# Analyses of Per- and Polyfluoroalkyl Substances in Water Using Ion Exchange Solid-Phase Extraction and LC–MS/MS with an Activated-Carbon Delay Column

Takumi Kunieda*, Reika Takahara, Kazuyuki Ishii, Manabu Takayanagi, Shigenori Ota, Hiroshi Hayashida,
GL Sciences Inc., Tokyo, Japan

## Introduction

The phrase per- and polyfluoroalkyl substances (PFAS) is a general term used for organofluorine compounds. These substances are known to degrade slowly; therefore, they persist in the environment for a long time. Their toxicity and the environmental pollution they cause have attracted worldwide attention, and research to mitigate these effects continues. The methods of solid-phase extraction (SPE) and liquid chromatography–tandem mass spectrometry (LC–MS/MS) have been used to analyze PFAS in drinking water under EPA methods 537.1 and 533. Additionally, some PFAS are known to leach from laboratory equipment materials, causing sample contamination. Therefore, precautions must be taken to accurately quantify PFAS. In particular, care must be taken to minimize the effects of PFAS background and contamination eluting from fluorinated resins such as polytetrafluoroethylene (PTFE), which are commonly used as components in LC systems. A known countermeasure for such effects is to delay the elution time of the blank peak by connecting a delay column packed with a C18 (ODS) material before the autosampler and shift the retention time relative to the peak derived from the sample. However, using a conventional C18 column, it is challenging to sufficiently increase the difference between the two retention times. Column sizes are limited due to the relationship between pressure rise and gradient delay time. Therefore, to perform stable PFAS analysis, we have developed a new delay column that is packed with high-purity activated-carbon beads. Additionally, two SPE cartridges of different sizes, 250 mg and 150 mg, were used and reference standard addition recovery tests were conducted for each cartridge.

## Methods

For LC–MS/MS, we used a 4000 QTRAP (AB SCIEX LLC, MA USA) instrument. For the delay column, we packed high-purity, spherical, activated carbon in the LC column hardware and installed in the HPLC system. We used an InertSustain C18–HP 150 mm × 2.1 mm analysis column with 3-µm-particle size (GL Sciences Inc., Tokyo Japan). We prepared a standard sample by diluting a PFAS 21 mixture standard PFAC-MXC (Wellington Laboratories, Ontario, Canada) and adding it to the sample water. We used 13 mixtures of MPFAC-C-ES (Wellington Laboratories, Ontario, Canada) as external standards. For sample preparation, we used SPE cartridge InertSep MA-2 250-mg (GL Sciences, Inc., Tokyo Japan) packed with a methacrylate polymer comprising a weak anion-exchange group (diethyl amine). We performed all the operations—from conditioning of the SPE cartridge to the evaporation of the elution solvent—using the automated SPE instrument AquaTrace ASPE899 (GL Sciences, Inc, Tokyo Japan). We passed a 500-mL sample through the SPE cartridge and then eluted it using 5 mL of 0.1% ammonia methanol. Subsequently, we heated the sample, exposed it to nitrogen gas, and concentrated it to a volume of 0.5 mL. For realizing a rapid SPE method, we used an InertSep MA-2 150-mg cartridge. We passed 30 mL of sample water through the cartridge and then used 1 mL of the eluting solvent. We did not distill off the solvent after elution. We added the mixture of standard MPFAC-C-IS (Wellington Laboratories, Ontario, Canada) to the eluate as an injection standard. To avoid contamination of the PFAS, we used a high-purity polypropylene vial in the autosampler and applied an aluminum foil and silicon septum cap on the vial. Before use, we soaked and washed all the glassware and pipette tips using methanol (Kanto Chemical Co., Inc, Japan). We also automatically washed the AquaTrace sample line tube with methanol.

**Table 1** Liquid Chromatograph Conditions

| | |
|---|---|
| System | Nexera UFLC (Shimadzu Corporation, Japan) |
| Column | InertSustain C18 (GL Sciences Inc, Japan)<br>3 µm HP, 150 × 2.1 mm I.D. |
| Delay Column | Delay Column for PFAS (GL Sciences Inc, Japan)<br>30 × 3.0 mm I.D. |
| Mobile Phase A | 10 mmol/L Ammonium acetate |
| Mobile Phase B | Acetonitrile |
| Flow Rate | 0.3 mL/min |
| Column Temp | 40 ℃ |
| Injection Vol | 1 µL |
| Gradient (A/B) | 80/20 – 2 min - 80/20 – 13 min - 0/100 – 2 min - 100/0-0.1 min - 80/20 – 6 min - 80/20 |

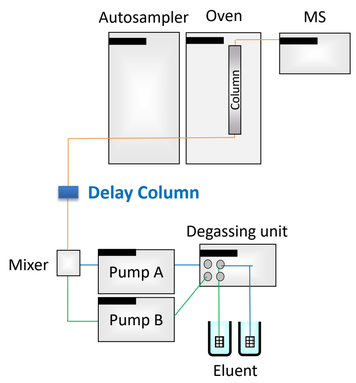


**Fig.1** Delay column installation position

**Table 2** Compound and Mass Spectrometer Conditions

| System | 4000 QTRAP (AB SCIEX LLC, MA USA) |
|---|---|

| Compounds | Transition Q1/Q3 | DP | EP | CE | CXP |
|---|---|---|---|---|---|
| PFBA | 213/169 | -45 | -10 | -14 | -9 |
| PFPeA | 263/219 | -50 | -10 | -11 | -9 |
| PFHxA | 313/269 | -50 | -10 | -15 | -9 |
| PFHpA | 363/319 | -55 | -10 | -14 | -9 |
| PFOA | 413/369 | -45 | -10 | -14 | -9 |
| PFNA | 463/419 | -65 | -10 | -16 | -9 |
| PFDA | 513/469 | -65 | -10 | -14 | -9 |
| PFUnDA | 563/519 | -65 | -10 | -16 | -9 |
| PFDoDA | 613/569 | -40 | -10 | -17 | -9 |
| PFTrDA | 663/619 | -50 | -10 | -19 | -9 |
| PFTeDA | 713/669 | -50 | -10 | -15 | -9 |
| PFHxDA | 813/769 | -65 | -10 | -17 | -9 |
| PFOcDA | 913/869 | -65 | -10 | -17 | -12 |
| PFBS | 299/80 | -80 | -10 | -62 | -3 |
| PFPeS | 349/80 | -100 | -10 | -70 | -13 |
| PFHxS | 399/80 | -80 | -10 | -80 | -3 |
| PFHpS | 449/80 | -100 | -10 | -104 | -15 |
| PFOS | 499/80 | -90 | -10 | -95 | -3 |
| PFNS | 549/80 | -105 | -10 | -116 | -13 |
| PFDS | 599/80 | -80 | -10 | -80 | -3 |
| PFDoDS | 699/80 | -115 | -10 | -126 | -13 |

| Extraction Standard | Transition Q1/Q3 | DP | EP | CE | CXP |
|---|---|---|---|---|---|
| $^{13}C_4$-PFBA | 217/172 | -30 | -10 | -14 | -31 |
| $^{13}C_5$-PFPeA | 268/223 | -25 | -10 | -12 | -11 |
| $^{13}C_5$-PFHxA | 318/273 | -30 | -10 | -14 | -47 |
| $^{13}C_4$-PFHpA | 367/322 | -30 | -10 | -14 | -19 |
| $^{13}C_8$-PFOA | 421/376 | -30 | -10 | -14 | -9 |
| $^{13}C_9$-PFNA | 472/427 | -30 | -10 | -14 | -11 |
| $^{13}C_6$-PFDA | 519/474 | -40 | -10 | -16 | -13 |
| $^{13}C_7$-PFUdA | 570/525 | -60 | -10 | -16 | -7 |
| $^{13}C_2$-PFDoA | 615/570 | -40 | -10 | -18 | -15 |
| $^{13}C_2$-PFTeDA | 715/670 | -45 | -10 | -18 | -17 |
| $^{13}C_3$-PFBS | 302/80 | -75 | -10 | -70 | -13 |
| $^{13}C_3$-PFHxS | 402/80 | -75 | -10 | -84 | -13 |
| $^{13}C_8$-PFOS | 507/80 | -110 | -10 | -90 | -13 |

| Injection Standard | Transition Q1/Q3 | DP | EP | CE | CXP |
|---|---|---|---|---|---|
| $^{13}C_3$-PFBA | 216/172 | -30 | -10 | -14 | -19 |
| $^{13}C_2$-PFOA | 415/370 | -30 | -10 | -14 | -9 |
| $^{13}C_2$-PFDA | 515/470 | -35 | -10 | -16 | -35 |
| $^{13}C_4$-PFOS | 503/80 | -105 | -10 | -120 | -13 |

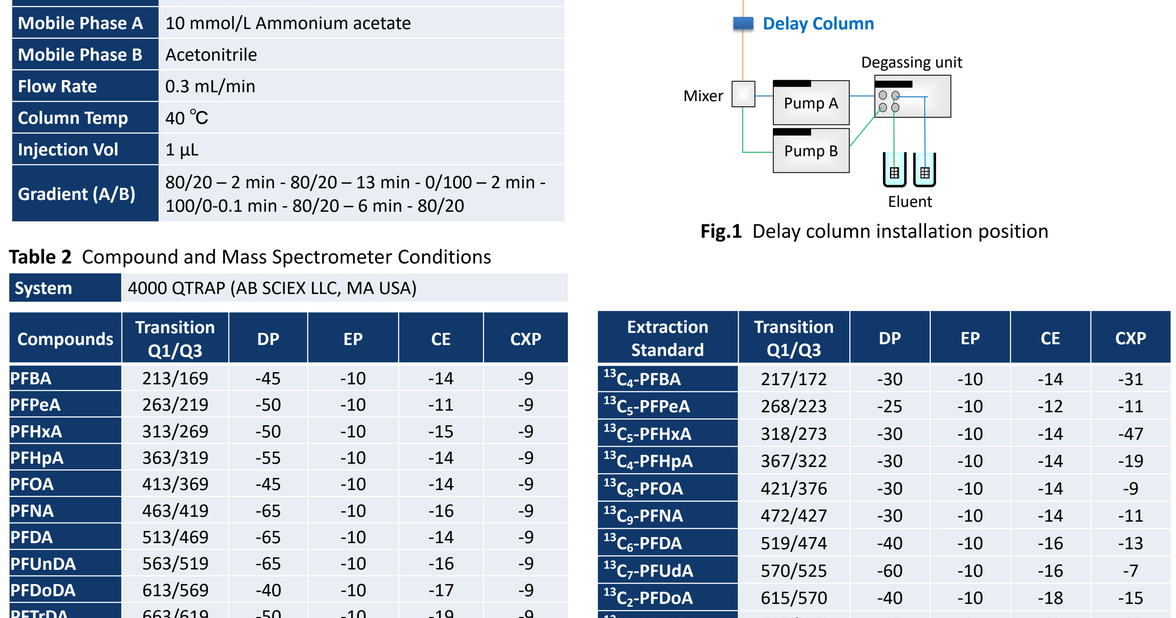


**Fig.2** Solid phase extraction method

## Results

Using our delay column packed with high-purity activated carbon to analyze the PFAS, we confirmed that the peak to be analyzed and the blank peak were separated sufficiently with respect to the retention time. Further, we confirmed that the PFAS 21 component in water can be extracted using SPE cartridge, InertSep MA-2. As a result of the recovery test and the use of extracted tap-water samples, we found the linearity of the measurements as 0.99 or better in the range 1–20 ng/L and the repeatability at 5 ng/L was ≤16%. When using a 150 mg SPE cartridge, the volume of sample water and the eluting solvent could be reduced, the evaporating operation of the elution solvent could be omitted, and the sample preparation time was shortened.

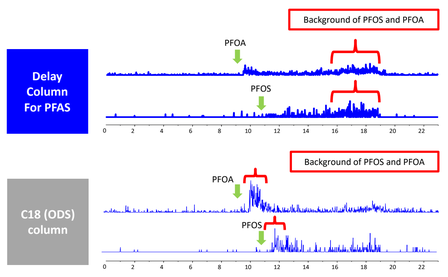


**Fig.3** Comparison chromatogram of Delay columns

**Table.3** Pressure Comparison of Delay Columns

| Analytical column | Delay Column | Pressure |
|---|---|---|
| InertSustain C18-HP (150 × 2.1 mm, 3 µm) | None | 19.8 MPa |
| | Delay Column for PFAS (30 × 3.0 mm) | 19.8 MPa |
| | C18 (ODS) column (50 × 2.1 mm, 3 µm) | 23 MPa |

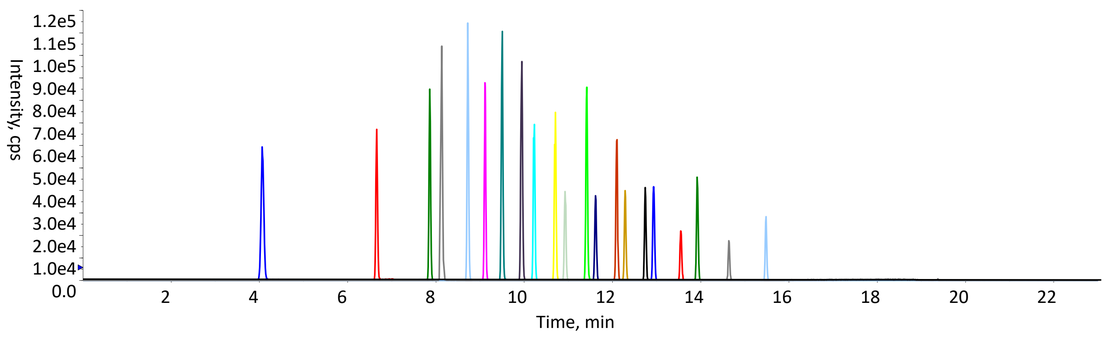


**Fig.4** PFAS 21 chromatogram

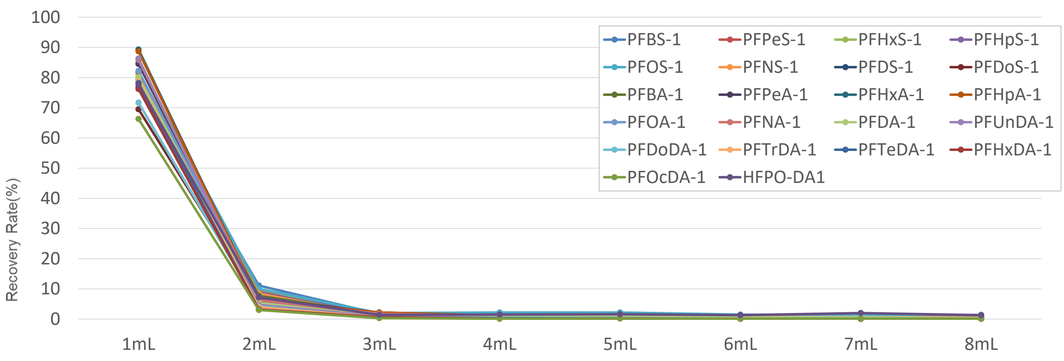


**Fig.5** SPE cartridge InertSep MA-2 250mg elution profile

**Table.4** Repeatability, Linearity, and Recovery

| Compounds | Repeatability (CV %, n = 5) | Calibration Range | Linearity (1 – 20 ng/L) | Recovery Rate (%) | R.T (min) |
|---|---|---|---|---|---|
| PFBA | 13 | 1-50 | 0.9999 | 80 | 4.11 |
| PFPeA | 8 | 1-50 | 0.9999 | 100 | 6.69 |
| PFHxA | 14 | 1-50 | 0.9999 | 96 | 7.88 |
| PFHpA | 7 | 1-50 | 0.9996 | 107 | 8.76 |
| PFOA | 10 | 1-50 | 0.9999 | 99 | 9.52 |
| PFNA | 10 | 1-50 | 0.9999 | 87 | 10.25 |
| PFDA | 7 | 1-50 | 1 | 101 | 10.95 |
| PFUnDA | 7 | 1-50 | 0.9997 | 104 | 11.65 |
| PFDoDA | 5 | 1-50 | 0.9999 | 96 | 12.32 |
| PFTrDA | 5 | 1-50 | 0.9997 | 108 | 12.96 |
| PFTeDA | 10 | 1-50 | 0.9999 | 88 | 13.58 |
| PFHxDA | 3 | 1-50 | 0.9999 | 119 | 14.67 |
| PFOcDA | 8 | 1-10 | 0.999 | 99 | 15.5 |
| PFBS | 12 | 1-50 | 0.9998 | 92 | 8.15 |
| PFPeS | 6 | 1-50 | 0.9998 | 95 | 9.13 |
| PFHxS | 8 | 1-20 | 0.9996 | 97 | 9.97 |
| PFHpS | 9 | 1-20 | 0.999 | 93 | 10.73 |
| PFOS | 16 | 1-20 | 0.9995 | 102 | 11.45 |
| PFNS | 9 | 1-10 | 0.996 | 95 | 12.13 |
| PFDS | 4 | 1-20 | 0.9992 | 86 | 12.77 |
| PFDoDS | 10 | 1-10 | 0.999 | 83 | 13.95 |

**Table.5** Repeatability Using a Small SPE (150 mg)

| Compounds | With surrogate correction | | Without surrogate correction | |
|---|---|---|---|---|
| | Recovery Rate (%) | Repeatability (CV %, n = 5) | Recovery Rate (%) | Repeatability (CV %, n = 5) |
| PFBA | 94 | 9 | 101 | 8 |
| PFPeA | 102 | 5 | 107 | 6 |
| PFHxA | 100 | 5 | 112 | 5 |
| PFHpA | 100 | 5 | 101 | 3 |
| PFOA | 97 | 11 | 103 | 10 |
| PFNA | 93 | 10 | 103 | 12 |
| PFDA | 96 | 5 | 104 | 5 |
| PFUnDA | 99 | 3 | 105 | 11 |
| PFDoDA | 101 | 5 | 111 | 6 |
| PFTrDA | 88 | 10 | 97 | 6 |
| PFTeDA | 90 | 5 | 105 | 13 |
| PFHxDA | 94 | 3 | 109 | 10 |
| PFOcDA | 96 | 6 | 111 | 12 |
| PFBS | 94 | 10 | 106 | 8 |
| PFPeS | 93 | 8 | 105 | 8 |
| PFHxS | 96 | 8 | 100 | 6 |
| PFHpS | 89 | 9 | 93 | 8 |
| PFOS | 98 | 10 | 106 | 16 |
| PFNS | 89 | 7 | 96 | 12 |
| PFDS | 88 | 6 | 95 | 9 |
| PFDoDS | 89 | 6 | 96 | 4 |

## Conclusions

For the delay column, two advantages of the delay column packed with high-purity activated carbon beads were confirmed compared to the previously used C18 column: first, there is no pressure increase, and second, the analyte peaks and the system-derived blank peaks can be sufficiently separated. This was thought to reduce the load on the instrument and the analytical column while simultaneously limiting the quantification error caused by the system-derived blank. For solid-phase extraction columns, it was confirmed that by switching to a smaller-scale column than the commonly used sizes, it was possible to elute with a smaller amount of solvent. This may be partly due to the fact that the columns are weak anion-exchange solid-phase extraction columns without a reversed-phase mode. This suggests that, depending on the concentration in the sample and the sensitivity of the instrument, smaller-scale and rapid processing may be possible.

## References

1. EPA method 537.1: Determination of selected per- and polyfluorinated alkyl substances in drinking water by solid-phase extraction and liquid chromatography/tandem mass spectrometry (LC/MS/MS),Version 1.0,November 2018
2. EPA method 533: Determination of per- and polyfluoroalkyl substances in drinking water by isotope dilution anion-exchange solid phase extraction and liquid chromatography/tandem mass spectrometry
3. 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)
4. Standard test method in water, Ministry of Health, Labor and Welfare, Japan
5. Water supply test method, 2020 Edition, Japan Water Works Association