

Development of an Analytical Method for PFAS in Environmental Water Using Online SPE-LC/MS

Ryoichi Sasano*¹, Mari Takazawa², Chiaki Maruyama¹, Mamoru Motegi², Shusuke Takemine²
¹Aisti Science Co., Ltd. - Wakayama (Japan), ²Center For Environmental Science in Saitama - Saitama (Japan)
E-mail: sasano@aisti.co.jp

Introduction

Per- and polyfluoroalkyl substances (PFAS) are persistent environmental contaminants of global concern because of their widespread occurrence, bioaccumulation potential, and potential adverse health effects. Online SPE-LC/MS has attracted increasing attention as a high-throughput technique for rapid, sensitive, and automated PFAS analysis. In our previous study (Takazawa et al., 2025), we developed an analytical method combining liquid-liquid partitioning with online SPE-LC/MS for 0.5 mL water samples. This approach effectively removed highly polar ionic matrix components prior to SPE and automated the analytical workflow. However, the small sample volume raised concerns regarding sampling variability. Therefore, this study developed a simple, rapid, and accurate method for PFAS analysis in environmental water using a 10 mL sample volume.

Material and Method

Thirty-five PFAS were selected as target analytes. Ultrapure water, hard water, river water, and brackish water were used as test matrices. A 10 mL water sample was mixed with 100 µL of 2 N hydrochloric acid and 5.8 mL of ethyl acetate, followed by manual shaking for 15 s to perform liquid-liquid partitioning. After standing for 1 min, approximately 1 mL of the organic phase was transferred to a vial and placed in the online SPE-LC/MS system.

Results and Discussion

Sample Volume and Extraction Solvent: In our previous study, liquid-liquid partitioning was performed by adding 1 mL of ethyl acetate-TBME to 0.5 mL of water sample. In the present method, the sample volume was increased to 10 mL, and 5 mL of ethyl acetate was initially added for liquid-liquid partitioning. Increasing the sample volume from 0.5 to 10 mL improved the applicability of the method to routine water quality testing and reduced concerns regarding sampling variability. In addition, whereas the previous method resulted in a two-fold dilution, the present method provided a two-fold enrichment, resulting in an overall four-fold improvement in sensitivity compared with the previous method. Initially, partitioning 10 mL of water with 5 mL of ethyl acetate resulted in aqueous and organic phase volumes of 10.8 and 4.2 mL, respectively. Therefore, the volume of ethyl acetate was increased to 5.8 mL to obtain approximately 5 mL of organic phase after partitioning, thereby maintaining a two-fold enrichment factor. In accordance with the increased sample volume, 100 µL of 2 N hydrochloric acid was added. The operational procedure remained as simple and rapid as that used in our previous study.

Effect of Liquid-Liquid Partitioning: Compares the proposed method with the conventional workflow, from field sampling and transportation to sample preparation in the laboratory. Unlike the conventional method, the proposed method uses the original sampling tube directly for liquid-liquid partitioning, eliminating the need to transfer the sample to additional laboratory vessels and thereby reducing the risk of contamination. Furthermore, as demonstrated in our previous study, highly hydrophobic PFAS that may adsorb onto the inner surface of the sampling tube or suspended particles can be recovered into the organic phase. Thus, liquid-liquid partitioning provides a simple and efficient sample preparation procedure.

Blank Reduction: An investigation of the sources of blank contamination revealed PFAS originating from the sealing material in the caps of the solvent bottles. Therefore, we developed PFAS-removal solvent-cleanup cartridges packed with ion-exchange resin to purify all solvents and ultrapure water used for sample preparation, mobile phases, and laboratory cleaning. This approach reduced PFAS blank levels to nearly negligible levels.

Calibration Curves: The mixed PFAS standard solution was diluted with ethyl acetate, the same solvent as the sample extract, to concentrations of 4, 10, 20, and 40 ng/L, corresponding to 2, 5, 10, and 20 ng/L in the original water samples, respectively. These calibration standards were subjected to SPE using the same online SPE-LC/MS procedure as the samples. Representative calibration curves are shown in Figure 5, and the coefficients of determination (R^2) for all PFAS are summarized in Table 1. Good linearity was obtained for all target compounds.

Spike-Recovery Test: Spike-recovery tests were performed by adding the mixed PFAS standard solution to each water sample at 10 ng/L. The recoveries and reproducibility ($n = 4$) are summarized in Table 1. Stable isotope-labeled internal standards were not used in this study, and recoveries were calculated from the absolute peak areas. Although recoveries above 120% or below 80% were observed for some compounds, generally satisfactory recoveries were obtained for most PFAS. The major exceptions were 4:2 FTS, a highly polar compound that is poorly transferred to the organic phase during liquid-liquid partitioning, and the neutral compounds FOSA, N-MeFOSA, and N-EtFOSA, which are poorly retained by the weak anion-exchange sorbent. Good reproducibility was obtained, with RSDs ($n = 4$) generally below 10%. This high reproducibility was attributed to the simple manual sample preparation procedure combined with automation of the entire process from SPE to LC/MS analysis.

Conclusion

By combining liquid-liquid partitioning with ethyl acetate and online SPE-LC/MS, a simple, rapid, and reliable method was developed for the determination of 31 PFAS in 10 mL water samples. The method reduced the risk of PFAS contamination, minimized manual sample handling, and maintained good analytical performance across diverse water matrices. These results demonstrate the potential of the proposed method as a practical and automated tool for routine monitoring of PFAS in environmental and drinking water.

Funding This research was performed by the Environment Research and Technology Development Fund (JPMEERF20255003) of the Environmental Restoration and Conservation Agency provided by Ministry of the Environment of Japan.

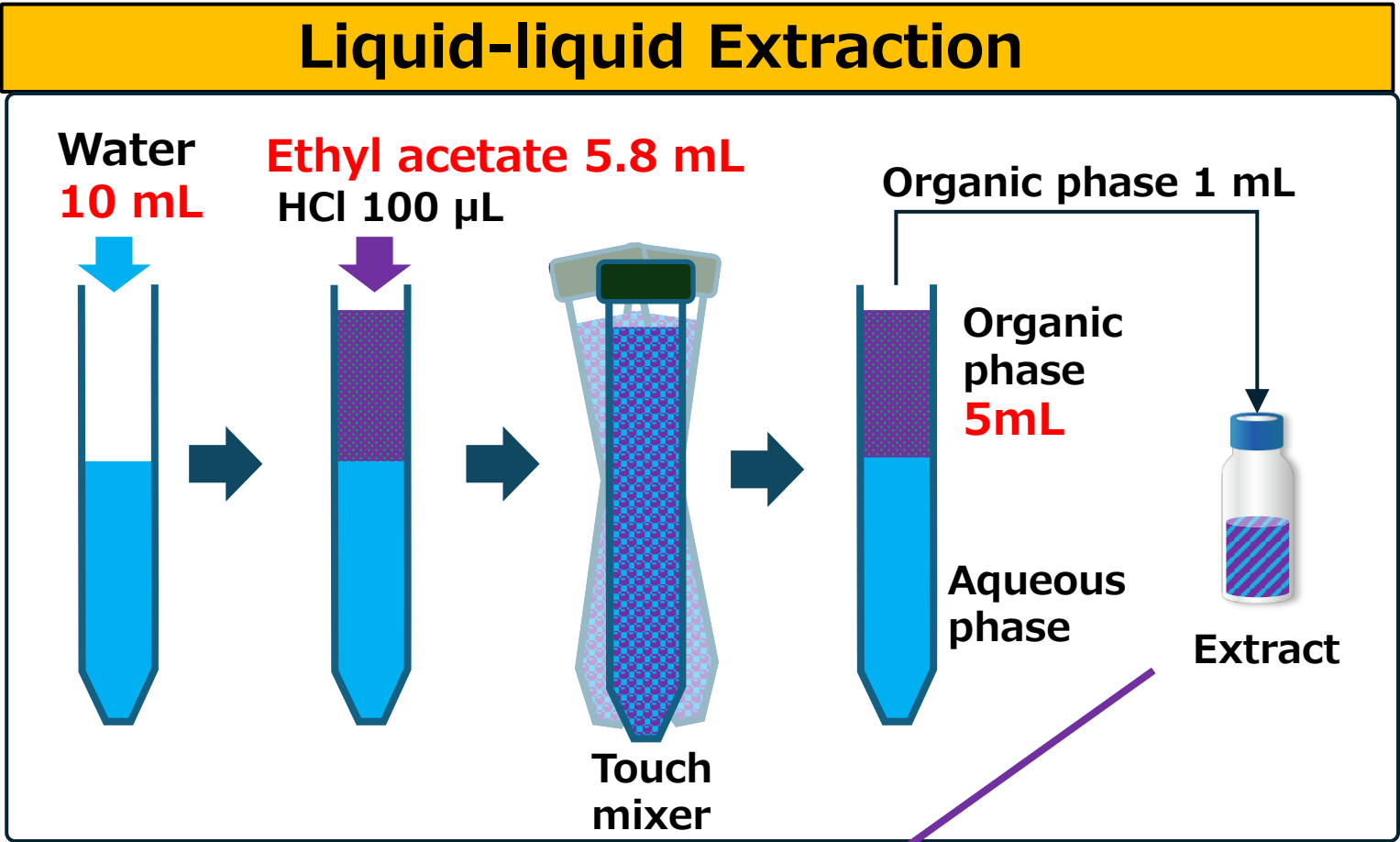


Fig. 1 Liquid-Liquid Extraction Procedure.

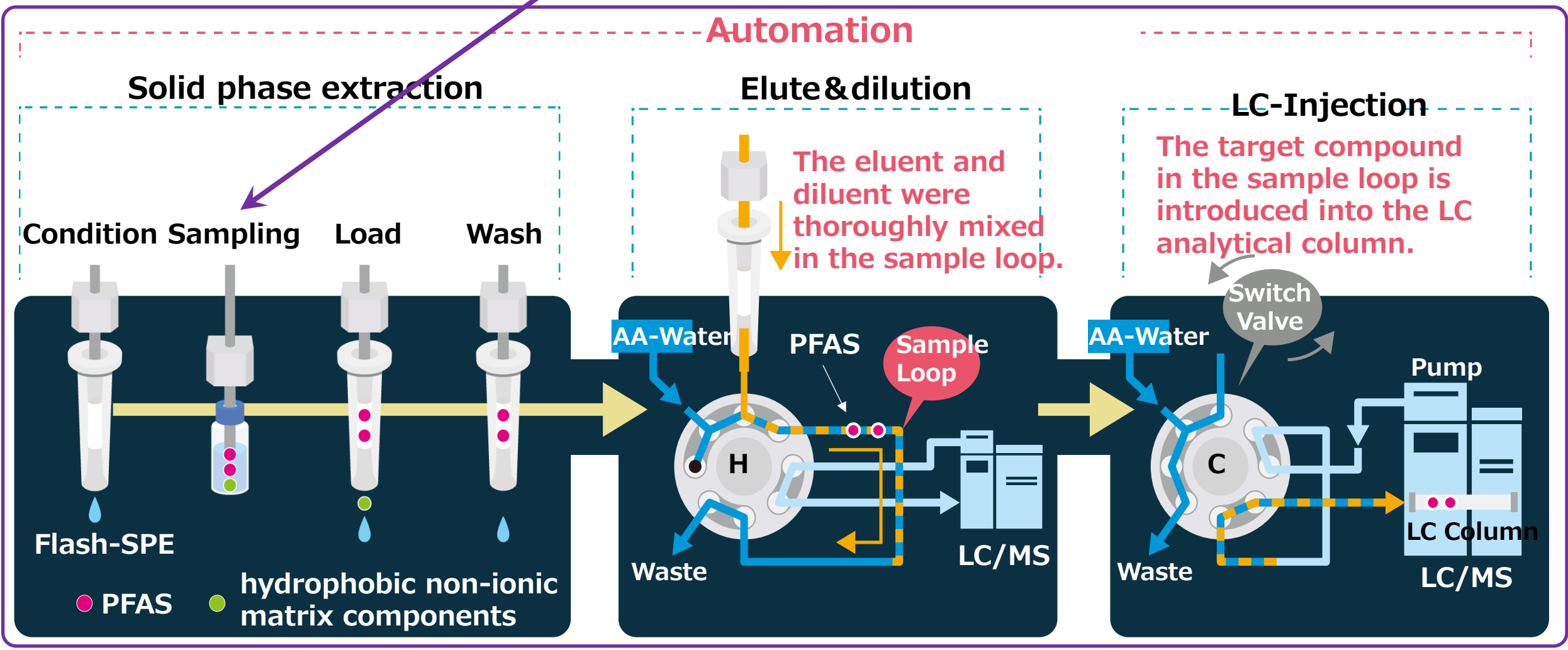


Fig. 2 The process of online SPE-LC-MS/MS.

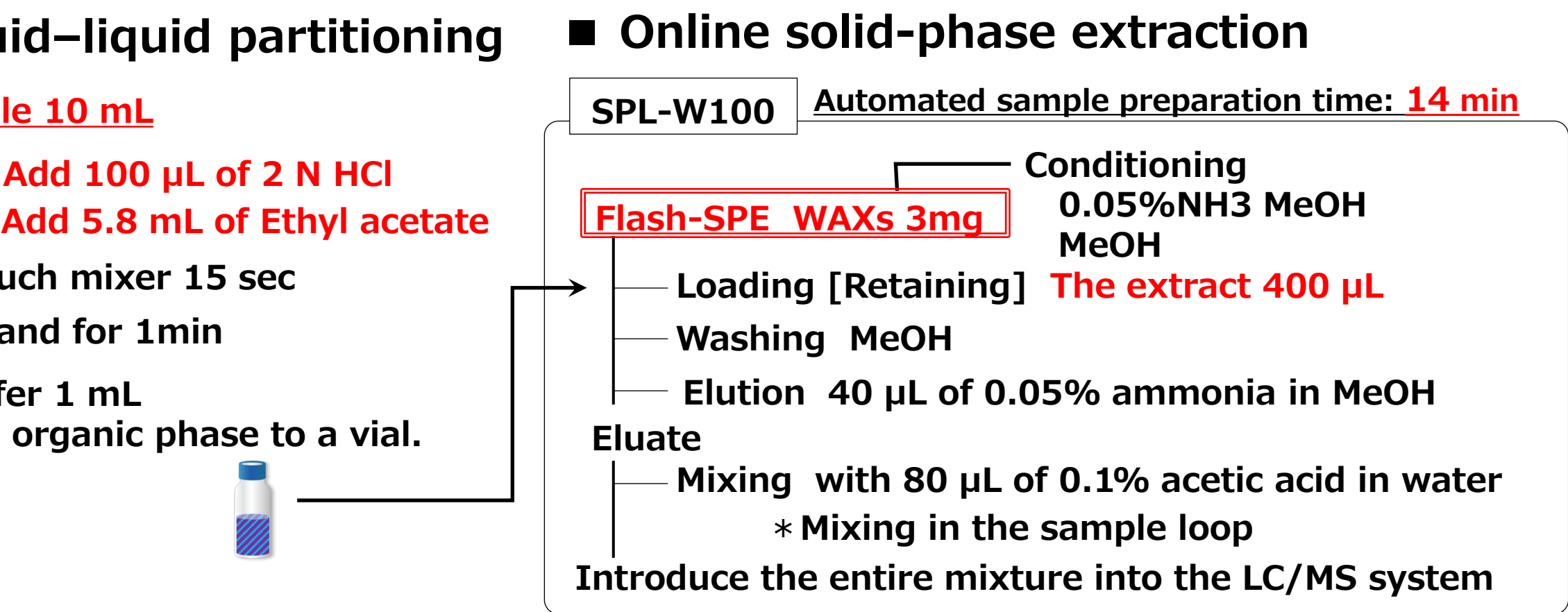
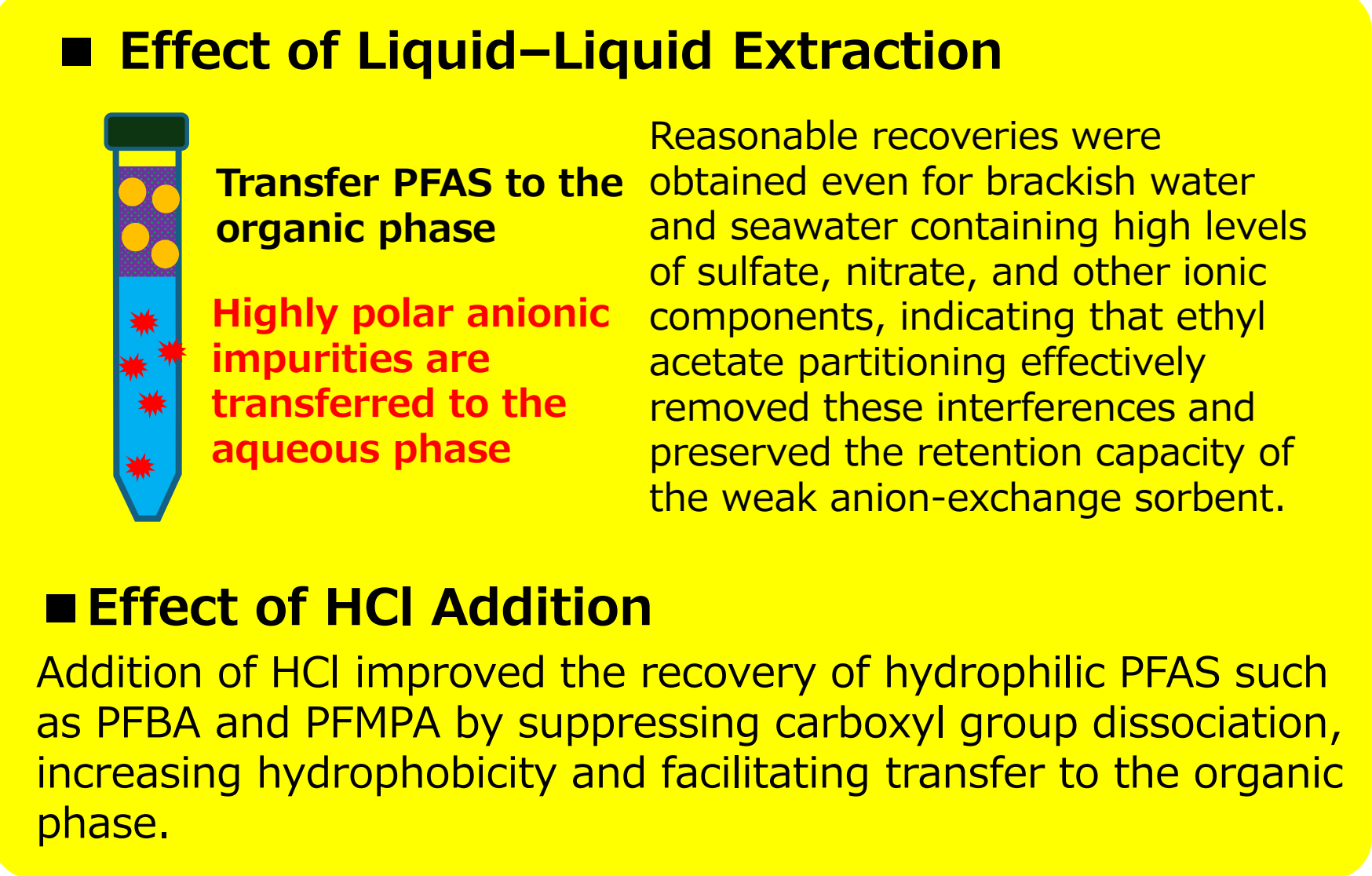
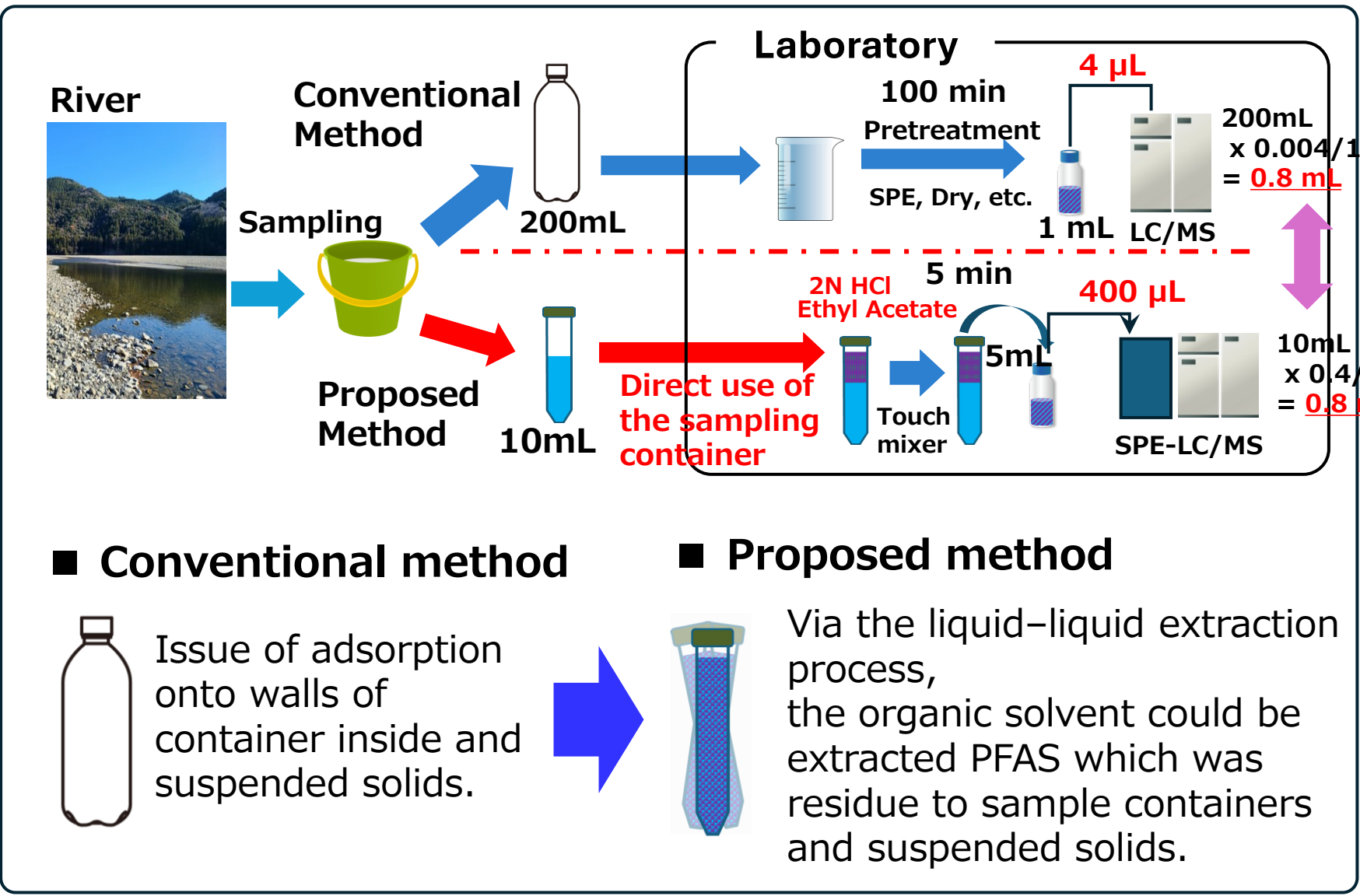


Fig. 3 Pretreatment procedure for PFAS analysis.



Online SPE-LC-MS/MS system
Online SPE-LC : SPL-W100 (AiSTI Science)
LC-MS: LCMS 8045 (Shimadzu)

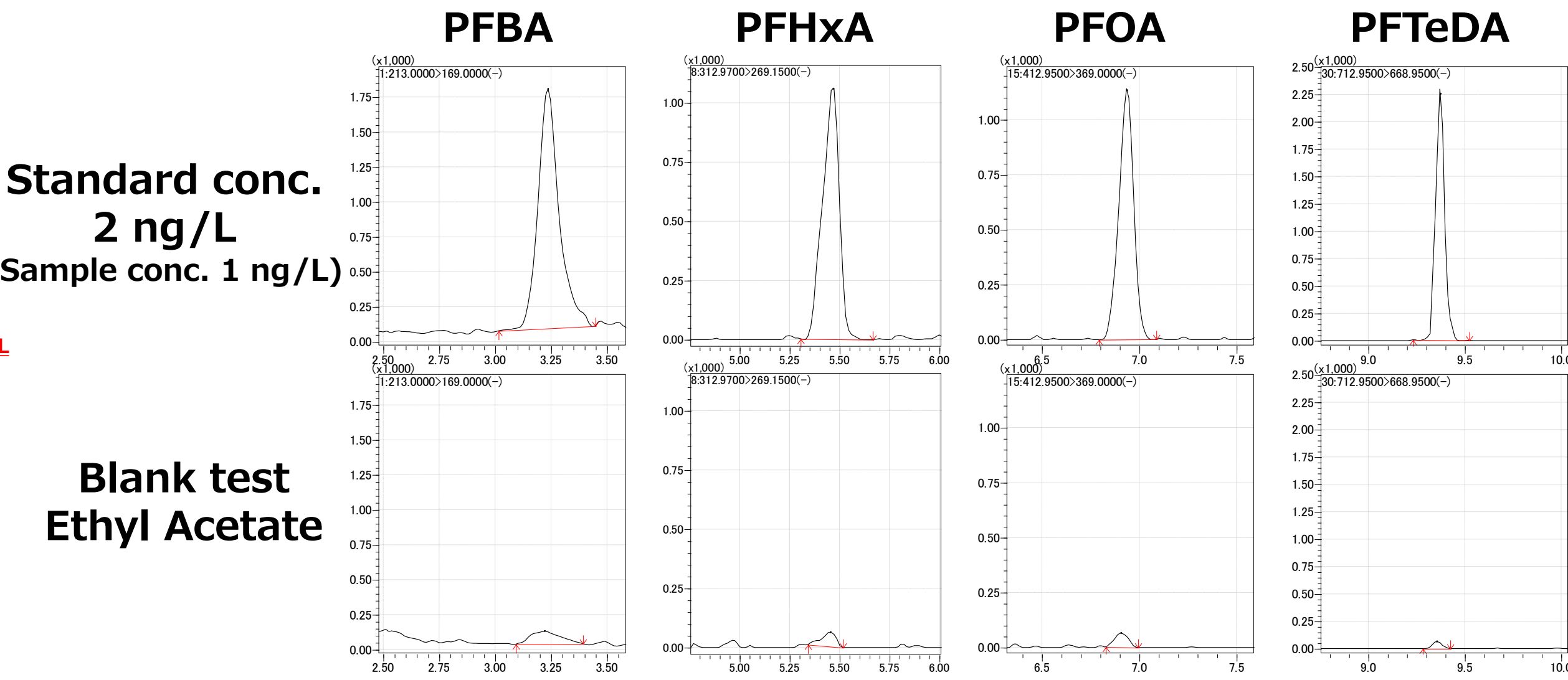


Fig. 4 Comparison of MRM Chromatograms Using Online SPE-LC/MS

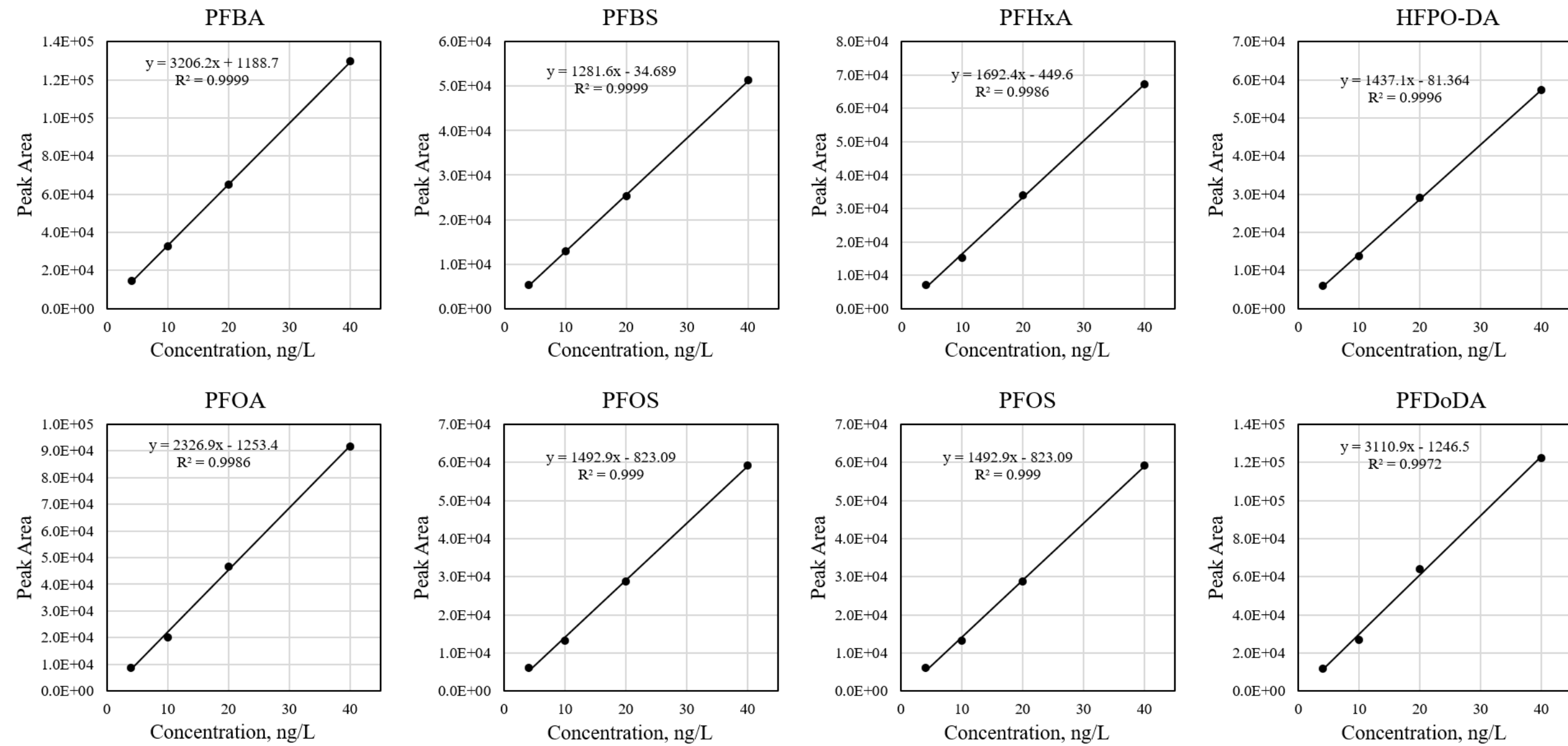


Fig. 5 Absolute calibration curve using the online SPE-LC-MS/MS system.

Table 1. Results of Spike-Recovery Tests: Recoveries and Reproducibility.

ID	Name	R ²	Spiked concentration, 10 ng/L, n = 4 Unit: %									
			River water		Brackish water		Seawater		Hard water		Tap water	
			Recovery	RSD	Recovery	RSD	Recovery	RSD	Recovery	RSD	Recovery	RSD
1	PFBA	0.9999	90	3	82	3	93	2	85	6	94	3
2	PFMPA	0.9988	102	5	94	4	108	1	98	4	107	7
3	PFPeA	0.9999	101	3	92	3	103	2	93	7	109	4
4	PFMBA	0.9942	106	12	101	6	113	8	101	15	111	11
5	PFBS	0.9999	70	4	62	4	68	2	71	5	74	2
6	NFDHA	0.9993	106	4	103	1	109	2	105	6	112	2
7	PFHxA	0.9986	100	6	95	1	107	1	96	7	117	4
8	PFEESA	0.9997	71	4	67	3	76	3	78	5	82	3
9	HFPO-DA	0.9996	96	2	96	2	102	2	95	3	100	2
10	PFPeS	0.9997	82	6	80	3	86	2	84	5	88	4
11	PFHpA	0.9992	100	4	95	2	110	2	99	7	118	3
12	PFHxS	0.9992	89	4	93	2	95	3	95	5	99	4
13	6:2FTSA	0.9950	84	4	80	2	86	4	86	6	91	3
14	PFOA	0.9986	114	3	105	2	117	1	103	7	126	4
15	PFHpS	0.9996	88	2	83	7	91	7	88	6	90	2
16	8:2 FTUCA	0.9993	110	5	113	3	113	2	113	6	125	2
17	PFNA	0.9984	115	2	103	3	114	2	102	6	122	5
18	PFOS	0.9990	90	3	90	3	96	4	93	3	93	3
19	8:2FTSA	0.9991	90	5	91	4	97	5	99	6	101	2
20	PFDA	0.9984	125	3	107	4	111	3	106	7	118	3
21	NMeFOSAA	0.9908	106	6	111	10	112	9	113	6	112	4
22	PFNS	0.9988	95	4	91	4	97	6	93	3	93	1
23	NEtFOSAA	0.9951	111	3	100	4	108	10	108	4	116	7
24	PFUnA	0.9977	116	4	103	1	112	6	106	6	116	5
25	PFDS	0.9984	88	9	91	7	90	5	90	9	97	5
26	PFDoDA	0.9972	111	5	103	4	110	6	112	5	116	3
27	PFTeDA	0.9987	114	3	104	5	113	6	113	7	121	2
28	PFTeDA	0.9952	119	5	101	3	114	4	110	6	119	2
29	PFHxD	0.9989	115	3	106	5	113	3	114	6	116	3
30	8:2 diPAP	0.9956	121	4	109	4	116	3	122	9	123	5
31	PFOcDA	0.9946	117	2	122	5	119	3	113	6	115	4

※ The recovery rate was calculated from the absolute peak area values (without using stable isotopes).