

## Analytical method development for widely targeted PFAAs in plasma using multi-gradient eluent system by LC-MS/MS

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### [Introduction]

Perfluoroalkyl acids (PFAAs) had been widely used because of their excellent surfactant property. While the product and usage of perfluorooctane sulfonic acid (PFOS), its salts and perfluorooctane sulfonyl fluoride (PFOS-F) were restricted by the Stockholm Convention in 2009, other PFAAs homologues and precursors which may break down to PFAAs are still used. PFAAs can be categorized into many types depending on their chemical compositions such as carboxylate acids, sulfuric acids, telomer acids, telomer sulfonate, amids, phosphoric acids and phosphoric acid esters. Those poses different chemical properties which makes them difficult to be analyzed simultaneously using LC-MS/MS. In this study, we developed a simultaneous analysis method for a wide range of PFAAs and their precursors in plasma using online-SPE-LC-MS/MS with multi-gradient eluent system.

### [Methods]

A standard mixture of PFCAs (C4-18), PFASs (C4-10), FOSAs, FTSs and PAPs (total of 28 compounds), was diluted to working concentrations with methanol. Plasma samples were pretreated by WAX cartridge (final solvent: 0.05% NH<sub>3</sub>, 0.15% HCOOH, 80% MeOH). The separation was achieved on Oasis WAX column (2 mm I.D. x 20 mm L.) and Triart C18 column (2 mm I.D. x 100 mm L.) maintained at 40°C on a UHPLC system (Shimadzu corporation, Kyoto, Japan), configured by four pumps and two 6-port valves. Then, 500 μL of sample was mixed with 250 μL of water and introduced to the LC. Data acquisition was performed on triple quadrupole mass spectrometer LCMS-8060 (Shimadzu Corporation, Kyoto, Japan). All samples were analyzed by multiple reaction monitoring (MRM). Fig. 1 shows the flow diagram of online-SPE-LC-MS/MS with multi-gradient eluent system.

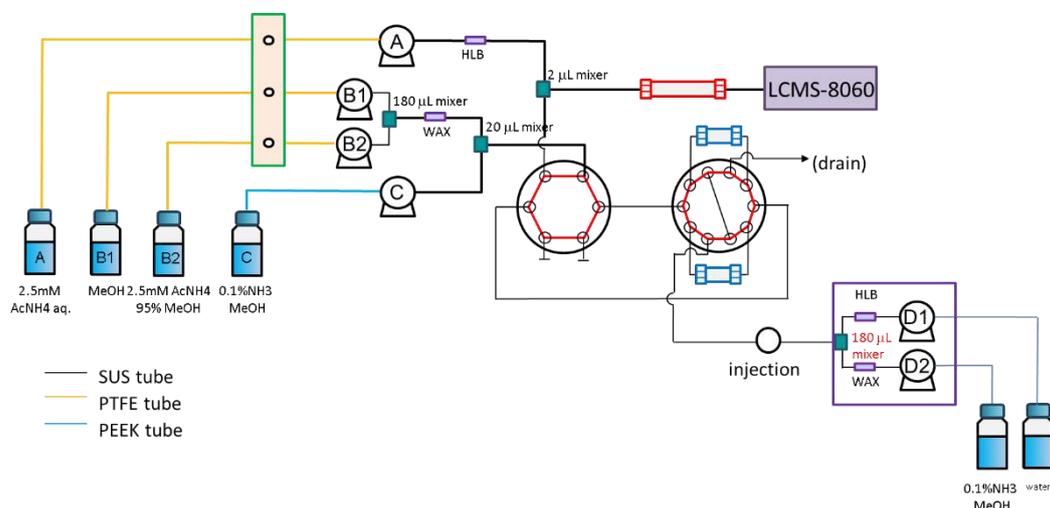


Fig. 1 Flow diagram of online-SPE-LC-MS/MS with multi-gradient eluent

[Results and discussion]

All compounds were successfully ionized by negative electrospray ionization (ESI) mode. Samples were loaded on a trap column (WAX) with 65% 2.5 mmol/L NH<sub>4</sub>Ac 95% MeOH. In this condition, MeFOSA and EtFOSA were hardly retained either on trap and analysis columns and eluted through these columns to mass spectrometer. As shown in Fig. 2, these two compounds came out early and other 26 compounds were eluted by tertiary gradient; A: 2.5 mmol/L NH<sub>4</sub>Ac aq., B: 2.5 mmol/L NH<sub>4</sub>Ac MeOH, C: 0.1% NH<sub>3</sub> MeOH, B 0% C 7.5% to B 80% C 7.5%. All compounds were eluted with excellent separation with peak width less than 30 minutes. Using 28 isotope-labelled PFAAs to examine recoveries, some compounds showed ion suppression or ion enhancement. Overall, this method resulted in good repeatability. Detailed results will be presented in the poster.

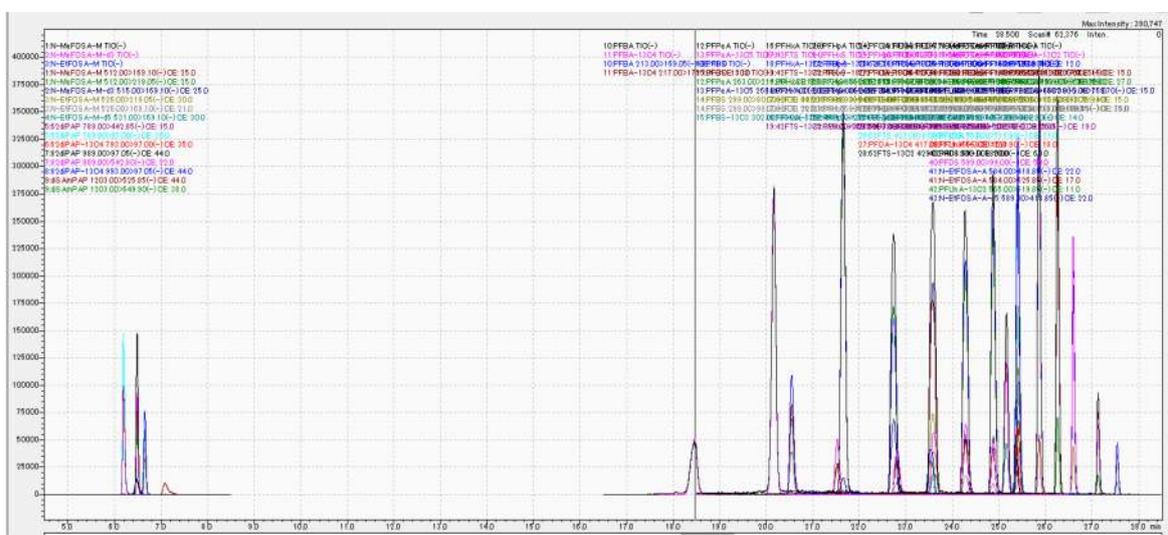


Fig. 2 MRM chromatograms of 28

[Conclusion]

We developed an online-SPE-LC-MS/MS method to capture a wide range of PFAAs and their precursors in plasma using multi-gradient eluent system. This research was conducted under a collaborative research agreement between Shimadzu and National Institute for Environmental Studies.