APPLICATION NOTE

Liquid Chromatography/ Mass Spectrometry

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# Direct Analysis of 17 Perfluorinated Compounds in Water at Low Parts-Per-Trillion Levels by LC/MS/MS Workflow

## Introduction

Perfluorinated compounds (PFCs) or perfluoroalkyl surfactants (PFASs) are human-made chemicals which are normally used in surfactants, fire-retardants, nonstick cookware coatings, and coatings for paper packaging for over half a century.[1, 2] In the last decade or so, PFASs have received lots of attention because they are highly stable and resistant to degradation in the environment. Reports of their occurrence in tap water, food or even human blood have led to concerns of their effect on human body as pollutants.[3-6] Hence, analysis of PFASs in biological and environmental matrices is critical to understanding their fate, persistence and toxicity.

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The two most commonly researched and most prevalent PFASs in the environment are perfluorooctane sulphonate (PFOS) and perfluorooctanoic acid (PFOA) because they are the end degradation products of several PFASs in commercial applications.[7] In addition, PFOA and PFOS are the two PFASs made in the largest amount.[8,9] The US EPA issued drinking water health advisory for PFOA and PFOS is 70 ng/L.[10] Meanwhile, the latest European Commission adopted proposal for PFAS is 100 ng/L for an individual PFAS and 500 ng/L for PFASs in total.[11] Regionally, the threshold is slightly different. For example, the Swedish National Food Agency has recently introduced a conservative "limit of action threshold" of 90 ng/L for total PFASs in the drinking water.[12] Several analytical techniques such as GC/MS, NMR, LC/MS/MS are available to detect PFAS. GC/MS analysis of perfluorosulfonyl compounds requires derivatization steps prior to measurements which limits its applicability to environmental monitoring. The specificity of NMR spectroscopy is excellent for fluorinated compounds and is suitable for structural characterization of PFCs in environmental samples. However, the low sensitivity is a drawback of these techniques. LC/MS/MS is the most commonly employed analytical technique for the measurement of PFASs in biological and environmental samples due to its high selectivity and sensitivity.[13]

The use of solid phase extraction (SPE) procedures before LC/MS/MS analysis is one of the most popular methods for PFASs extraction from aqueous environmental matrices.[14] In this study, a LC/MS/MS direct sample injection method was developed, and the results indicate that this simple LC/MS/MS workflow provides an excellent sensitivity and specificity for the analysis of PFASs in drinking and surface water samples.

## Experimental

### Hardware/Software

The chromatographic separation was conducted by a PerkinElmer UHPLC System and detection was achieved using a PerkinElmer QSight® 220 triple quadrupole mass spectrometer, equipped with ESI and APCI ionization sources. All instrument control, data acquisition and data processing were performed using the Simplicity™ 3Q software.

## Method

### Standard and Sample Preparation

Water samples including some household and nearby surface waters were obtained locally in Ontario, Canada. The PFASs mixture and internal standard (IS) for the analytes were obtained from Wellington Laboratories (Guelph, Ontario). LC/MS grade water was purchased from Thermo Fisher (MA, USA). The stock solution was serially diluted with LC/MS grade water to make calibration standards ranging from 0.5 to 2000 ng/L (ppt). Water samples were first filtered through a 0.45 µm nylon filter and centrifuged, then 50 µL of the supernatant was injected directly onto a PerkinElmer Brownlee™ SPP C18 (100 x 2.1 mm, 2.7 µm) reverse phase analytical column for further analysis.

To check for any carryover, a LC/MS grade water blank was used.

### LC Conditions and MS Parameter Settings

The LC conditions are shown in Table 1. The sample is first flushed with 95% mobile phase A for one minute at 1.0 mL/min, then slowly ramp up to 98% mobile phase B to elute all the analytes of interest at a flow rate of 0.5 mL/min with total run time of about 18 minutes, Table 2.

*Table 1.* LC conditions.

| | |
|---|---|
| **Analytical Column** | Brownlee™, SPP C18, 100 x 2.1 mm, 2.7 µm (PN: N9308404) |
| **Delay Column** | Brownlee™, SPP C18, 50 x 3 mm, 2.7 µm (PN: N9308408) |
| **Mobile Phase A** | 5-mM ammonium acetate |
| **Mobile Phase B** | 5-mM ammonium acetate in methanol |
| **Needle Wash 1** | $H_2O$/MeOH 50:50 (v/v) |
| **Needle Wash 2** | MeOH |
| **Column Oven Temperature** | 30 °C |
| **Auto Sampler Temperature** | 15 °C |
| **Injection Volume** | 50 µL |

*Table 2.* LC time program.

| Time (min) | Mobile Phase A (%) | Mobile Phase B (%) | Flow Rate (mL/min) |
|---|---|---|---|
| 1 | 95 | 5 | 1.0 |
| 1.3 | 95 | 5 | 0.5 |
| 2.1 | 55 | 45 | 0.5 |
| 9.5 | 30 | 70 | 0.5 |
| 11.0 | 15 | 85 | 0.5 |
| 12.3 | 2 | 98 | 0.5 |
| 13.5 | 2 | 98 | 0.5 |
| 13.6 | 95 | 5 | 0.5 |
| 17.0 | 95 | 5 | 1.0 |

The MS source settings are shown in Table 3. Source parameters including gas flows, temperature and position settings, were optimized for maximum sensitivity. In addition, optimized compound-dependent parameters for the list of the multiple reaction monitoring (MRM) transitions for all analytes of PFASs including nine internal standards are shown in Table 4. The MS acquisition method was generated automatically by defining the expected retention time and its corresponding time window for the analytes of interest in the time-managed-MRM module of the Simplicity software.

*Table 3.* MS source settings.

| | |
|---|---|
| **ESI Voltage (Negative)** | -4000V |
| **Drying Gas** | 140 |
| **Nebulizer Gas** | 400 |
| **Source Temperature** | 350 °C |
| **HSID Temperature** | 250 °C |

## Results and Discussion

Representative chromatograms for LC/MS grade water fortified at 125 ng/L of 17 PFASs, are shown in Figure 1 for perfluorinated acetic acids and Figure 2 for perfluorinated sulfonates. Chromatographic analysis showed that, as the retention time increased, the signal response in terms of peak area decreased in general except for the PFODA which somehow showed better response than that for C12 to C16 perfluorinated acetic acids. The chromatographic separation of 17 PFASs on a Brownlee™ SPP C 18 column was reasonably good (Figure 1, 2 and Table 4). However, the PFUnDA and PFDS were observed to coelute under present LC conditions. Different LC gradients were also tested but no improvement on separation for these two analytes was achieved due to their similar chemical structures. Further investigation is needed to resolve such issue in the future.

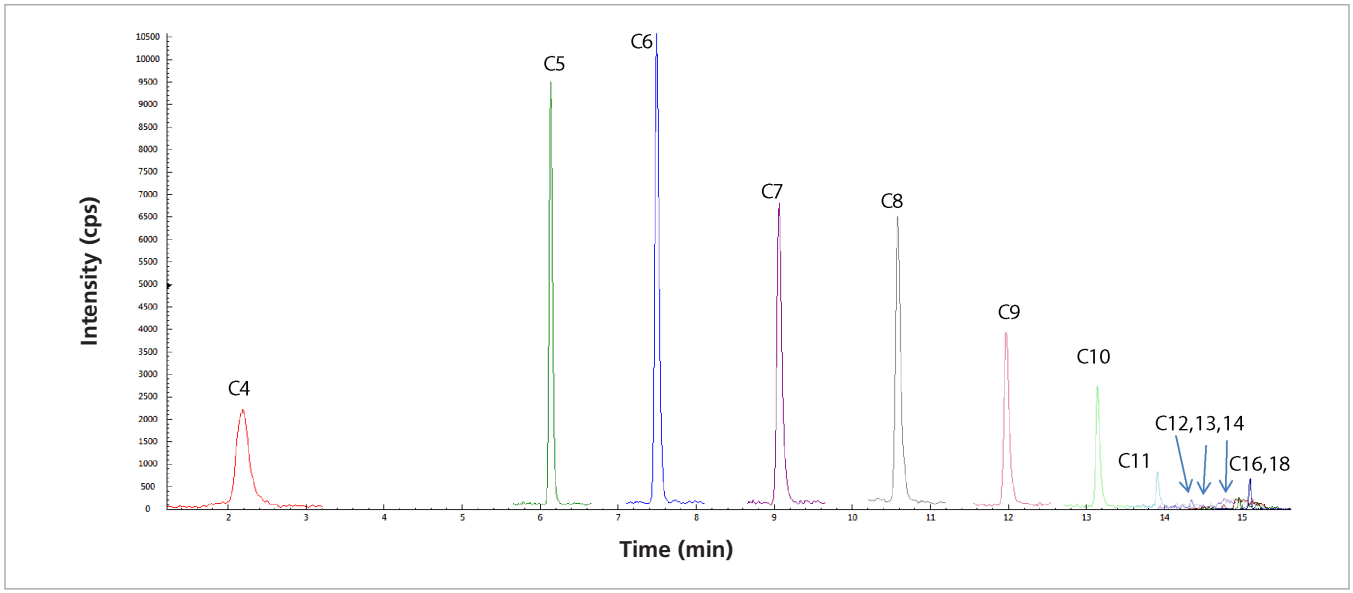


*Figure 1.* Chromatogram of perfluorinated acetic acids spiked at 125 ng/L.

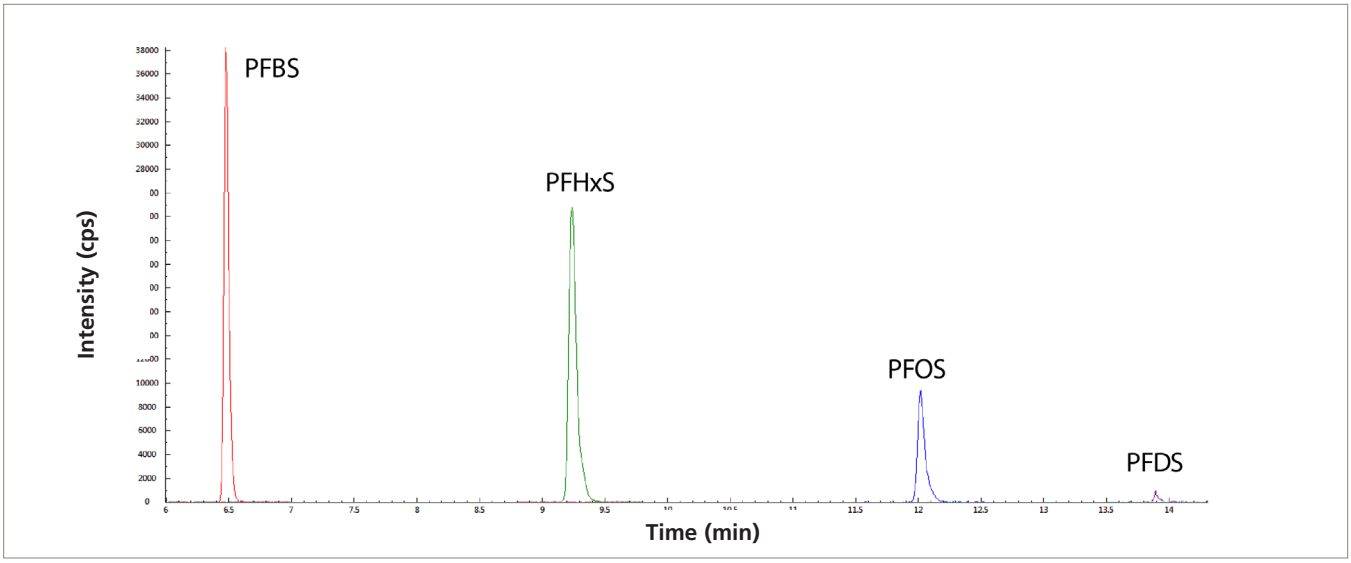


*Figure 2.* Chromatogram of perfluorinated sulfonates spiked at 125 ng/L.

*Table 4.* Optimized MRMs and compound-dependent parameters for all PFASs.

| Compound Name | Acronym | Q1 (amu) | Q2 (amu) | RT (min) | CE | EV | CCL2 |
|---|---|---|---|---|---|---|---|
| Perfluorobutanoate | PFBA2 | 213.0 | 69.0 | 2.22 | 80 | -20 | 100 |
| | PFBA1 | 213.0 | 169.0 | 2.22 | 14 | -20 | 100 |
| Perfluoropentanoate | PFPeA2 | 263.0 | 69.0 | 6.15 | 57 | -20 | 100 |
| | PFPeA1 | 263.0 | 219.0 | 6.15 | 14 | -20 | 100 |
| Perfluorobutylsulfonate | PFBS2 | 299.0 | 80.0 | 6.49 | 80 | -20 | 100 |
| | PFBS1 | 299.0 | 99.0 | 6.49 | 42 | -20 | 100 |
| Perfluorohexanoate | PFHxA2 | 313.0 | 119.0 | 7.60 | 30 | -20 | 100 |
| | PFHxA1 | 313.0 | 269.0 | 7.60 | 14 | -20 | 100 |
| Perfluoroheptanoate | PFHpA2 | 363.0 | 169.0 | 9.15 | 28 | -20 | 100 |
| | PFHpA1 | 363.0 | 319.0 | 9.15 | 18 | -20 | 100 |
| Perfluorohexylsulfonate | PFHxS2 | 399.0 | 80.0 | 9.30 | 83 | -20 | 100 |
| | PFHxS1 | 399.0 | 99.0 | 9.30 | 44 | -20 | 100 |
| Perfluorooctanoate | PFOA2 | 413.0 | 169.0 | 10.70 | 33 | -20 | 100 |
| | PFOA1 | 413.0 | 369.0 | 10.70 | 18 | -20 | 100 |
| Perfluorononanoate | PFNA2 | 463.0 | 169.0 | 12.05 | 30 | -20 | 100 |
| | PFNA1 | 463.0 | 419.0 | 12.05 | 19 | -20 | 100 |
| Perfluorooctylsulfonate | PFOS2 | 499.1 | 80.0 | 12.05 | 85 | -20 | 100 |
| | PFOS1 | 499.1 | 99.0 | 12.05 | 57 | -20 | 100 |
| Perfluorodecanoate | PFDA2 | 513.1 | 169.0 | 13.20 | 30 | -20 | 100 |
| | PFDA1 | 513.1 | 469.0 | 13.20 | 17 | -20 | 100 |
| Perfluoroundecanoate | PFUnDA2 | 563.1 | 169.0 | 14.00 | 32 | -20 | 110 |
| | PFUnDA1 | 563.1 | 519.0 | 14.00 | 17 | -20 | 100 |
| Perfluorododecanoate | PFDoDA2 | 613.1 | 169.0 | 14.40 | 42 | -20 | 110 |
| | PFDoDA1 | 613.1 | 569.0 | 14.40 | 18 | -20 | 100 |
| Perfluorodecylsulfonate | PFDS1 | 599.1 | 80.0 | 14.00 | 110 | -20 | 140 |
| | PFDS2 | 599.1 | 99.0 | 14.00 | 57 | -20 | 130 |
| Perfluorotridecanoate | PFTriA1 | 663.1 | 619.0 | 14.70 | 16 | -20 | 120 |
| | PFTriA2 | 663.1 | 169.0 | 14.70 | 44 | -20 | 140 |
| Perfluorotetradecanoate | PFTA1 | 713.1 | 669.0 | 14.80 | 18 | -20 | 110 |
| | PFTA2 | 713.1 | 169.0 | 14.80 | 48 | -20 | 110 |
| Perfluorohexadecanoate | PFHxDA1 | 813.1 | 769.0 | 14.96 | 16 | -20 | 140 |
| | PFHxDA2 | 813.1 | 169.0 | 14.95 | 48 | -20 | 140 |
| Perfluorooctadecanoate | PFODA1 | 913.1 | 869.0 | 15.10 | 18 | -20 | 160 |
| | PFODA2 | 913.1 | 169.0 | 15.10 | 48 | -20 | 160 |
| Perfluoro[$^{13}$C ] butanoate | PFBA-$^{13}$C4 | 217.0 | 172.0 | 2.23 | 14 | -20 | 100 |
| Perfluoro[$^{13}$C ] hexanoate | PFHxA-$^{13}$C2 | 315.0 | 270.0 | 7.60 | 14 | -20 | 100 |
| Perfluoro[$^{13}$C ] octanoate | PFOA-$^{13}$C4 | 417.0 | 372.0 | 10.70 | 18 | -20 | 100 |
| Perfluoro[$^{13}$C ] nonanoate | PFNA-$^{13}$C5 | 468.0 | 423.0 | 12.05 | 19 | -20 | 100 |
| Perfluoro[$^{13}$C ] decanoate | PFDA-$^{13}$C2 | 515.1 | 470.0 | 13.20 | 17 | -20 | 100 |
| Perfluoro[$^{13}$C ] undecanoate | PFUnDA-$^{13}$C2 | 565.1 | 519.9 | 14.00 | 17 | -20 | 100 |
| Perfluoro[$^{13}$C ] dodecanoate | PFDoDA-$^{13}$C2 | 615.1 | 569.9 | 14.40 | 18 | -20 | 100 |
| Perfluorohexane[$^{18}$O ] Sulphonate | PFHxS-$^{18}$O2 | 403.0 | 103.0 | 9.30 | 44 | -20 | 100 |
| Perfluorooctane[$^{13}$C ] Sulphonate | PFOS-$^{13}$C4 | 503.1 | 99.1 | 12.05 | 57 | -20 | 100 |

## Analytical Challenges for Testing Multi-Residues of PFASs from Drinking Water Samples

Sample matrix effect was negligible after simple filtration and centrifugation of water sample because drinking water or surface water is relatively cleaner as compared to industrial waste water. However, system contamination can be a main concern for LC/MS/MS analysis of PFASs. The contamination might be caused by tubing, filters, fittings, or even solvents used in LC system. For example, Polytetrafluoroethylene (PTFE) is a commonly used material in tubing and filters for almost all LC system, it can be potentially a source of PFAS contamination during analysis. To remedy this contamination issue, a delay column (Brownlee, SPP C18, 50 x 3 mm, 2.7 µm) was inserted between the mixing valve and the autosampler to trap PFASs from the pump. Figure 3 shows examples of the system background contamination from the LC pumping system for PFDoDA and PFTriA in this case, indicated by a small bump at a retention time delayed by the delay column. The identity of these well separated contamination peaks can be confirmed by both the quantifier and qualifier ions and their corresponding ion ratios. In present study, PFHxDA, PFTA, PFUnDA, PFDA, PFOS, PFOA, PFHxS, PFPeA, PFBA were also identified qualitatively to be present in the background contamination.

Another source of contamination was possibly coming from the internal standard mixed solution. As indicated in IS spiked water blank sample (not shown), the IS stock solution might have been contaminated somehow by some of the analytes, namely, PFHxA, PFHxS, PFOA, PFNA, PFDA, PFUnDA at various amount of concentration up to about 40 ppt for unknown reason, which were also confirmed by both the quantifier and qualifier ions. Such contamination could possibly lead to quantitation error at low concentration (explained in detail below). Therefore, all results discussed here, unless stated otherwise, are obtained from calibrations without using internal standards.

Finally, another possible contamination might come from the LC/MS grade water solvent. To verify this, the LC/MS grade water was injected directly into the system and showed no MS response for all analytes except PFoDA which was identified and estimated to be less than 2 ppt (below the quantitation limit for PFoDA in present study). Such low concentration interference has little or no effect on the validity of the present study, therefore, can be ignored.

### Carryover

The carryover was investigated by injecting a highest concentration calibration standard (2000 ppt in this case) followed by a blank water injection. No analyte peaks were observed after a high concentration injection under the detection limit of current LC/MS/MS method. The overlaid TIC chromatograms for these two injections are shown in Figure 4, demonstrating the robustness of the studied method.

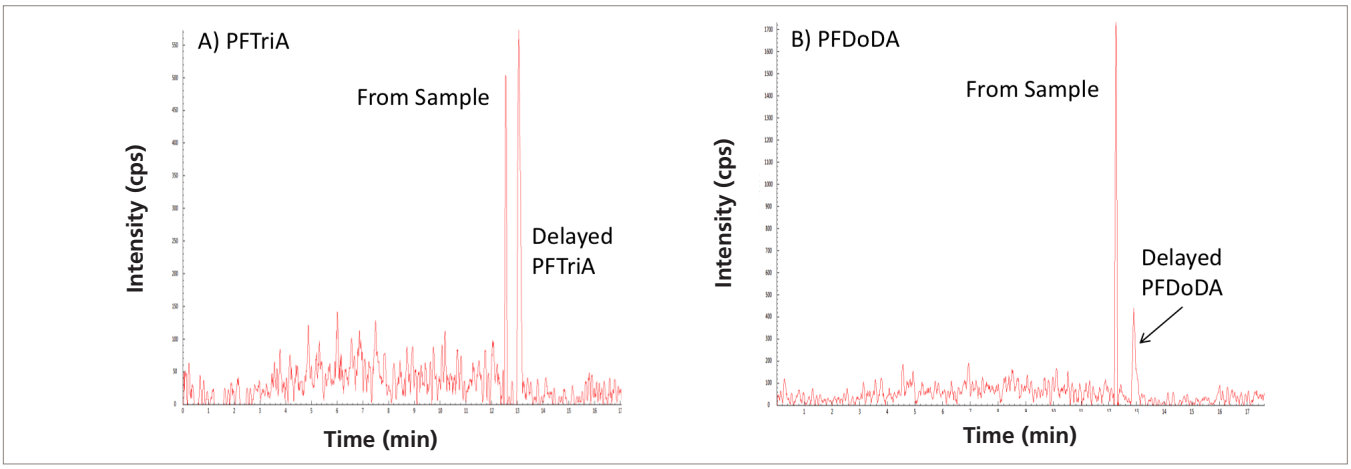


*Figure 3.* Example of system background contamination that separated by delay column.

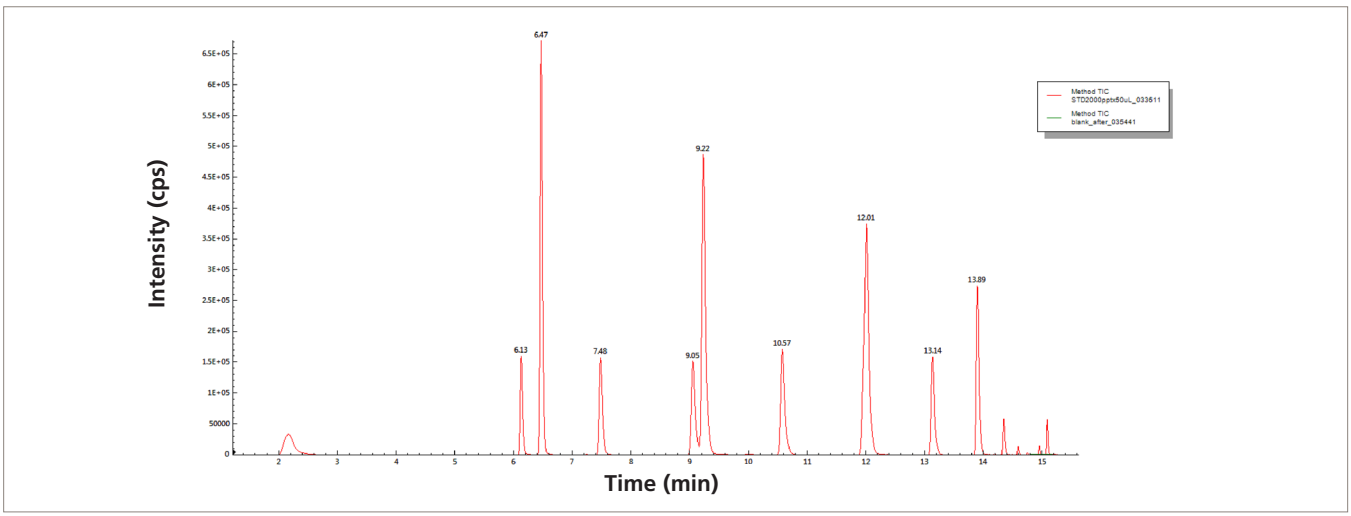


*Figure 4.* Overlay of TICs from 2000 ppt standard (red) and blank water (green).

## Linearity

Calibration was performed by preparing and running thirteen concentration levels of analyte standards in neat solution (pure solvent) with and without adding internal standards. No significant difference was seen for medium to high concentration calibration standards in terms of peak height or peak area. Because of the IS contamination problem mentioned above, the calibration curve loses its linearity at low concentration range when using internal standards for calculation, which possibly leads to false positive when quantifying unknown samples. In addition, the sample matrix, either clean drinking water or surface water, is relatively simple. Therefore, using internal standards in clean drinking water sample is not as critical as that in other complex matrices. Therefore, all results discussed here are obtained from those calibrations without using internal standards. Example calibration curves for PFBS, PFOA, and PFODA in present study are shown in Figure 5. Overall, the calibration curves for all analytes showed good linearity, with regression coefficient of $R^2 \geq 0.99$ for all the analytes. Quantitation precision (%RSD, n = 3) for all analytes at 250 and 500 ng/L (or ppt) were all found to be between 0.4 to 8.3%.

## Limit of Quantification and Sample Results

The limits of quantification (LOQs) were determined based on the signal to noise ratio of ≥10 for analyte's quantifier ion. The identity of each PFASs analyte is confirmed by ensuring that the product ion ratios (qualifier vs. quantifier) were within 20% tolerance windows of the expected ratio. The LOQs are listed in Table 5 along with water sample results. Majority of the tested PFASs with carbon chain length of nine or less have a LOQ of ≤10 ng/L (or ppt) in water matrix. Other PFASs with longer chain were found to have a LOQ of ≤63 ng/L in present study except PFODA whose LOQ is 2 ng/L because of good signal intensity which is possibly a result of reasonably good ionization efficiency.

The developed LC/MS/MS method was applied for the analysis of PFASs in 12 water samples. Example chromatograms for the positively identified PFASs in sample S1 and S6 are shown in Figure 6 and 7, respectively. The calculated concentrations along with the corresponding method LOQs for all investigated samples are summarized in Table 5. No PFASs of equal or longer than C10 were detected for all real water samples. PFOS was found in household water samples (S1, S2, S7, S11), tap water samples (S3, S8), and small river or pond samples (S5, S9). The other commonly seen contaminant, PFOA, was found in seven of the water samples. This is consistent with many literature's findings. Lake water (S6) and fountain water (S10) samples were found to have high PFBA, a short-chain perfluorinated acid, which is the degradation product of long-chain perfluorinated acids. Recent studies reveal that the short-chain PFASs might have higher bio-accumulative effect than that from the long-chain ones.[15] However, no PFAS peak was found from a commercially available bottled drinking water sample (S12) under current experimental conditions.

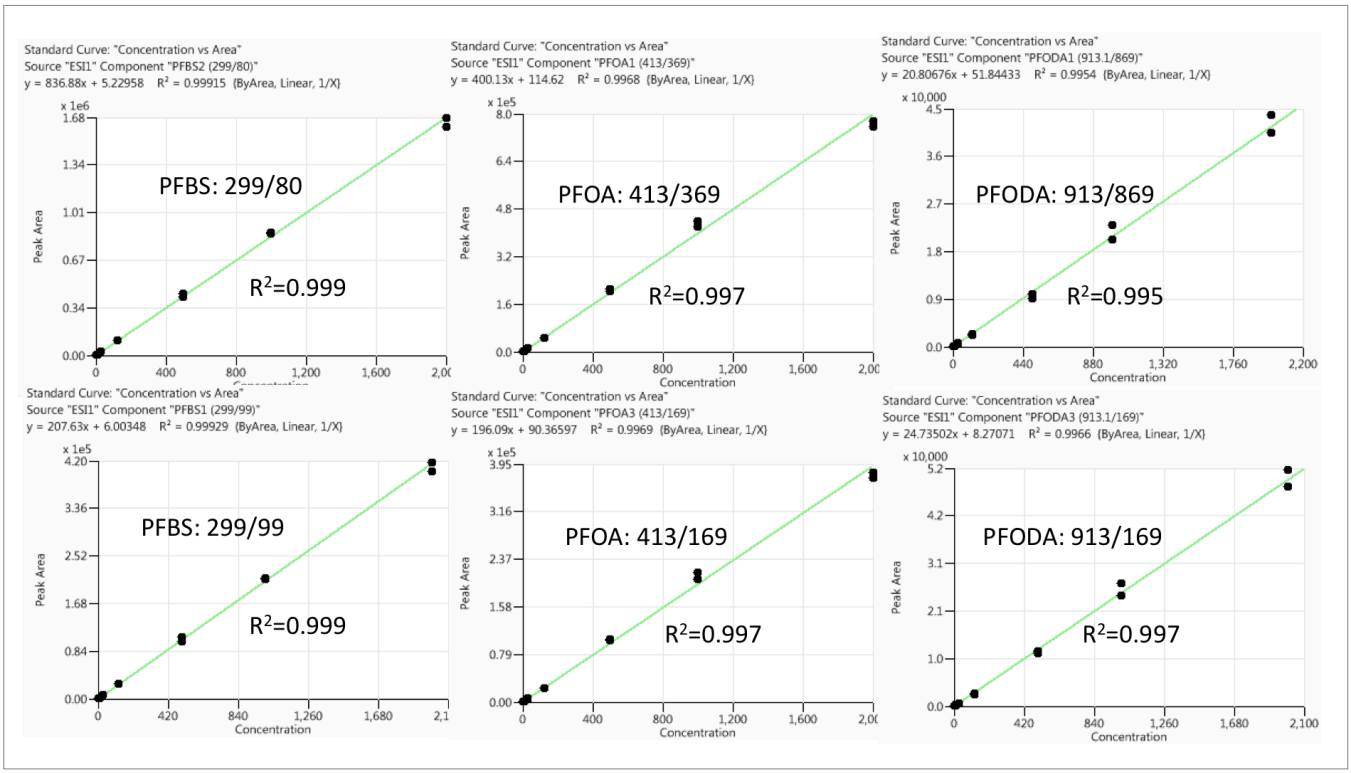


*Figure 5.* Example calibration curves for PFBS, PFOA, and PFODA in pure water.

*Table 5.* Summary results for the positively identified PFASs in water in ng/L and method LOQs.

| Analyte | C Chain | S1 | S2 | S3 | S4 | S5 | S6 | S7 | S8 | S9 | S10 | S11 | S12 | LOQ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| PFBA | C4 | 22 | - | - | - | - | 15 | - | - | - | - | - | - | 8.0 |
| PFPeA | C5 | - | - | - | - | - | - | - | - | - | 8 | - | - | 1.0 |
| PFBS | C4 | - | 1 | - | - | 1 | 1 | - | 1 | 8 | 1 | 2 | - | 0.5 |
| PFHxA | C6 | 7 | - | - | - | - | 2 | - | - | 5 | 18 | 4 | - | 1.0 |
| PFHpA | C7 | - | - | - | 1 | - | - | - | - | - | - | - | - | 1.0 |
| PFHxS | C6 | 2 | 1 | 1 | - | - | - | 1 | 1 | - | - | 2 | - | 0.5 |
| PFOA | C8 | 5 | 3 | - | 2 | 1 | 2 | - | 1 | - | - | 4 | - | 1.0 |
| PFNA | C9 | - | - | - | - | - | - | - | - | 21 | - | - | - | 8.0 |
| PFOS | C8 | 17 | 15 | 11 | - | 10 | - | 13 | 13 | 11 | - | 14 | - | 8.0 |
| PFDA | C10 | - | - | - | - | - | - | - | - | - | - | - | - | 31.3 |
| PFUnDA | C11 | - | - | - | - | - | - | - | - | - | - | - | - | 31.3 |
| PFDoDA | C12 | - | - | - | - | - | - | - | - | - | - | - | - | 62.5 |
| PFDS | C10 | - | - | - | - | - | - | - | - | - | - | - | - | 31.3 |
| PFTriA | C13 | - | - | - | - | - | - | - | - | - | - | - | - | 62.5 |
| PFTA | C14 | - | - | - | - | - | - | - | - | - | - | - | - | 62.5 |
| PFHxDA | C16 | - | - | - | - | - | - | - | - | - | - | - | - | 31.3 |
| PFODA | C18 | - | - | - | - | - | - | - | - | - | - | - | - | 2.0 |

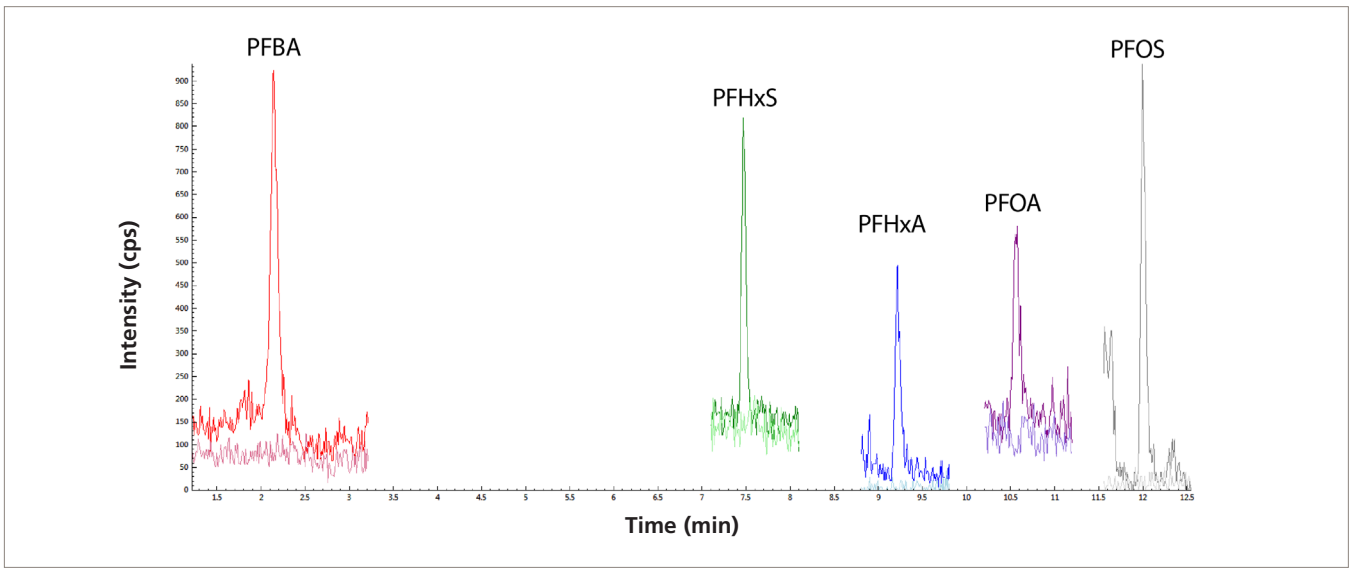


*Figure 6.* Detected PFASs from S1 sample as compared to baseline from the blank sample.

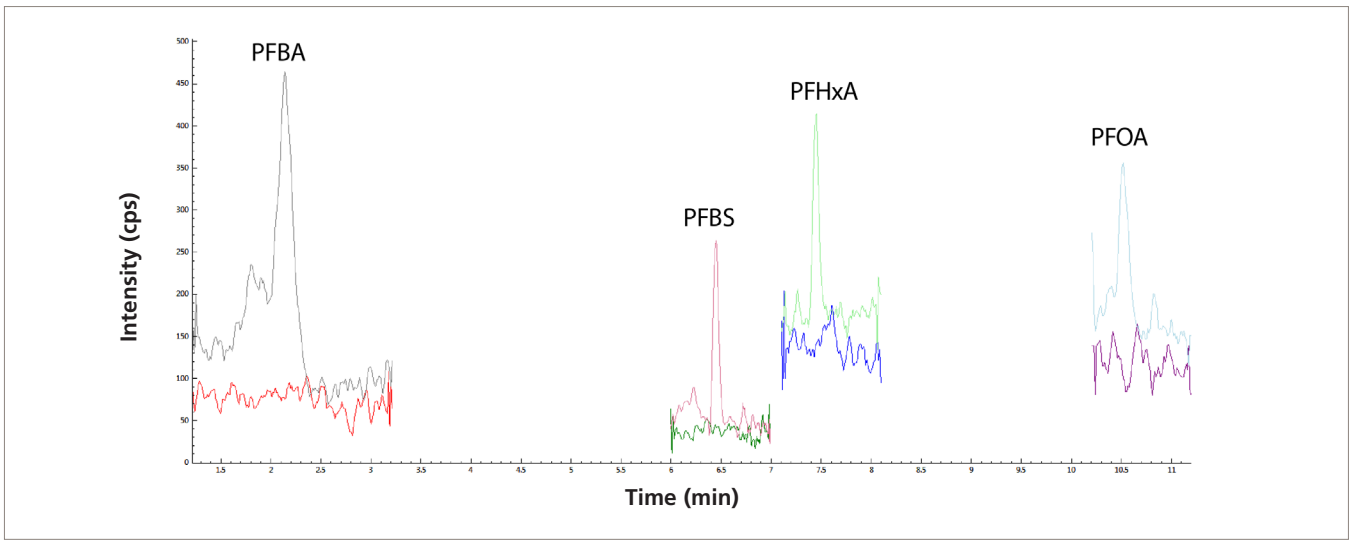


*Figure 7.* Detected PFASs from S6 sample as compared to baseline from the blank sample.

## Conclusions

A simple and robust LC/MS/MS method for PFASs analysis in drinking or surface water at low ng/L (or ppt) level was developed by coupling a LX-50 UHPLC system to a QSight 220 triple quadrupole mass spectrometer. This method can be applied for determination of PFASs, with LOQs below the limits set by many regulatory boards.

The time-managed-MRM module in the Simplicity software was effectively used in this study for monitoring 17 PFASs in drinking or surface water samples. This feature simplified the optimization of MS method on optimum dwell time for monitoring many analytes in samples.

Good linearity of calibration curves (with $R^2$>0.99) were obtained from ng/L to µg/L (or ppt to ppb) level for most of the 17 PFASs with LOQs of 1 to 63 ng/L (or ppt) depending on the analyte.

For several real water samples analyzed, it was found that at least two of the 17 PFASs were positively detected except for a commercially available bottled drinking water sample.

## References


1. Schultz, M. M.; Barofsky, D. F.; Field, J. A., Fluorinated alkyl surfactants. Environ. Eng. Sci. 20, (5), 487-501(2003).
2. 3M Environmental and health assesment of Perfluorooctane sulfonate and its salts.; U.S. Evironmental Protection Agency Public Docket AR226-1486.: Washington, DC (2003).
3. Boulanger, B.; Peck, A. M.; Schnoor, J. L.; Hornbuckle, K. C., Mass budget of perfluorooctane surfactant in Lake Ontario. Environ. Sci. Technol. 39, (1), 74-79 (2005).
4. Giesy, J. P.; Kannan, K., Global distribution of perfluorooctane sulfonate in wildlife. Environ. Sci. Technol. 35, (7), 1339-1342 (2001).
5. Kannan, K.; Corsolini, S.; Falandysz, J.; Fillmann, G.; Kumar, K. S.; Loganathan, B. G.; Mohd, M. A.; Olivero, J.; Van Wouwe, N.; Yang, J. H.; Aldous, K. M., Perfluorooctanesulfonate and related fluorochemicals in human blood from several countries. Environ. Sci. Technol. 38, (17), 4489-4495 (2004).
6. Schultz, M. M.; Barofsky, D. F.; Field, J. A., Fluorinated alkyl surfactants. Environmental Engineering Science, 20, (5), 487-501 (2003).
7. Ellis, D. A.; Mabury, S. A.; Martin, J. W.; Muir, D. C. G., Thermolysis of fluoropolymers as a potential source of halogenated organic acids in the environment. Nature 412, (6844), 321-324 (2001).
8. PUBLIC HEALTH STATEMENT Perfluoroalkyls, Public Health Service Agency for Toxic Substances and Disease Registry (2015)
9. PERFLUORINATED CHEMICALS (PFCs): PFOA & PFOS INFORMATION PAPER, Association of State and Territorial Solid Waste Management Officials (2015)
10. FACT SHEET PFOA and PFOS Drinking Water Health Advisories, US Environmental Protection Agency (2016).
11. EU targets EDCs in drinking water, ChemicalWatch (2018).
12. S. Englund, Evaluation of the Removal Efficiency of Perfluoroalkyl Substances in Drinking Water (2015).
13. Villagrasa, M.; de Alda, M. L.; Barcelo, D., Environmental analysis of fluorinated alkyl substances by liquid chromatography-(tandem) mass spectrometry: a review. Anal. Bioanal. Chem. 386, (4), 953-972 (2006).
14. Van Leeuwen, S. P. J.; de Boer, J., Extraction and clean-up strategies for the analysis of poly- and perfluoroalkyl substances in environmental and human matrices. Journal of Chromatography A 2007, 1153, (1-2), 172-185
15. Kjølholt, J.; A Jensen, A.A. ; Warming, M., Short-chain Polyfluoroalkyl Substances (PFAS). The Danish Environmental Protection Agency (2015)



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