Extended Abstract - Simultaneous determination of 10 types of per- and polyfluoroalkyl substances with wide range of pKa values in human serum without pretreatment

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Introduction

Per- and polyfluoroalkyl substances (PFASs) are highly versatile synthetic substances compounds that have been frequently utilized in industrial production and social life since the 1950-60s.[1] PFASs include perfluoroalkyl acids (PFAAs) and their precursors, and a number of fluoropolymers. Perfluoroalkyl sulfonates (PFSAs) and perfluorocarboxylates (PFCAs) are two typical classes of PFAAs, and perfluorooctanesulfonate (PFOS, C8 PFSA) and perfluorooctanoate (PFOA, C8 PFCA) been widely detected in human serum, urine and milk. [2] In 2009, PFOS was included as a persistent organic pollutant (POP) in the Stockholm Convention. Moreover, in 2015, a proposal to list PFOA, its salts, and PFOA-related substances in the Stockholm Convention was submitted by the European Union. Several main manufacturers have phased out the production of PFOS and PFOA and related compounds since 2000, and declining trends of PFOS and PFOA concentrations have been observed in human blood/serum collected from the USA, Sweden, Norway, China and Germany.[3-6]

On-line SPE methods could overcome shortcomings existing in traditional analytical methods like manual operation is complex, blank contamination is serious, sample usage is large, and detection time is long. Till now, several on-line SPE coupled with LC-MS methods have been applied to the analysis of PFAS in serum[2,7]. For large-scale human monitoring study, it is especially advantageous to use a rapid and accurate method with low LODs, and fully automatic without sample preparation before injection, and could detect a broad range of PFASs with varied PFASs simultaneously in a short run time utilized small sample volume. Turbulent flow online SPE column combines size exclusion and traditional stationary phase to separate target analytes in biological fluids from matrix components, and has been used for the clean-up of serum, urine, hair and tissue extracts, for the analysis of PFASs [2,8].

The aim of present work is to develop and validate a rapid, fully automatic and sensitive analytical method for the simultaneous analysis of 10 types PFASs totally 43 PFASs including PFCAs (C4-C14, C16, C18), PFSAs (C4-C10, C12), Cl-PFESAs (6:2, 8:2-), FTSs (4:2, 6:2, 8:2, 10:2) , PFPAs (PFHxPA, PFOPA), PFPiAs (6:6, 6:8, 8:8), monoPAPs (6:2, 8:2), diPAPs(6:2, 8:2, 6:2/8:2), FOSAs (PFOSA, N-Me-FOSA, N-Et-FOSA) and FOSAAs (FOSAA, N-Me-FOSAA, N-Et-FOSAA) in human serum. Moreover, our method was applied to detect a human serum standard reference materials 1957 (SRM 1957) for accuracy verification, and we further expanded the reference value that did not available in NIST reference values and literatues data.

Materials and methods

PFAC-MXB (98% purity in methanol); MPFAC-MXA (>98%); 6:2-, 8:2-, $^{13}C_2$ 6:2- and 13C2 8:2-monoPAPs, 6:2-, 8:2-, $^{13}C_4$ 6:2-, and $^{13}C_4$ 8:2- diPAPs, 4:2-, 6:2-, 8:2-FTSs, $^{13}C_2$ 4:2-, 6:2-, 8:2-FTSs, and C6-, C8-, C10-6:6-, 6:8-, 8:8- PFPiAs; FOSA, $^{13}C_8$ FOSA, PFPAs were supplied by Wellington Laboratories, Canada. 6:2 Cl-PFESA and 8:2 Cl-PFESA were purified from the commercial F-53B product purchased from Shanghai Synica Co., Ltd. LC-MS-grade solvents, acetonitrile (ACN), methanol(MeOH), isopropanol (IPA), were purchased from Fisher Scientific (Ottawa, ON, Canada). Ammonium acetate (NH4OAc, >97%), ammonium hydroxide (28%), acetic acid (>99.8%, HPLC grade), and formic acid (>98%, HPLC grade) were obtained from Alfa Aesar (Ward Hill, MA, USA). Water (>18.2 MV cm) used in the present study was purified on a Milli-Q Advantage A10 system (Millipore, USA). The purity of all standards was over 98%.

The UltiMate™ 3000 system (Thermo Scientific, USA) consisted of a dual- gradient rapid separation pump DGLC-3600RS, a TCC-3200 column oven with a two-position, six-port (2P-6P) valve, and a WPS-3000 TLS auto-sampler. The entire analysis process is controlled by a Chromeleon 6.70 chromatography workstation. A TSQ Quantiva triple quadrupole mass spectrometer (Thermo Scientific, USA) was used to measure the target compounds. The data was recorded using the Xcalibur 3.0 software.

Results and discussion

1 Optimization of analytical procedures

Zorbax Extend-C18 is specially designed for applications at high pHs (2-11.5), and Zorbax Extend-C18 column 3.0 ×150mm, 3.5 μm shown excellent peak shapes for PFPAs and monoPAP. Thus Zorbax Extend-C18 was selected for proper detection.

For PFPAs detection, and the standard supplier recommends elevating the pH of the eluent to 9 for optimal chromatography, and Esparza et al studied the pH of the aqueous phase adjusting with ammonium hydroxide between 6 and 10.5, and drew the conclusion that 2 mM ammonium acetate buffer with pH of 6.2 was used as optimal for PFPA detection, while the standards stored in MeOH: water (75:25) at pH 9. As for monoPAP detection, supplier recommends that the response of the monoPAPs can be optimized, and peak tailing minimized, by utilizing a methanol: water gradient where the pH of the water has adjusted to 11 with ammonium hydroxide, and Ding et al investigated water containing 0.1% NH_3 H_2O was the optimal conditions as mobile phase for monoPAP detection.

Based on the conclusions in the above literature, in present study, we optimized and pHs (9.0, 9.5, 10.0, 10.25, 10.5, 10.75, 11.0) of 2 mM ammonium acetate buffer solution using as aqueous mobile phase (shown in Fig 1). Value pH=10.50 might be the best choice for both PFPAs and monoPAPs. The different optimal pH conditions of PFPA and monoPAP observed in our study were consisting with some literatures that PFASs (including PFCAs, PFSAs and PFPAs) and PAPs were analyzed using two different gradient elution runs.

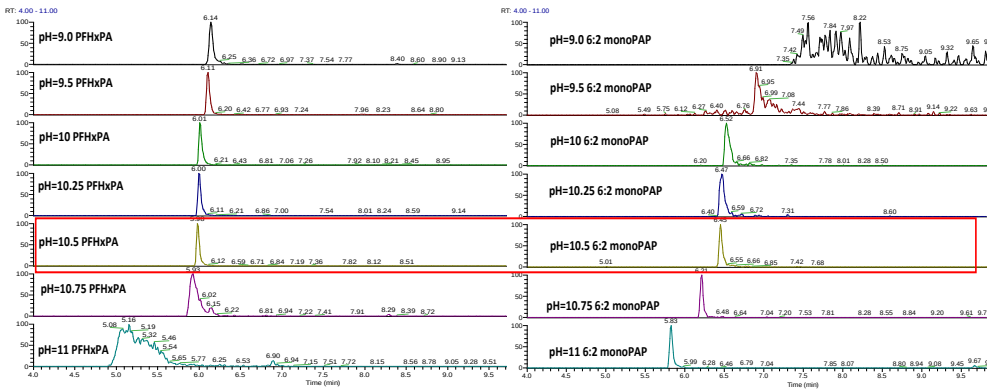

Fig. 1 Chromatographic peaks of PFHxPA and 6:2monoPAP standard solution (5 ng mL-1) at several pHs (9.0, 9.5, 10.0, 10.25, 10.5, 10.75, 11.0)

As for organic phase, MeOH or ACN or a mixture of ACN and MeOH (V:V=1:1) blend was tested for the best elution efficiency (shown in Fig. 2). Mixture of ACN and MeOH was better than MeOH or ACN utilized only, it might due to elution capability of ACN was stronger than MeOH, while cleaning capability was weaker than MeOH. Also, mixture of ACN and MeOH (V:V=1:1) also could minimize background noise and maintain appropriate sensitivity of both LC and MS detector. Additionally, 1-MP as an ion pair reagent, has been used to improve the chromatographic peak tailing of PFPAs and PAPs, ACN and MeOH (V:V=1:1) with 5 mM 1-MP could further increase the peak intensities of selected PFASs (Fig. 2), profit from 1-MP could generate ion-pairs that mask the negative charges of the phosphonate group, leading to an increase in the retention of PFPAs, PAPs on a C18 stationary phase through hydrophobic interaction. Eventually, ACN and MeOH (V:V=1:1) with 5 mM 1-MP were utilized as mobile phase B.

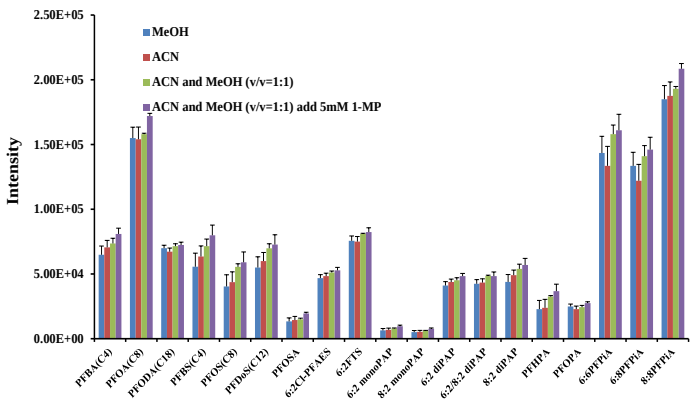

Fig. 2 Intensities of representative compounds PFASs by different organic mobile phase (5 ng mL-1 standard solution spiked in serum)

2 Validation of the method and performance with serum samples

The matrix matched curve displayed good linearity ($r^2 > 0.99$) over the concentration range of 0.05 to 100 ng mL-1 for most targeted PFASs, and 0.1 to 100 ng mL-1 for PFPAs and monoPAPs (shown in Table 3). Sensitivity was evaluated by MLODs and LOQs, which were determined using the chromatographic peaks obtained from samples fortified with 0.01, 0.02 and 0.05 ng mL-1 of standards solution. The MLODs of PFPAs, PFPiAs, monoPAP, diPAP and FTSs were ranging from 0.023 to 0.089 ng/mL, and our results are considerably lower than the little data found in the literatures.

Conclusion

A rapid, accurate, and fully automatic method was established to simultaneously analyze 10 types PFASs including PFCAs, PFSAs, PFPAs, PFPiAs, FTSs, monoPAPs, diPAPs and FOSAs, FOSAAs, Cl-PFESAs in human serum. Serum samples were directly analyzed without pre-treatment before injection. Optimized experiments were conducted to determine the analytical column, mobile phase composition and pH, and cleaning procedures. Matrix effects were corrected due to the matrix removal efficiency of the Turboflow column, matrix matched curve and abundant internal isotope standards that were used in present study. MLODs, linearity, accuracy and precision, and spiked recoveries of the method satisfied the requirements for determining PFASs well. Especially, the method showed comparable MLODs, satisfactory spiked recoveries for the analysis of PFPAs (PFHxPA, PFOPA) and monoPAP (6:2monoPAP, 8:2monoPAP) compared with literatures, with MLODs ranged from 0.13 to 0.89 ng mL-1 and recoveries from 84.3% to 91.6%.

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References

1 Olsen G W, Lange C C, Ellefson M E (2012) *Environmental science & technology* **46** 6330-6338
2 Gao K, Gao Y, Li Y (2016) *Journal of Chromatography A*, **1471** 1-10
3 Yeung L W Y, Mabury S A (2016) *Environmental Chemistry* **13** 102-110
4 Poothong S, Lundanes E, Thomsen C (2017) *Analytica Chimica Acta*
5 F. Perez, M. Llorca, M. Farré, D. Barceló (2012) **402** 2369-2378
6 Yeung L W Y, Robinson S J, Koschorreck J (2016) *Environmental Chemistry* **47** 3875-3882
7 Lee H, Mabury S A (2011) *Environmental Chemistry* **45** 8067-8074