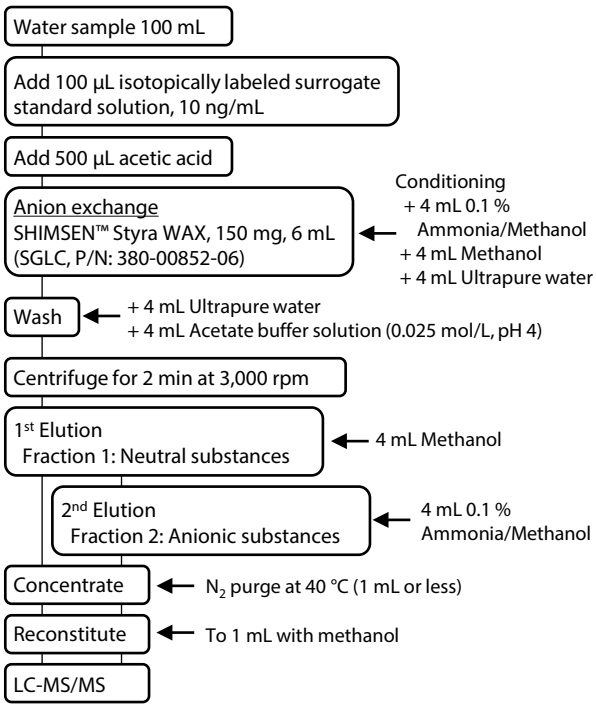

Fig. 2 Sample Preparation Workflow

■ Analytical Conditions

HPLC and MS analytical conditions are indicated in Table 2.

Since PFAS may be present in the analytical system, mobile phases, and other sources, it is essential to minimize system-derived contamination to ensure quantitative accuracy. Therefore, a delay column was installed between the mixer and autosampler, and mobile phases were prepared using reagents specifically intended for PFOS/PFOA analysis. Additionally, a guard column was installed directly before the analytical column to prevent column degradation caused by sample-derived impurities.

Table 2 Analytical Conditions

HPLC Conditions: Nexera™ X3		MS Conditions: LCMS-8060RX	
Analytical Column:	Shim-pack Scepter™ C18-120 (100 mm × 2.1 mm I.D., 1.9 µm, P/N: 227-31012-05)	Ionization:	ESI (Negative mode)
Guard Column*1:	Shim-pack Scepter C18-120 (5 mm × 2.1 mm I.D., 1.9 µm, P/N: 227-31120-01)	Interface Temp.:	250 °C
Delay Column:	Delay column for PFAS (GL Science, P/N: 5020-90005)	Interface Voltage:	-1.0 kV
Mobile Phases:	A) 2 mmol/L Ammonium Acetate in H_2O B) Methanol	Focus Voltage:	-2.0 kV
Gradient Program:	B conc. 5 % (0 min) → 50 % (2 min) → 100 % (19.0-23.0 min) → 5 % (23.01-28.0 min)	ESI Probe Position:	+3 mm
Flowrate:	0.3 mL/min (0-19.0 min) → 0.6 mL/min (19.01-23.00 min) → 0.3 mL/min (23.01-28.00 min)	Nebulizing Gas:	3 L/min
Column Temp.:	40 °C	Drying Gas:	5 L/min
Injection Volume:	5 µL	Heating Gas:	15 L/min
		DL Temp.:	200 °C
		Heat Block Temp.:	300 °C

*1 Shim-pack™ EXP Guard Column Holder (P/N: 227-32041-01)

■ Measuring Standard Solutions

A standard mixture of 30 PFAS (Cat. No. ISO 21675-NSS, Wellington Laboratories) and an internal standard mixture (Cat. No. ISO 21675-LSS, Wellington Laboratories) were diluted with methanol for PFOS/PFOA analysis (P/N: 130-15941, FUJIFILM Wako Pure Chemical) to prepare calibration standards at 0.01, 0.05, 0.2, 1, 5, and 10 ng/mL (internal standard concentration: 1 ng/mL). Each calibration standard was measured three times.

MRM chromatograms obtained from the mixed standard solution (0.05 ng/mL in solution) are shown in Fig. 3. Good peak shapes were obtained for each PFAS.

The linear range, correlation coefficient (R) of each PFAS calibration curve, and the peak area repeatability of the lowest concentration used for calibration (0.05 ng/mL for 6:2 FTSA and 8:2 FTSA, 0.01 ng/mL for all others) are shown in Table 3. The calibration curve linearity was good, with R > 0.996 for all PFAS. Peak area repeatability at the lowest concentration used for calibration was also good, with %RSD (n = 3) <16 % for all PFAS.

Table 3 Linear Range and Correlation Coefficient of PFAS Calibration Curves and Repeatability at Lowest Concentration Used for Calibration

Compound Name	Calibration Range (ng/mL in solution)	Correlation Coefficient R	Area %RSD (%, n = 3)
PFBS	0.01 - 10	0.99979	9.8
PFHxS	0.01 - 10	0.99987	15.3
PFHpS	0.01 - 10	0.99996	13.2
PFOS	0.01 - 10	0.99990	11.3
PFDS	0.01 - 10	0.99991	7.0
FOSA	0.01 - 10	0.99958	2.2
N-MeFOSA	0.01 - 10	0.99948	11.0
N-EtFOSA	0.01 - 10	0.99944	0.4
N-MeFOSAA	0.01 - 10	0.99937	6.4
N-EtFOSAA	0.01 - 10	0.99960	6.2
6:2FTSA	0.05 - 5	0.99639	3.9
8:2FTSA	0.05 - 5	0.99639	3.5
9ClPF3ONS	0.01 - 10	0.99996	14.0
PFBA	0.01 - 10	0.99985	6.9
PFPeA	0.01 - 10	0.99971	10.9
PFHxA	0.01 - 10	0.99985	3.0
PFHpA	0.01 - 10	0.99984	15.8
PFOA	0.01 - 10	0.99979	11.7
PFNA	0.01 - 10	0.99976	5.7
PFDA	0.01 - 10	0.99988	12.5
PFUnDA	0.01 - 10	0.99987	3.9
PFDoDA	0.01 - 10	0.99977	9.5
PFTrDA	0.01 - 10	0.99978	3.5
PFTeDA	0.01 - 10	0.99960	6.9
PFHxDA	0.01 - 10	0.99916	7.2
PFOcDA	0.01 - 10	0.99967	4.9
8:2FTUCA	0.01 - 10	0.99960	8.3
8:2diPAP	0.01 - 10	0.99962	1.7
HFPO-DA	0.01 - 10	0.99956	2.6
DONA	0.01 - 10	0.99989	5.4

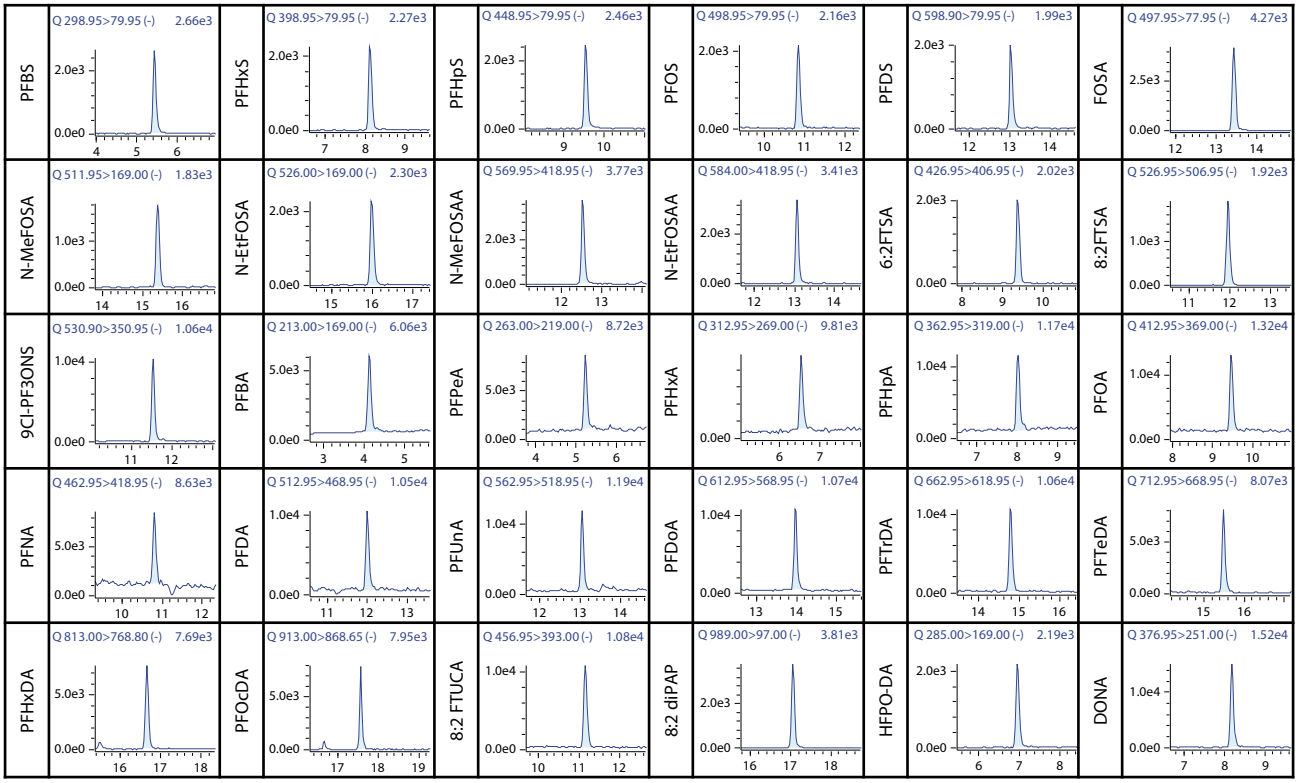

Fig. 3 MRM Chromatograms from Mixed PFAS Standard Solution (0.05 ng/mL in Solution)

■ Spike Recovery Tests Using Ultrapure Water

A procedure blank test was conducted using ultrapure water (P/N: 212-01363, FUJIFILM Wako Pure Chemical), and a spike recovery test was performed by spiking ultrapure water with either a "Low" concentration (1 ng/L in water sample, 0.1 ng/mL in solution) or a "High" concentration (10 ng/L in water sample, 1 ng/mL). Samples were prepared for analysis (Fig. 2), and the results of the spike recovery test using ultrapure water are shown in Table 4.

Good results were obtained, with percentage recoveries ranging from 80 to 119 % for "Low" spiked samples and 93 to 116 % for "High" spiked samples.

Table 4 Spike Recovery Test Using Ultrapure Water

Compound	Concentration (ng/L in ultrapure water)			Recovery Rate*2 (%)	
	Blank	Low	High	Low	High
PFBS	0.03	0.97	10.01	97	100
PFHxS	-	1.09	9.58	109	96
PFHpS	-	0.96	9.83	96	98
PFOS	-	0.99	9.60	99	96
PFDS	-	0.94	9.44	94	94
FOSA	0.01	0.96	10.57	96	106
NMeFOSA	-	0.93	10.07	93	101
NEtFOSA	-	1.00	10.37	100	104
NMeFOSAA	-	0.88	10.17	88	102
NEtFOSAA	-	0.94	10.48	94	105
6:2FTSA	-	0.91	11.23	91	112
8:2FTSA	-	0.80	11.63	80	116
9Cl-PF3ONS	-	0.90	9.50	90	95
PFBA	0.18	1.06	9.90	106	99
PFPeA	0.17	1.12	10.66	112	107
PFHxA	0.15	1.17	9.86	117	99
PFHpA	0.17	1.02	9.37	102	94
PFOA	0.19	1.11	9.78	111	98
PFNA	0.17	1.19	9.90	119	99
PFDA	0.14	1.05	9.82	105	98
PFUnA	0.09	0.90	9.29	90	93
PFDoA	0.09	1.05	10.13	105	101
PFTrDA	0.07	1.02	10.42	102	104
PFTeDA	0.04	0.97	10.17	97	102
PFHxDA	0.03	1.00	10.22	100	102
PFOcDA	-	0.89	9.85	89	99
8:2 FTUCA	-	0.99	9.95	99	100
8:2 diPAP	-	0.93	9.96	93	100
HFPO-DA	-	0.99	10.30	99	103
DONA	-	0.95	9.29	95	93

*2 As prescribed by ISO 21675, percentage recoveries were calculated without considering the procedure blank results.

■ Spike Recovery Tests Using Industrial Wastewater

A matrix blank test was conducted using industrial wastewater, and a spike recovery test was performed by spiking industrial wastewater with either a "Low" concentration (1 ng/L in wastewater, 0.1 ng/mL in solution) or a "High" concentration (10 ng/L in wastewater, 1 ng/mL in solution). Samples were prepared for analysis (Fig. 2), and the results of the spike recovery test using industrial wastewater are shown in Table 5.

For the "Low" concentration samples, percentage recoveries ranged from 59 to 170 %. Recovery rates were not sufficient for compounds detected in the matrix blank at concentrations exceeding the spike level, suggesting interference from the blank. In contrast, the "High" concentration samples showed generally good recoveries, ranging from 81 to 116 %.

Table 5 Spike Recovery Test Using Industrial Wastewater

Compound	Concentration (ng/L in wastewater)			Recovery Rate*3 (%)	
	Blank	Low	High	Low	High
PFBS	0.78	1.68	10.36	90	96
PFHxS	0.38	1.32	9.95	95	96
PFHpS	-	1.00	9.84	100	98
PFOS	1.65	2.49	11.58	85	99
PFDS	-	0.80	8.49	80	85
FOSA	0.12	1.12	11.15	100	110
NMeFOSA	-	0.96	10.30	96	103
NEtFOSA	-	0.92	10.97	92	110
NMeFOSAA	0.03	0.91	10.65	88	106
NEtFOSAA	0.18	1.06	10.23	88	100
6:2FTSA	3.70	4.30	15.02	59	113
8:2FTSA	-	0.84	11.19	84	112
9Cl-PF3ONS	-	0.92	9.29	92	93
PFBA	16.02	17.29	24.17	127	81
PFPeA	7.97	9.43	19.06	147	111
PFHxA	12.77	13.39	23.08	62	103
PFHpA	6.49	7.19	16.46	69	100
PFOA	9.99	11.50	20.15	151	102
PFNA	9.17	10.86	20.74	170	116
PFDA	5.86	6.63	15.75	77	99
PFUnA	3.38	4.20	12.56	82	92
PFDoA	1.68	2.69	12.08	101	104
PFTrDA	0.50	1.33	8.96	83	85
PFTeDA	0.29	1.23	10.59	93	103
PFHxDA	0.09	0.96	10.23	100	101
PFOcDA	0.02	0.84	9.96	82	99
8:2 FTUCA	0.03	0.96	10.04	93	100
8:2 diPAP	-	0.83	9.81	95	98
HFPO-DA	0.75	1.64	11.44	90	107
DONA	-	0.87	9.82	87	98

*3 High concentrations of PFAS were detected in the blank wastewater. Recovery was calculated using the difference between the blank concentrations and the spiked sample concentrations.

■ Conclusion

PFAS analysis in wastewater was conducted using the LCMS-8060RX, in accordance with ISO 21675.

Analysis of calibration standards showed good peak shapes, good repeatability at the limit of quantification (0.01 ng/mL or 0.05 ng/mL in solution), and good calibration curve linearity for all the compounds.

The spike recovery test in ultrapure water also showed good percentage recoveries from samples spiked at 1 ng/L and 10 ng/L in water. In contrast, the spike recovery test using industrial wastewater showed insufficient percentage recoveries at lower concentrations (1 ng/L in water) when the unspiked industrial wastewater contained relatively high concentrations of PFAS. However, good percentage recoveries were obtained at the higher concentration (10 ng/L in water).

<References>

1) ISO 21675:2019, Water quality — Determination of perfluoroalkyl and polyfluoroalkyl substances (PFAS) in water — Method using solid phase extraction and liquid chromatography-tandem mass spectrometry (LC-MS/MS)

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