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An integrated method for simultaneously determining 10 classes of per- and polyfluoroalkyl substances in one drop of human serum

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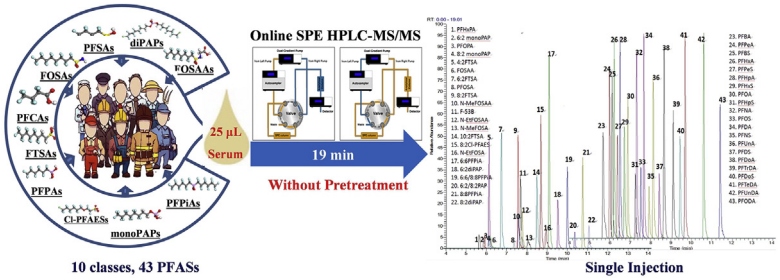
HIGHLIGHTS

- An automated online SPE method was developed for detecting 10 classes of PFASs in 25 uL serum.
- PFASs detected by the present method were consistent with the reference values of SRM 1957.
- Several PFASs in SRM 1957 without reference concentrations were determined for the first time.
- The method is promising for epidemiologic studies and degradation kinetics studies of PFASs precursors.

GRAPHICAL ABSTRACT



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ABSTRACT

Per- and polyfluoroalkyl substances (PFASs) represent a group of synthetic chemicals, and they have quite different physicochemical properties, which result in difficulties of their simultaneous determination in a single injection. A sensitive, reliable, and fully automated method was developed for simultaneously detecting 10 classes of PFASs (total of 43) in human serum using online Turboflow SPE-UHPLC-MS/MS. This method provided high linearity of matrix-matched calibration standards ($R > 0.99$), excellent method limits of detection (MLODs) (0.013–0.089 ng mL^{-1}), satisfactory matrix spiked recoveries (84.3–109%) and relative standard deviations (RSDs) (intra-day RSDs: 1.3–12.6%, inter-day RSDs: 1.7–13.8%, inter-week RSDs: 1.8–13.5%, inter-month RSDs: 3.1–12.4%), short analysis time (19 min per sample) and small sample amount requirement (25 μL), which were suitable for large-scale epidemiologic studies. Moreover, the method provided the feasibility of real-time monitoring for the degradation kinetics of PFASs precursors both *in vitro* and *in vivo*. The quality of the present method was further verified by repetitive analysis of a standard reference material (SRM 1957), with the deviations of the targeted PFAS concentrations ranging from 1.9% to 14.2% ($n = 5$) between the detected and reference values. The present study also determined values for several PFASs in SRM 1957 other than those on the certificate, for the first time, such as N-EtFOSA, 6:2 Cl-PFESA, and PFBA. Finally, the established method

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was applied to detect PFASs in serum samples of 15 ordinary people and 15 occupational workers, and 6:2 FTSA was found as the dominant precursor.

1. Introduction

Per- and poly-fluoroalkyl substances (PFASs) are highly versatile synthetic compounds that have been frequently applied in industrial production and social life since the 1950–60s due to their unique physicochemical properties. Increasing global concern has been directed toward PFASs due to their persistence in the environment, bioaccumulation, long-range transport behavior, and potential toxicological effects [1–5]. Perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) are the two most predominant PFASs, and they were considered persistent organic pollutants (POPs) in the Stockholm Convention in 2009 [6] and 2015 [7], respectively. The production and application of these legacy PFASs have been restricted in several countries or regions, and declining trends of PFOS and PFOA concentrations have been observed in human blood/serum collected from the USA [8], Sweden [9], Norway [10], China [11] and Germany [12,13]. However, in recent years, shorter chain PFASs, chlorinated polyfluorinated ether sulfonates (Cl-PFAESs), etc., have been widely applied in industrial production as alternatives of legacy PFASs, and they have been detected in environmental media and human bodies, with some PFASs even showing a large-scale increasing trend [12–15].

Notably, fluorinated precursors should also be considered when evaluating the total PFAS exposure to humans [8,12,13]. Studies have reported that polyfluoroalkyl phosphoric acid monoesters (monoPAPs), polyfluoroalkyl phosphate diesters (diPAPs), perfluoroalkyl phosphonic acids (PFPAs), perfluoroalkyl phosphinic acids (PFPiAs), and fluorotelomer sulfonic acids (FTSAs) could biodegrade to perfluoroalkyl carboxylic acids (PFCAs) in rats, rainbow trout and the activated sludge of wastewater treatment plants [16–18], and perfluorooctane sulfonamides (FOSAs) and perfluoroalkane sulfonamidoacetic acids (FOSAAs) could be transformed to PFOS in rat liver microsomes [19]. Although the metabolism of fluorinated precursors is closely related to the total PFASs body burden in humans, little information on the fluorinated precursors in the human body is available, let alone the degradation kinetics of the fluorinated precursors. Further research to investigate and characterize the levels, profiles, and degradation kinetics of these emerging PFASs in human serum is urgently needed.

One of the reasons for the lack of data for PFASs precursors is the absence of an accurate, sensitive and rapid analytical detection method for the different classes of PFASs, especially their simultaneous determination. The physiochemical properties of the different PFASs vary, with pKa values ranging from 0.14 for PFSAs [20], 0.2–2.8 for PFCAs [21], 2.1–5.6 for PFPAs [22], 3.9 for FOSAA [23], 6.3–6.8 for FOSAs [24] to 3.0–7.0 for monoPAPs [22]. Accordingly, the detection conditions of the different PFAS classes and components are closely related to their corresponding pKa values, complicating the pH adjustment of extracts and HPLC mobile phase using one single extraction and injection. Compared with that of PFCAs and PFSAs, the concentrations of PFPAs, PFPiAs, monoPAPs, diPAPs, FOSAs, and FOSAAs in reported data were generally within the range of 10^{-3} to 1 ng mL^{-1} in blood, plasma or serum from the general population [8,12,13,25]. The low concentration might be due to the degradation or transformation of the PFASs precursors in the long time process of sample pretreatment, which resulted in the inaccuracy of both PFASs precursors (less than the true value) and PFASs (higher than the true value). Moreover, the LODs of these PFASs precursors were higher than that of legacy PFASs by the same analytical method, and the excellent condition for legacy PFASs might not be a satisfactory detecting condition for PFASs precursors, and vice versa. Thus, many previous studies have employed two or more different extraction methods and/or analytical procedures in order to determine a broad range of PFASs [8,12,13,25], resulting in an increase of the amount of sample (generally 0.5–3 mL) and prolonged analysis time [8–13,25]. Some novel detecting methods have achieved PFASs detection using a small sample amount, e.g., our previous study developed a new SELDI probe for the rapid screening of trace toxic chemicals in a single drop human blood [26]. However, the MLODs of several PFASs were not satisfactory. Moreover, fluorochemicals such as polytetrafluoroethene (PTFE) are widely used in laboratory equipment, and PFPAs are prone to stick to metal [22], while fluorinated precursors are ubiquitous in indoor air in the laboratory [27], which makes it hard to eliminate blank contamination and leads to interference for certain PFASs during the pretreatment process.

An online SPE method could overcome these shortcomings mentioned above to a great extent. It could reduce the sample amount, shorten the analysis time, minimize manual operations as well as processing contamination, and could guarantee that PFASs enter into the detecting system without any loss. More importantly, the online SPE system could adjust the pH of the mobile phase more automatically and effectively, to achieve simultaneous detection of PFASs with varying pKa values. Although several methods utilizing online SPE coupled with LC-MS/MS have been applied for the analysis of PFASs in serum, the simultaneous detection was still limited to less than 5 classes of PFASs, and the sample amount was generally 50–150 μL. Moreover, protein precipitation with organic reagents (methanol or acetonitrile) and/or dilution with 0.1 M formic acid were/was needed before injection into the online SPE system [28–32].

The present study aimed to develop an integrated method for simultaneously detecting 10 classes of PFASs (targeting a total of 43 PFASs; the structures and molecular formula are shown in the Supplementary material, Fig. S-1) in human serum, reducing sample requirement, analysis time, manual operation and processing contamination. A human serum standard reference material (SRM 1957) was further employed to verify the accuracy of the developed method. Moreover, serum samples of ordinary people (n = 15) and occupational workers (n = 15) were utilized for practical tests.

2. Materials and methods

2.1. Chemicals and materials

A list of the 43 targeted PFASs and 27 internal standards with their abbreviations and nomenclature are given in Table 1. All native and isotope-labeled PFASs except 6:2 Cl-PFESA and 8:2 Cl-PFESA were obtained from Wellington Laboratories (Guelph, Ontario, Canada). 6:2 Cl-PFESA and 8:2 Cl-PFESA were purified from the commercial F-53B product purchased from Shanghai Synica Co. Ltd. The purity of all standards was over 98%. LC-MS-grade solvents,