SHIMADZU

Application News

Liquid Chromatography Mass Spectrometry No.C67A

LC/MS Analysis of Perfluorochemicals (PFCs) Using Impurity Delay Method

The 4th Conference of the Parties (COP4) of the Stockholm Convention concerning Persistent Organic Pollutants (POPs) was held in May, 2009, and nine additional chemicals were newly listed as persistent organic pollutants (new POPs). Perfluorooctane sulfonic acid (PFOS), its salts, and perfluorooctane sulfonyl fluoride (PFOSF) were additionally listed under Annex B. Due to the concern that there are applications in which a possible alternative is not available at this time, certain applications have been specified as essential under the categories of Acceptable Purposes and Specific Exemptions. These include photosensitive materials and other semiconductor-related applications, photo masks, certain medical devices, metal plating, fire-fighting foam, electrical and electronic parts for color printers, CCD color filters for medical devices, etc. Thus, while advancing the development of alternative technologies, steps have been taken toward future abolition of these uses. Moreover, in Japan, PFOS and perfluorooctanoic acid (PFOA) are designated as Type 2 Monitoring Chemical Substances in the Law Concerning the Evaluation of Chemical Substances and Regulation of Their Manufacture, etc., which obligates manufacturers to report to the Ministry of Economy, Trade and Industry the amounts of these substances manufactured and sold in the previous year. Thus, the qualitative and quantitative analysis of these compounds is becoming more and more necessary.

PFOA and PFOS and its related substances can be detected with high sensitivity by LC/MS.

Here we introduce the simultaneous analysis of 6 related salts of PFOS and PFOA, which differ in their carbon chain length and branches. Fig. 1 shows the structural formula for PFOA and for the 6 related salt compounds of PFOS. The deprotonated molecule $[M-H]^-$ of these compounds was detected using electrospray ionization.

Depending on the analytical conditions used, there could be a problem in the analysis of PFOA because PFOA in the mobile phase and any PFOA originating from the online degasser and flow line will be concentrated in the analytical column, and further, will elute and be detected at the same time as the injected PFOA analysis sample. It is extremely difficult to eliminate these multiple sources of PFOA contamination, especially considering the possibility of contamination to the mobile phase from PFOA which is always present in the ambient air. Here, by installing a delay column between the mixer and sample injector as shown in Fig. 2, we were able to separate the PFOA peak generated due to detection of the PFOA analyte in the sample from the PFOA peak originating from PFOA in the mobile phase and HPLC system. The sample-derived PFOA was detected with high sensitivity. We have termed this technique, in which the impurity is eluted later than the analyte, the impurity delay method.

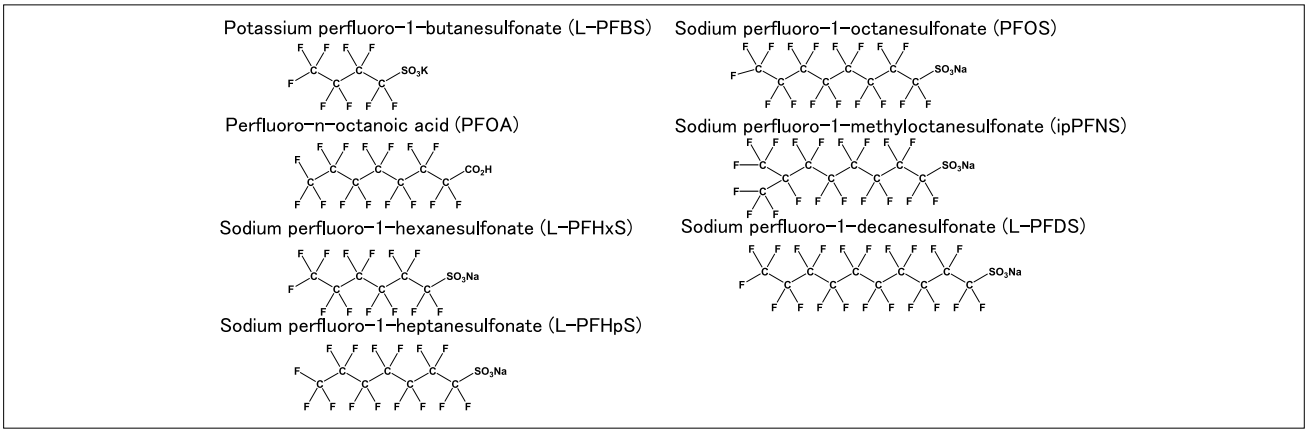

Fig. 1 Structures of Perfluorochemicals

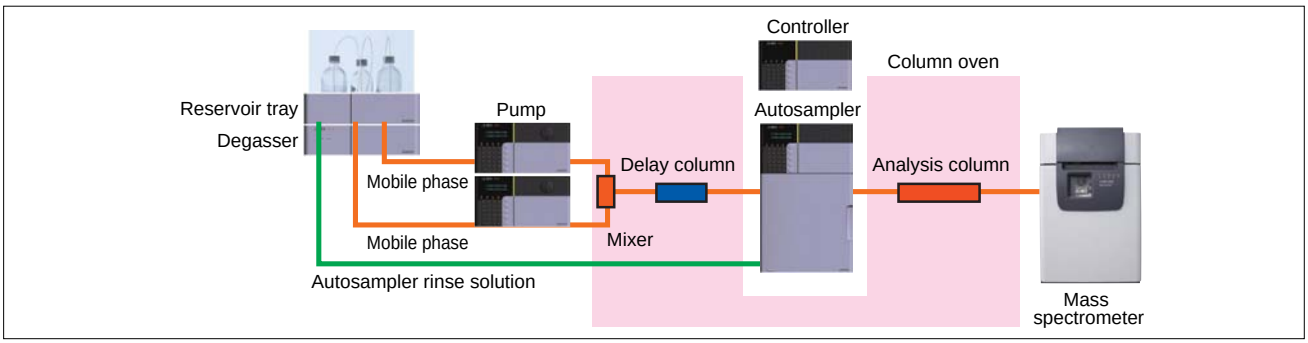

Fig. 2 Flow Diagram of LC/MS System

Fig. 3 shows SIM chromatograms obtained with the delay column installed and not installed.
PFOA was monitored using *m/z* 412.90. With the delay column not installed, the impurity-related PFOA was detected at a retention time of 11.5 min when no sample was injected (Fig. 3a) and when a blank sample was injected (Fig. 3b). Next, when PFOA standard solution was injected, a single overlapping peak was generated due to detection of both the impurity and PFOA standard sample at the same time (Fig. 3c). On the other hand, after installing the delay column, elution of the PFOA present in the mobile phase and flow line as an impurity was delayed due to retention in the delay column, and was therefore eluted after the PFOA that was injected as a sample. Fig. 3d and Fig. 3e show SIM chromatograms obtained with injection of a blank sample and injection of the PFOA standard sample, respectively. The PFOA injected as a sample was detected at a retention time of 13.5 min. In this experiment, the water used for the mobile phase was ultra pure water produced at our laboratory, the acetonitrile was HPLC grade, and the methanol used for the 50 % methanol dilution of the standard sample was also HPLC grade. The HPLC tubing, etc. all conformed to the Prominence Series standard specifications. In addition, based on our results of investigation of autosampler needle rinse solutions, we recommend that 50 % methanol be used.

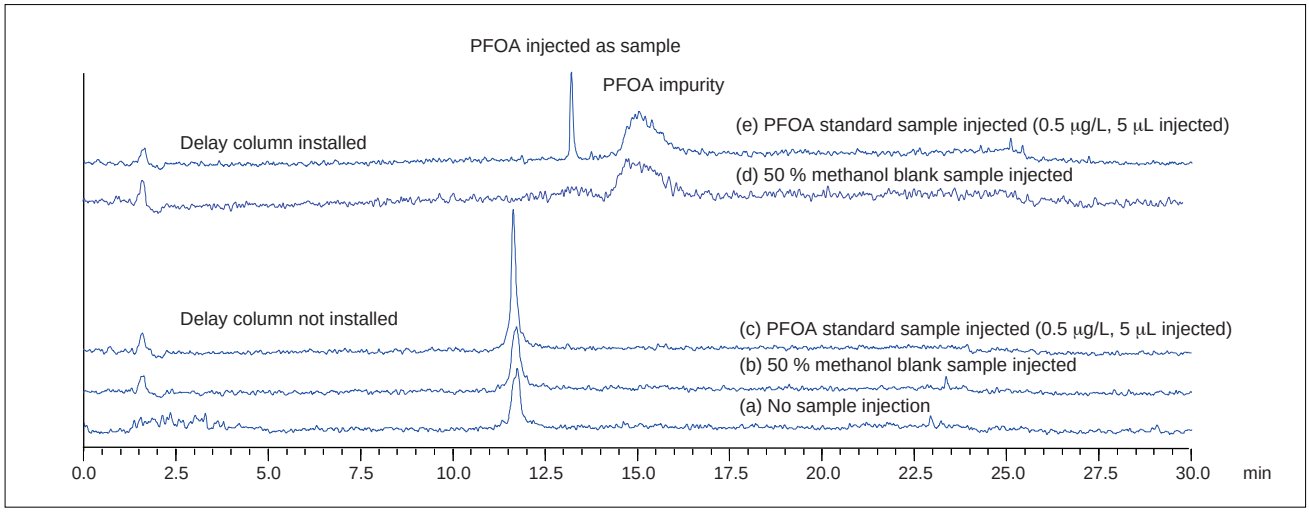

Fig. 3 SIM Chromatograms of PFOA (*m/z* 412.90)

Fig. 4 shows SIM chromatograms of PFOA and PFOS-related substances. To ensure retention of L-PFBS, which has the shortest carbon chain, the starting concentration of the acetonitrile mobile phase was set at 25 %. Regarding the concentration notations, the anion concentration was used for PFOA and PFOS, and the potassium salt (L-PFBS) or sodium salt concentration was used for the other substances. The concentration used for each of the SIM chromatogram substances of Fig. 4a was 2.5 μg/L, and for those of Fig. 4b, a concentration of 0.5 μg/L was used (5 μL injected, respectively).

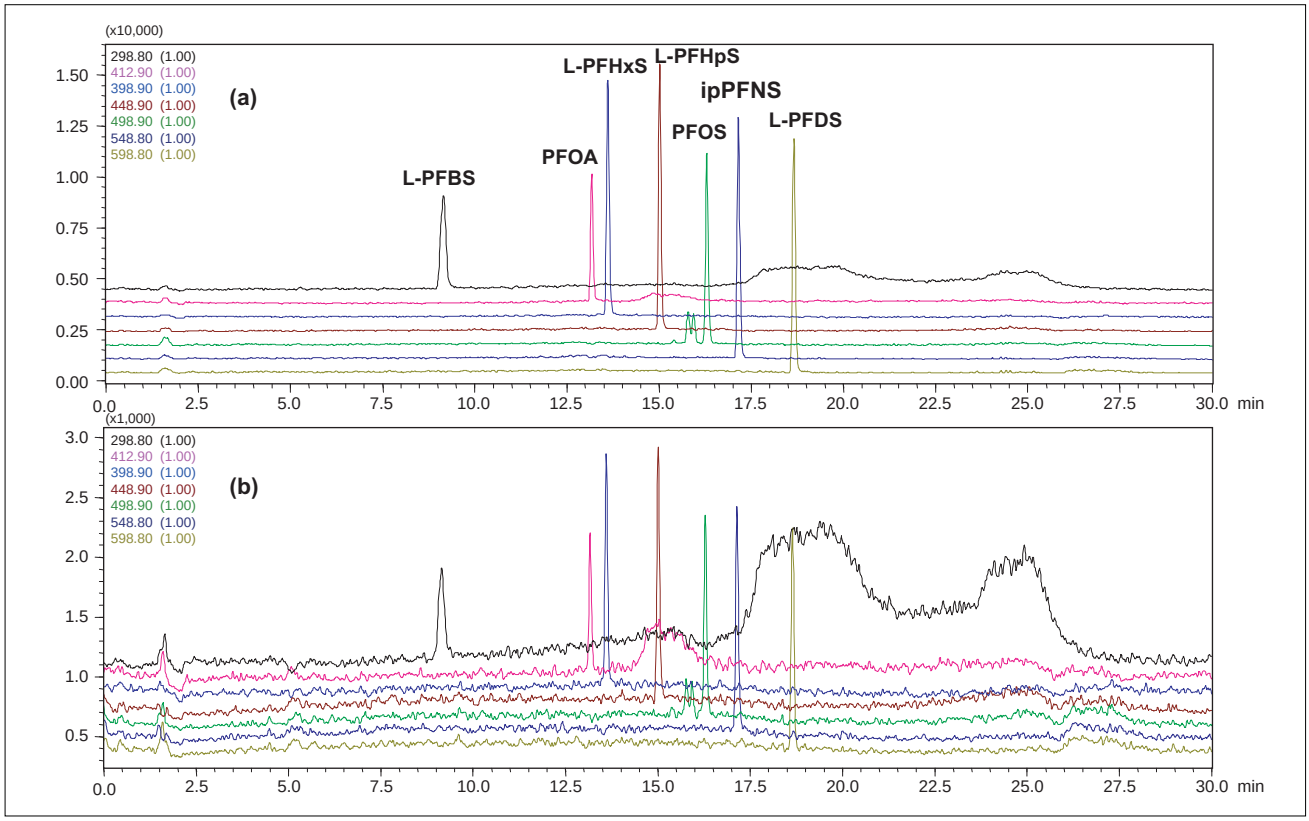

Fig. 4 SIM Chromatograms of a Standard Mixture*1 (2.5 μg/L (a) and 0.5 μg/L (b) each, 5 μL injected)

*1 PFOA standard sample: Wako Pure Chemical Industries, Ltd. 163-09542 Chemicals, PFOS standard sample: Fluka 77282, PFOS-related substances: Wellington Laboratories

Fig. 5 shows the calibration curves (0.25 – 5 μg/L, n = 5) for all the substances. Excellent linearity was obtained for each substance. In addition, Fig. 6 shows the SIM chromatograms obtained from 5 successive measurements of each substance, using a concentration of 0.5 μg/L. The area repeatability RSD (%) ranged from a minimum of 2.05 to a maximum of 6.18. Thus, high sensitivity quantitative analysis of perfluorochemicals can be conducted by LC/MS to a concentration as low as 0.5 μg/L.

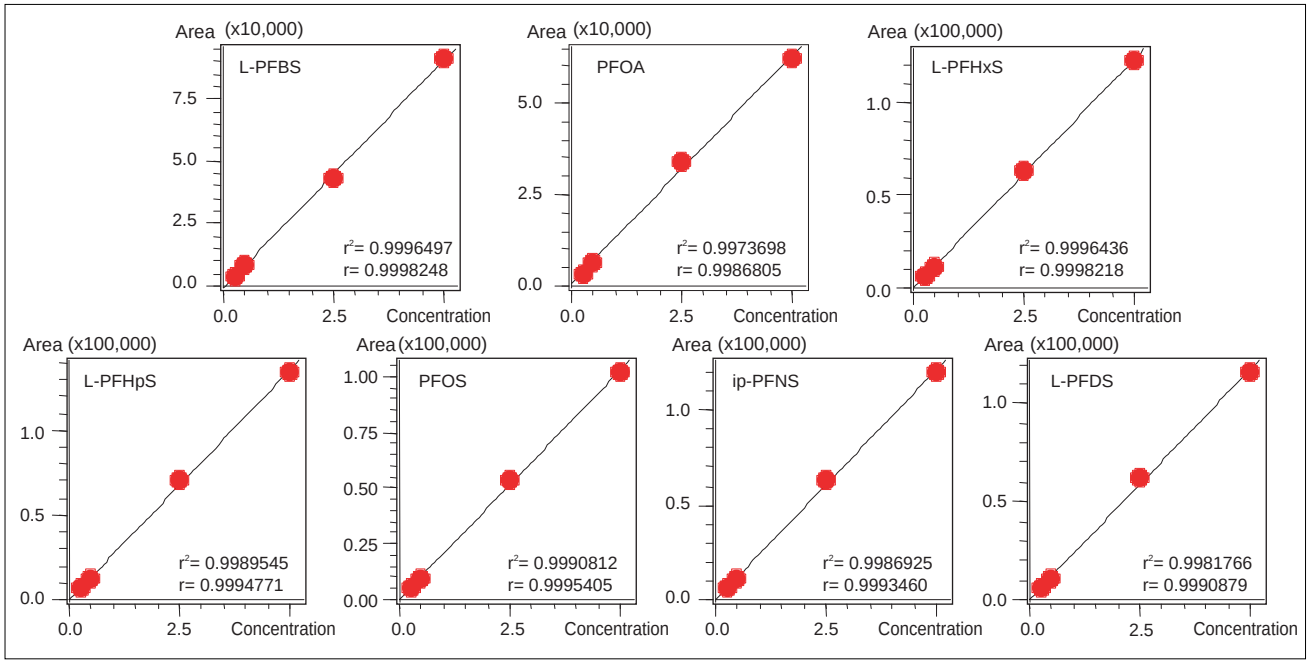

Fig. 5 Calibration Curves of Standard Solutions (0.25 – 5 μg/L)

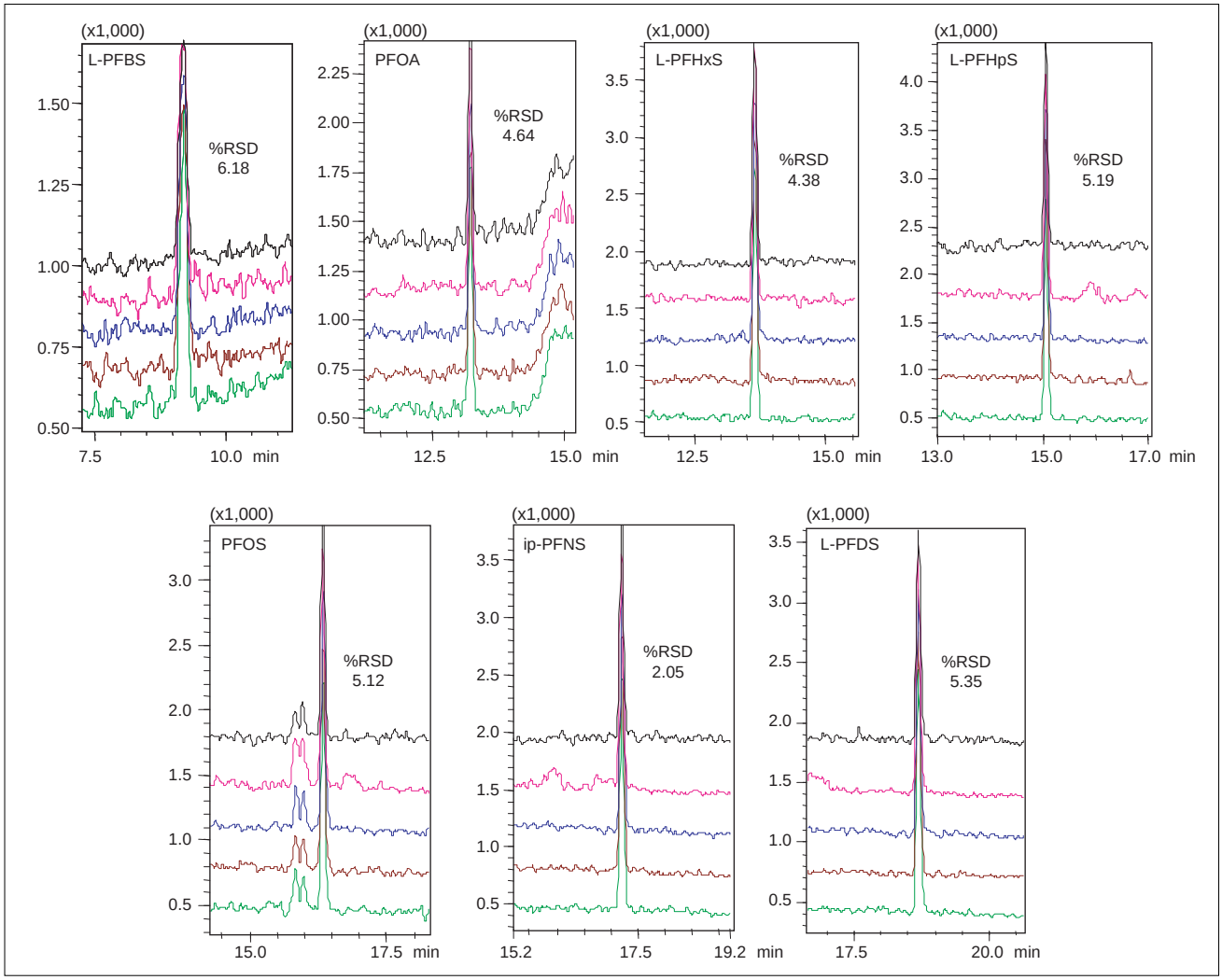

Fig. 6 Peak Area Repeatability (0.5 μg/L each, n = 5)

Next, we introduce an analysis of fabric extract, simulating an actual sample screening analysis. A 2 cm-square piece of fabric possibly coated with water-repellant PFOA and PFOS was cut out and placed in a polypropylene centrifuge tube containing 5 mL methanol. After shaking for 30 minutes followed by centrifugation, 5 µL of the supernatant was injected into the LC/MS. Fig. 7 shows the SIM chromatograms of the fabric extract. Both PFOA and PFOS were detected in fabric extract, but other PFOS-related compounds were not detected. A rough calculation estimated that PFOA was coated on the fabric at a concentration of about 5 ng/cm^2, and PFOS was coated at about 0.1 ng/cm^2. Thus, simple methanol extraction can be used for screening of possible PFOA and PFOS coating on a sample surface.

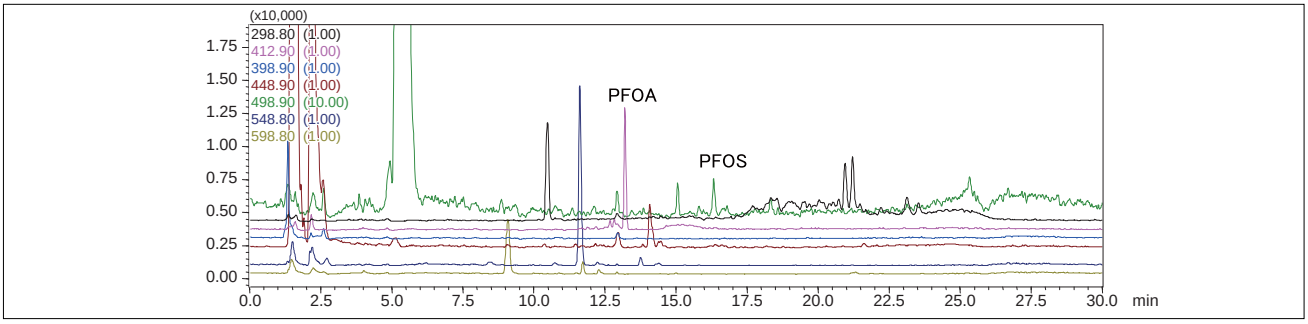

Fig. 7 SIM Chromatograms of PFOA and PFOS in Fabric Extract

Fig. 8 shows an example of analysis of a PFOA standard solution using a Prominence UFLC + LCMS-2020. A single high-speed, high-resolution analysis was performed in 10 minutes. The impurity delay method was able to be applied even using high-speed analysis. SIM chromatograms of PFOA are shown for the cases where the delay column is installed and when it is not installed. The sample-associated PFOA peak (0.5 µg/L, 10 µL injected) and impurity-related peak are clearly separated (Fig. 8f).

Fig. 9 shows the calibration curves for PFOA and PFOS within the range of 0.1 – 50 µg/L. Both show excellent linearity with a coefficient of determination greater than 0.9999. In addition, using the LCMS-2020, the peak area repeatability (RSD (%), n = 6) with a concentration of 0.1 µg/L was 1.59 for PFOA, and 1.26 for PFOS, demonstrating that analysis can be conducted with excellent repeatability at low concentrations.

Thus, use of the LCMS-2020 with its excellent sensitivity and repeatability and its newly developed QoQ ion optical system, together with the impurity delay method, allows 0.1 µg/L trace-level quantitative analysis of PFOA and PFOS with excellent repeatability.

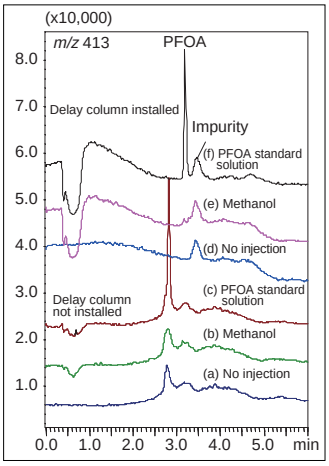

Fig. 8 SIM Chromatograms of PFOA

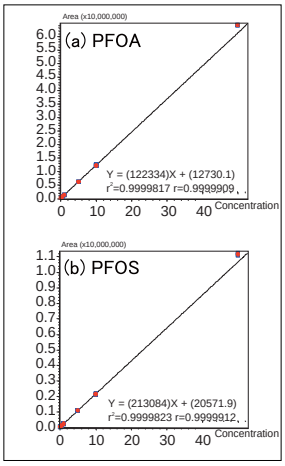

Fig. 9 Calibration Curves for PFOA and PFOS

Table 1 Analytical Conditions

Delay Column	: Develosil Packed Column C30-UG-5 (35mmL. × 4.0mmI.D.)	, Shim-pack XR-ODS(30mmL. ×3.0mmI.D.,2.2 µm)
Analysis Column	: Shim-pack FC-ODS (150 mmL. × 2.0 mmI.D., 3 µm)	, Shim-pack XR-ODS (75 mmL. × 2.0 mmI.D., 2.2 µm)
Mobile Phase A	: 5 mmol/L Ammonium acetate-water	, ←
Mobile Phase B	: Acetonitrile	, ←
Time Program	: 25 %B (0 min) → 85 %B (20 min) → 25 %B (20.01 – 30 min)	, 25 %B (0 min) → 85 %B (4 – 5 min) → 25 %B (5.01 – 10 min)
Flow Rate	: 0.2 mL/min	, 0.4 mL/min
Injection Volume	: 5 µL	, 10 µL
Column Temp.	: 40 °C	, ←
MS	: LCMS-2010EV	, LCMS-2020
Probe Voltage	: -3.5 kV (ESI-Negative mode)	, ←
CDL Temp.	: 200 °C	, 250 °C
Block Heater Temp.	: 200 °C	, 450 °C
Nebulizing Gas Flow	: 1.5 L/min	, ←
CDL Voltage	: Default values	, ←
Q-array DC & RF Voltages	: Default values	, ←
Drying Gas Flow	: 10 L/min (0.1 MPa)	, 10 L/min
SIM Monitoring Ion	: *m/z* 298.80(L-PFBS), 412.90(PFOA), 398.90(L-PFHxS), 448.90(L-PFHpS), 498.90(PFOS), 548.80(ipPFNS), 598.80(L-PFDS)	, *m/z* 412.90(PFOA), 498.90(PFOS)

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