

Analysis of Per- and Polyfluoroalkyl Substances (PFAS) in Drinking Water and Milk using LC-MS/MS coupled with Online-SPE Interface

Maho Tanaka¹, Nozomi Maeshima¹, Manami Kobayashi¹ (1) Shimadzu Corporation, Kanagawa, Japan

1. Introduction

- ◆ Per- and Polyfluoroalkyl Substances (PFAS) are a chemical group of organic fluorinated compounds, and perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) are representative compounds. They have been used in applications such as water repellents, surface treatment agents, fire extinguishers, and coatings. PFAS are known to be widely present in the environment and wildlife due to their stable structure. Concerns about human exposure through diet have led various countries to conduct investigations into food contamination by PFAS.
- ◆ We have examined a quantitative analysis method for 43 PFAS in tap water, drinking water, and milk, and developed a pretreatment and analysis method that realized labor and time savings using LC-MS/MS and automatic pretreatment equipment (Fig.1). For extraction from milk, the QuEChERS method was used with reference to the FDA method.



Fig. 1 Automatic pretreatment equipment : SPL-W100 (AiSTI SCIENCE) LC-MS/MS : LCMS-8060RX (Shimadzu)

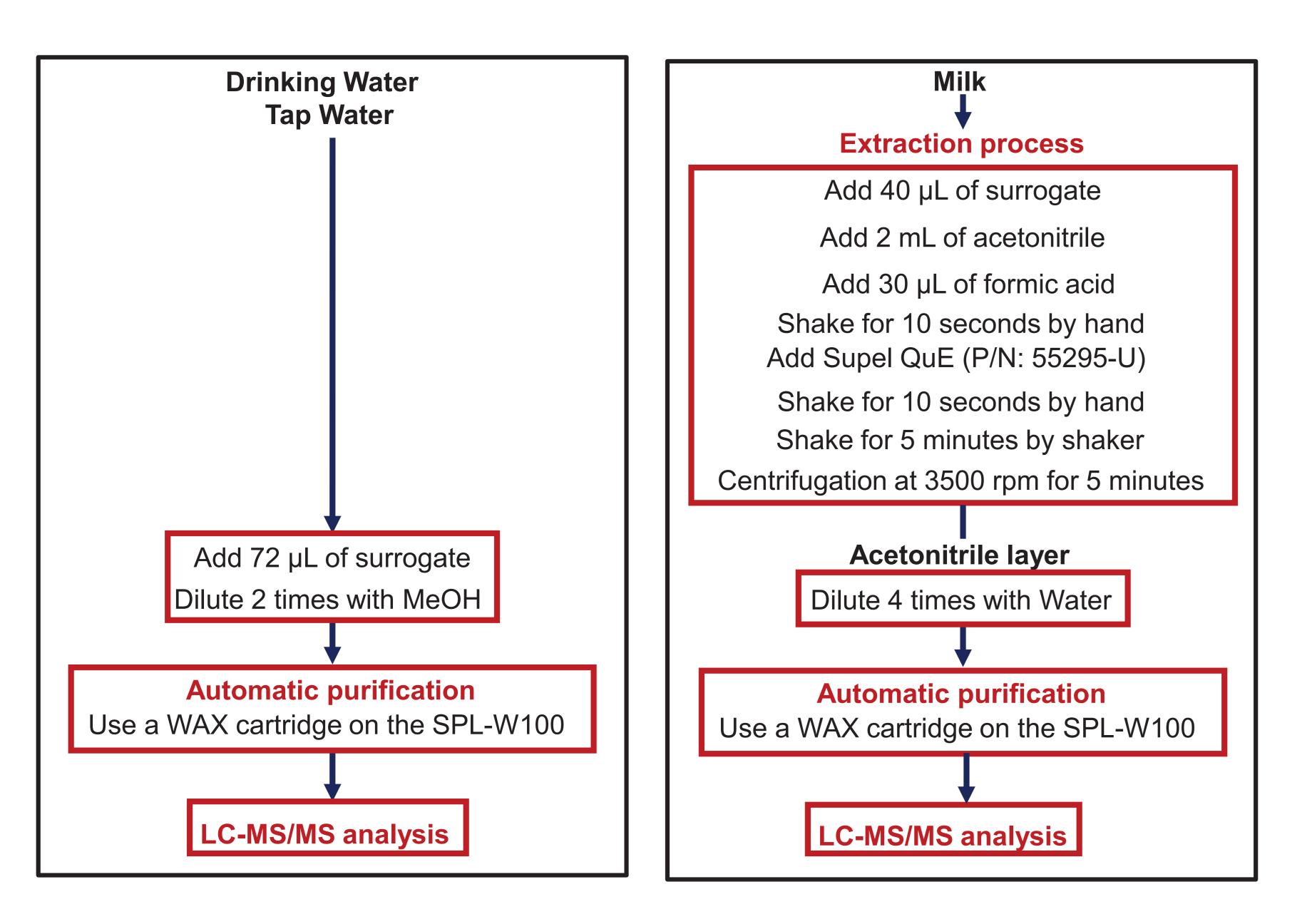


Fig. 2 Workflow of pretreatment process

2. Methods

- ◆ PFAC-MXG, PFAC-MXI, PFAC-MXJ, PFAC30PAR and MPFAC-HIF-ES purchased from Wellington Laboratories were used as standards and ISTDs.
- 600 μL of tap water or drinking water from a plastic bottle was mixed with 600 μL of methanol in a vial, then placed on the auto sampler of the SPL-W100 (AiSTI SCIENCE) (Fig. 2 and 3).
- ♦ 2 g of commercially purchased milk was weighed, and 2 mL of acetonitrile and 30 μL of formic acid were added. The sample was shaken by hand for 10 seconds, after which 1/5 of Supel QuE (P/N: 55295 U) was added, and immediately shaken by hand for 10 seconds. After being shaken with a shaker for 5 minutes, the milk was centrifuged at 3,500 rpm for 5 minutes. A mixture of 100 μL of an acetonitrile layer diluted 4 times with water was automatically purified on the SPL-W100 (Fig. 2 and 3).
- ◆ The analysis conditions for LC-MS/MS are shown in Table 1.

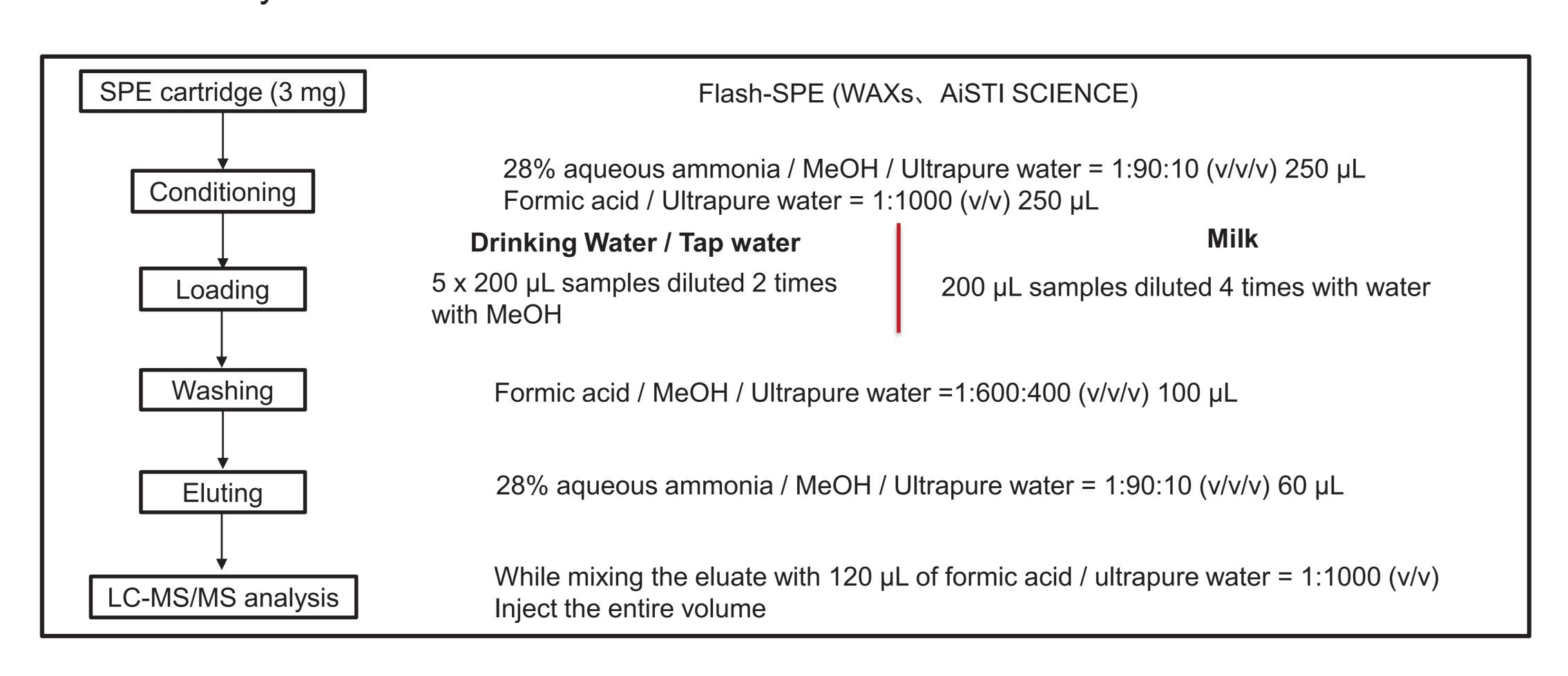


Fig. 3 Automated concentration and solid phase purification flow

Table 1 Analysis conditions

[HPLC conditions]	(Nexera TM X3)	[MS conditions]	(LCMS-8060RX)
Analytical Column:	Shim-pack Scepter C18-120	Interface:	ESI Negative mode
	(100 mm \times 2.1 mm I.D., 3 μ m)	Mode:	MRM
Delay Column:	Shim-pack Scepter C18-120	Interface voltage:	-1 kV
	(50 mm \times 2.1 mm I.D., 3 μ m)	Ion focus voltage:	-1 kV
Mobile phase A:	2 mM Ammonium acetate in water	Interface temp.:	300°C
	/ Acetonitrile=95:5	Nebulizer gas flow:	3 L/min
Mobile phase B:	Acetonitrile	Drying gas flow:	10 L/min
Gradient program:	B conc. 20%(0-1 min) - 100%(1	Heating gas flow:	10 L/min
	min-14 min) - 20%(14.01 min-16		
	min)	Heating block temp.:	500°C
Flow rate:	0.3 mL/min(0-11 min) - 0.6	DL temp.:	200°C
	mL/min(11.10 min-14 min) - 0.3	Probe position:	+2 mm
	mL/min(14-16 min)		
Column temp '	40 °C		

3. Results

- ◆ Fig. 4 shows the MS chromatograms for simultaneous analysis of 43 PFAS compounds, and fig. 5 shows the calibration curves for representative compounds. All compounds eluted within 11 minutes, indicating good separation.
- In water, good calibration curves can be obtained in the range of 0.0004 to 0.01 μg/kg for 4 components (PFOA, PFNA, PFHxS, PFOS) and 0.001 to 0.1 μg/kg for all other component.
- In milk, good calibration curves can be obtained in the range of 0.005 to 1 μg/kg for 4 components (PFOA, PFNA, PFHxS, PFOS) and 0.05 to 1 μg/kg for all other compounds.
- In water, the recovery tests were conducted at concentrations of 0.0004, 0.004, and 0.001 μg/kg to verify the recovery rates and repeatability. Table 2 and fig. 6 shows the criteria in drinking-water by EPA and recovery rate and reproducibility in tap water and drinking-water obtained from actual analysis.
- ♦ In milk, the recovery tests were conducted for 4 compounds at the spiked concentrations of 0.01, 0.1, and 1 μg/kg, and for all other compounds at the spiked concentrations of 0.1 and 1 μg/kg. Table 3 and fig. 7 shows the criteria in milk by AOAC SMPR and recovery rate and reproducibility in milk obtained from actual

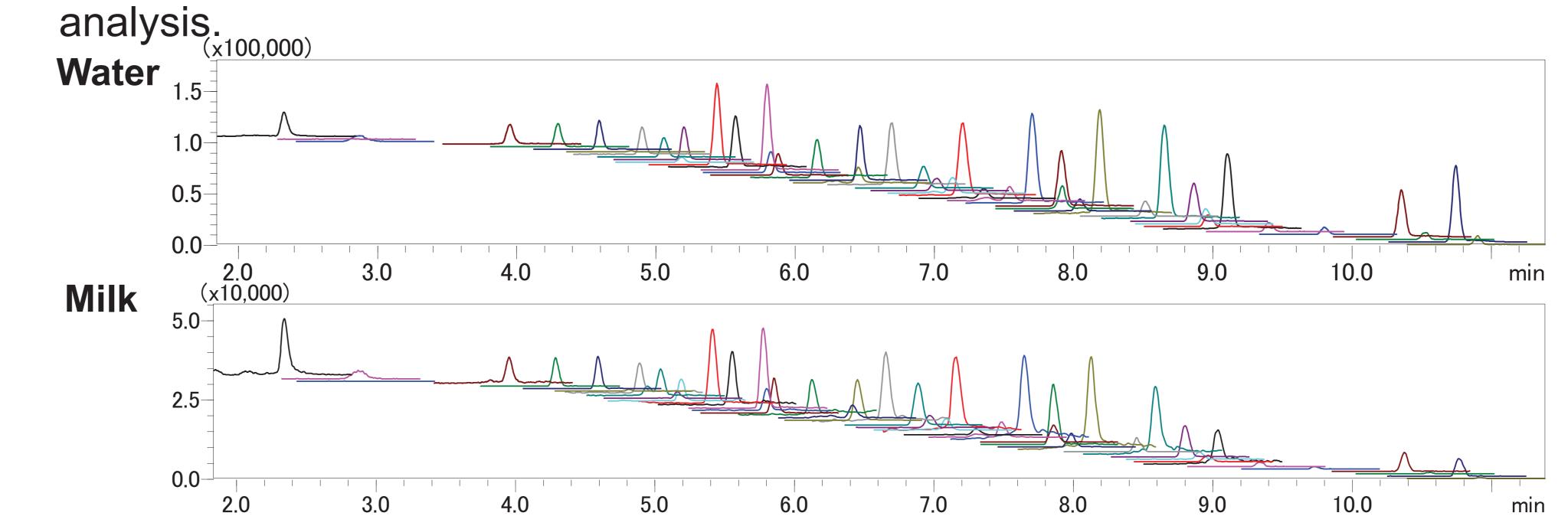


Fig. 4 MS chromatograms of samples spiked with 43 PFAS compounds

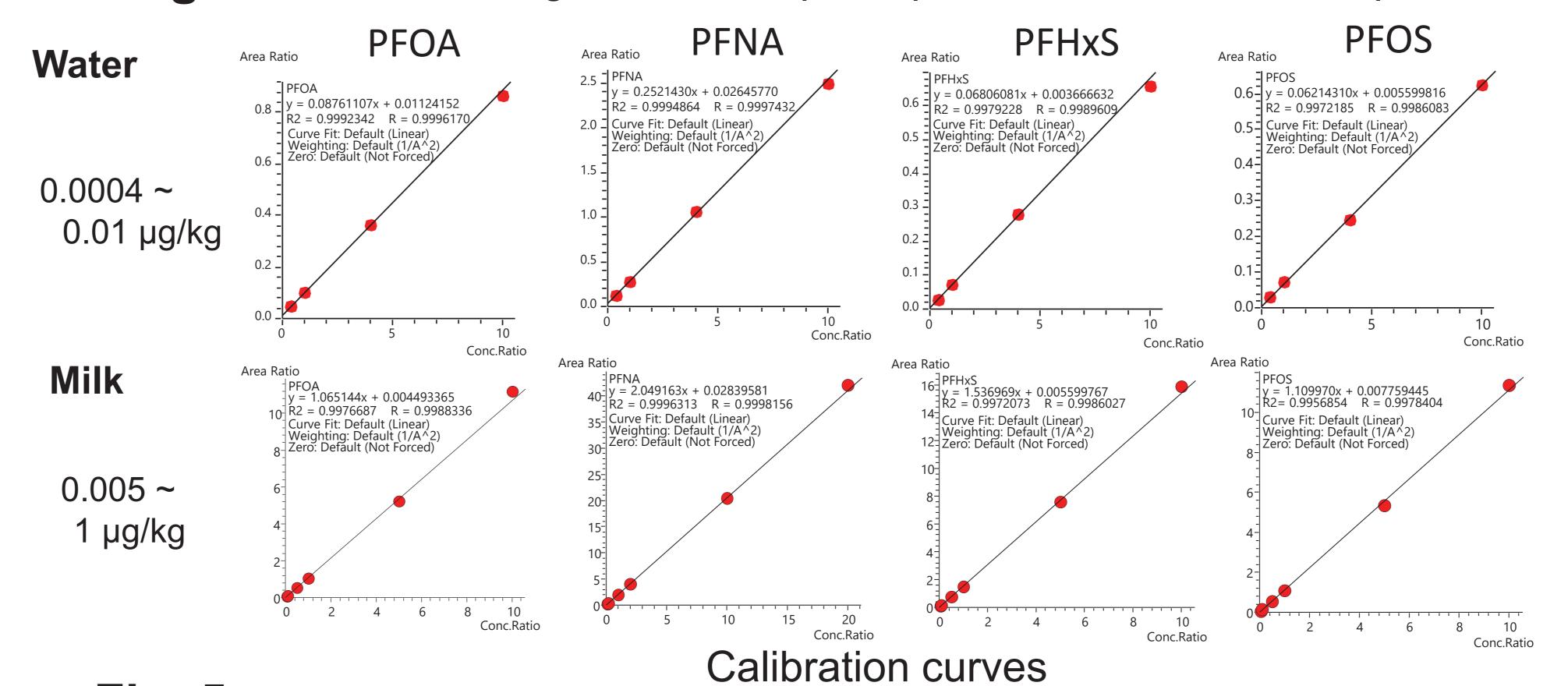


FIG. 5 (Water: absolute calibration curve, Milk: matrix calibration curve)

Table 2 EPA criteria in drinking-water and recovery rate in tap and drinking-water

The Criteria of drinking-water Recovery rate of PFAS in tap water and drinking water

ompound	LOQ (µg/kg)	Compound	Conc.	Recovery (%)				
		Compound	(µg/L)	tap	Drinking water	ta		
PFOS	≦0.004	PFOS		•		10		
PFOA	≦0.004	PFU3	0.0004	93.1	98.3	18		
PFNA	≦0.01	PFOA	0.0004	85.0	94.6	18		
PFHxS	≦ 0.01	PFNA	0.0004	83.8	113.6	15		
IEPO-DA	≦ 0.01	PFHxS	0.0004	119.4	97.0	10		
		HEPO-DA	0.001	110.4	108.6	15		
		Other PFAS	≦0.004	92.3-131.9	91.8-138.1	≦1		

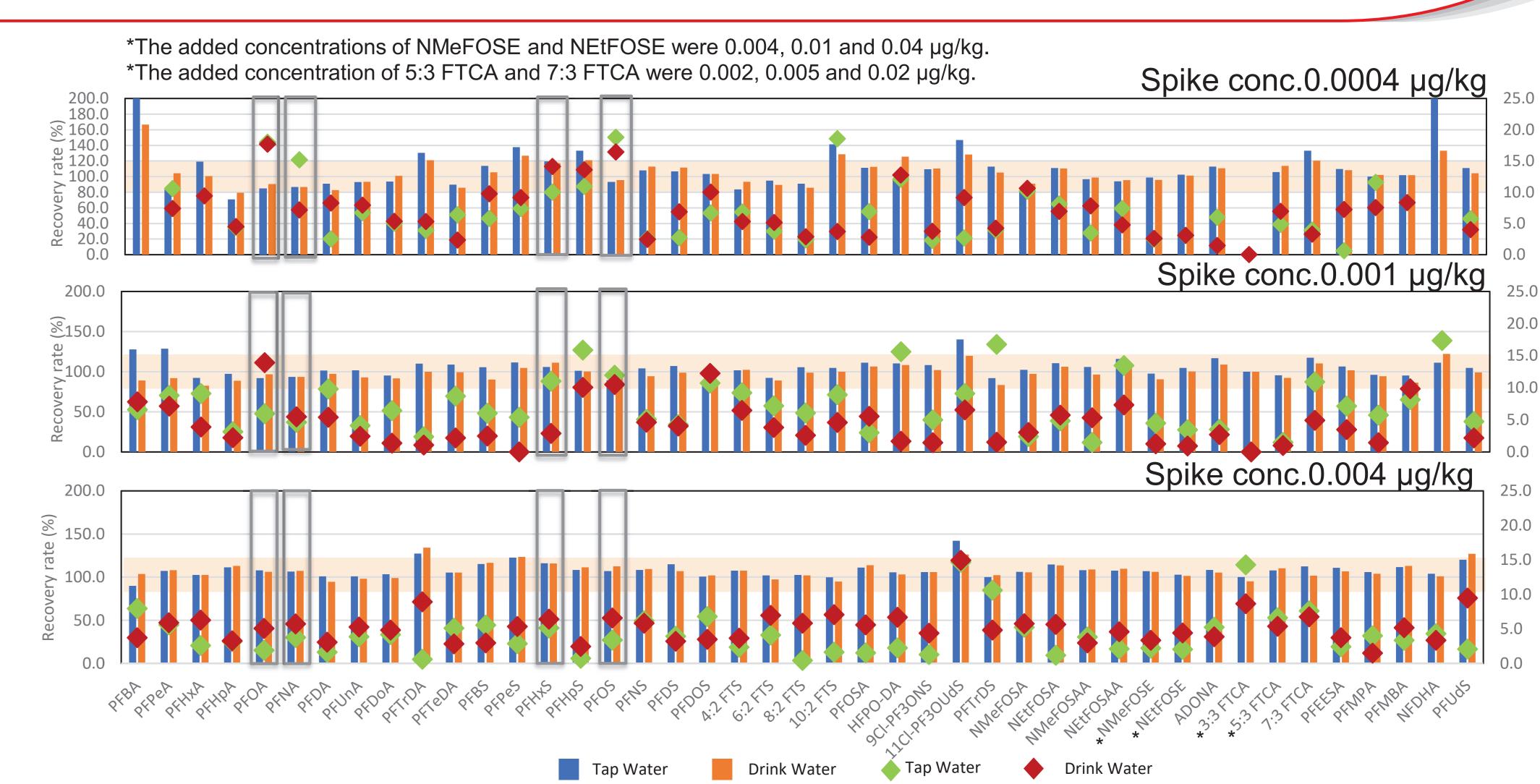
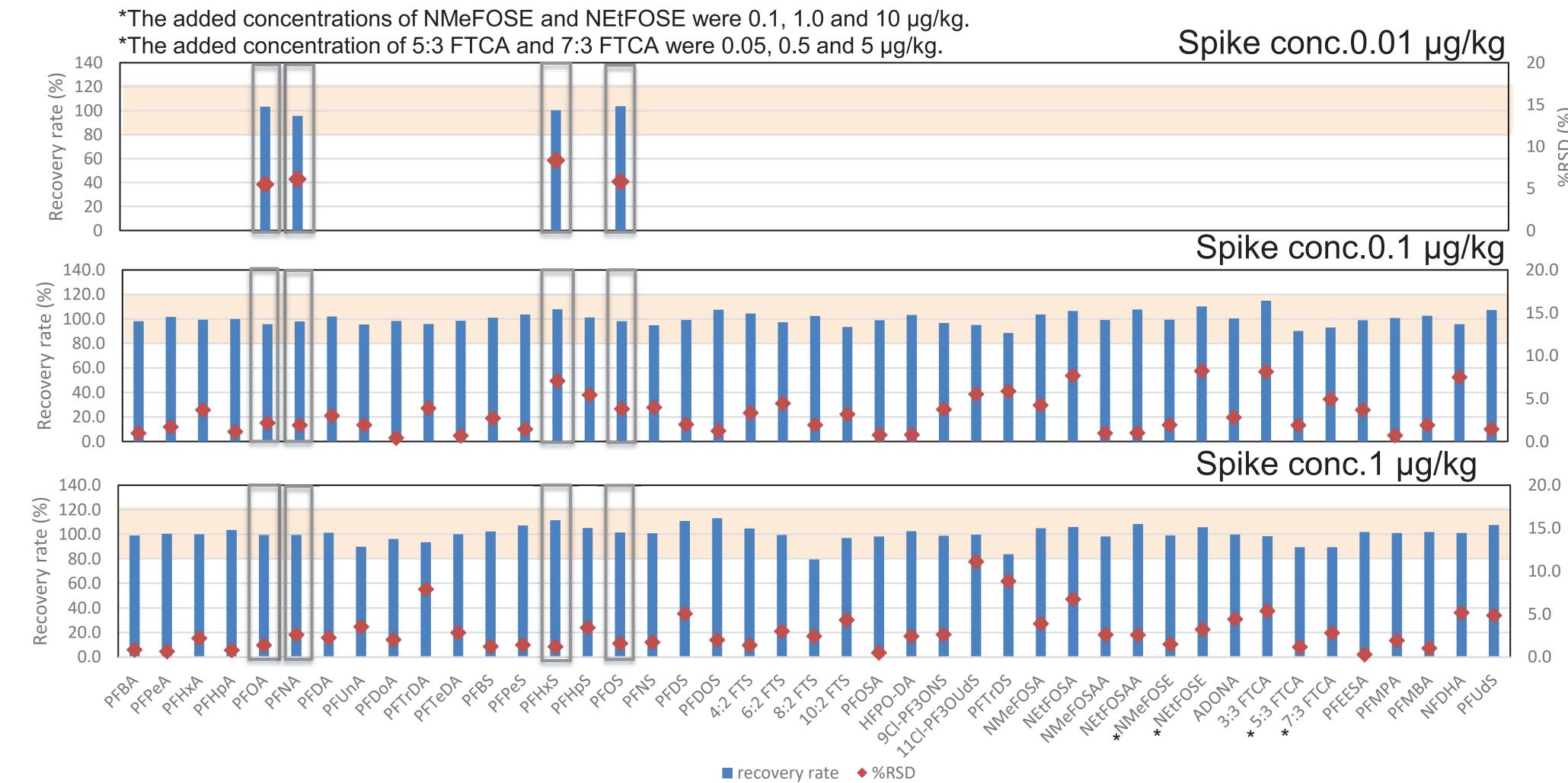


Fig. 6 Recovery rate and repeatability (n=3) of PFAS in tap and drinking-water

 Table 3
 The criteria value in milk by AOAC SMPR and recovery rate in milk

The criteria of milk				Recovery rate of PFAS in milk			
Compound	LOQ (µg/kg)	Recovery (%)	Repeatability (%)	Compound	Conc. (µg/kg)	Recovery (%)	Repeatability (%)
PFOS	≦0.01	65-135	≦25	PFOS	0.01	103.7	5.8
PFOA	≦0.01	65-135	≦25	PFOA	0.01	103.3	5.5
PFNA	≦0.01	65-135	≦25	PFNA	0.01	95.7	6.1
PFHxS	≦0.01	65-135	≦25	PFHxS	0.01	100.3	8.4
PFBA and PFPeA	≦ 1.0	-	-	PFBA	0.1	98	1.0
Other PFAS	≦ 0.1	-	-	PFPeA	0.1	101.6	1.7
				Other PFAS	0.1	79.4-113.1	≦ 8.2



Recovery rate and repeatability (n=3) of PFAS in milk

4. Conclusion

- ◆ LC-MS/MS methods were developed to analyze 43 PFAS in 11 minutes using automatic pretreatment, saving time and labor.
- ◆ We conducted recovery tests by adding samples to tap water, drinking water, and milk, and achieved the LOQ set by EPA regulations and AOAC criteria for all compounds.
- The quantitative values of the four main components in tap water were PFOA 0.00109 μg/kg, PFNA 0.00044 μg/kg, PFHxS 0.0004 μg/kg and PFOS 0.00063 μg/kg. PFAS was not detected above the detection limit in drinking water and milk.

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