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A simple and rapid extraction method for sensitive determination of perfluoroalkyl substances in blood serum suitable for exposure evaluation

Noelia Luque [a], Ana Ballesteros-Gómez [a], Stefan van Leeuwen [b], Soledad Rubio [a,*]

[a] Department of Analytical Chemistry, Institute of Fine Chemistry and Nanochemistry, Agrifood Campus of International Excellence, Edificio Anexo Marie Curie, Campus Rabanales, 14071 Córdoba, Spain
[b] RIKILT-Institute of Food Safety, PO Box 230, 6700 AE Wageningen, The Netherlands

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ABSTRACT

In this work, we propose a microextraction method based on a new supramolecular solvent (SUPRAS) made up of reverse aggregates of hexanoic acid, combined with liquid chromatography/triple quadrupole mass spectrometry (LC/QQQ MS–MS) for the determination of the perfluoroalkyl substances (PFASs) in blood serum. A SUPRAS is a nano-structured liquid made up of surfactant aggregates synthesized through a self-assembly process. The method involved the acidification of 765 μL of blood serum (600 μmol of hydrochloric acid per mL of serum) followed by the addition of hexanoic acid (97 μL) and tetrahydrofuran (THF) (600 μL), conditions under which the supramolecular solvent (~360 μL) formed in situ after vortex-shaking and centrifugation. Parameters affecting extraction efficiency and concentration factors were studied. The overall sample treatment took only 20 min and several samples (20–30) can be simultaneously analyzed using conventional lab equipments, making additional investments unnecessary. Recoveries for the internal standards in samples ranged from 75 to 89% with relative standard deviations between 1 and 15%. Calibration was based on the use of internal standards. The method was very sensitive with detection limits ranging from 2 to 20 pg mL^{-1} for PFASs. The approach developed was successfully applied to the determination of PFASs in different blood serum samples. The concentration of PFASs found in samples of animal origin ranged between 17 and 197.3 pg mL^{-1} and between 84 and 5168 pg mL^{-1} in samples of human origin. Both the analytical and operational features of this method make it suitable for the evaluation of exposure to PFASs.

1. Introduction

Per- and polyfluoroalkyl substances (PFASs) are emerging contaminants that comprise a large group of chemicals [1]. Attention has been focused on these chemicals due to the discovery of their global biospheric distribution explained by their persistence and bioaccumulation in biota [2,3].

The marketing and use of perfluorooctane sulfonate (PFOS) were restricted by the European directive 2006/122/EC [4]. PFOS has been included in the annex III of the Directive 2008/105/EC as part of the list of substances subject to review for identification as priority hazardous compounds in the framework of the European water policies [5]. PFASs have been also listed as contaminants of relevance to be monitored in fish and other seafood for human consumption for determining good environmental status of marine waters in the Marine Strategy Framework Directive [6]. In addition, PFOS, its salts and perfluorooctane sulfonyl fluoride (PFOSF) have been included in Annex B to the Stockholm Convention on persistent organic pollutants (POPs) [7].

The presence of organic fluoride in humans was first reported by over 30 years ago [8] but studies to determine the concentration of PFASs in serum of workers with an occupational exposure (in the order of 1000–2000 ng mL^{-1}) and general population (about 100 times lower) did not begin until the 1990s and 2000s, respectively [9,10]. The exposure to PFASs is likely to occur via several routes e.g. ingestion (e.g. food, drinking water, dust, etc.), dermal contact and inhalation [11] and they are widely distributed in the body and especially in blood, liver and kidney [12]. Major PFASs found in human serum are PFOS, perfluorooctanoic acid (PFOA) and perfluorohexane sulfonate (PFHxS), although long chain perfluoroalkyl carboxylates (e.g. C_9–C_{14}) are also found in these biological matrices at relevant concentrations [13–16].

The determination of PFASs in blood samples (i.e. whole blood, serum and plasma) is mostly made by liquid chromatography (LC)–tandem mass spectrometry (MS/MS) with negative electrospray ionization (ESI-) interfaces [16,17]. Comparison of extraction and quantitation methods for PFASs in human plasma, serum and whole blood has been reported [18]. Current sample

* Corresponding author. Tel.: +34 957 218643; fax: +34 957 218644.
E-mail address: qa1rubrs@uco.es (S. Rubio).

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treatments for PFASs analysis are mostly based on ion-pair extraction employing methyl-tert-butyl-ether after reaction with tetrabutylammonium [10], solvent extraction with acetonitrile [3,19] or solid phase extraction (SPE), both off-line [15,20,21] or column switching [16,22,23]. Organic solvent volumes around 10–15 mL [3,13,18] and times between 15 min and 16 h are spent by extraction [13,18,20,21]. Acidification [20,22], alkaline digestion [21] or addition of organic solvent to samples [3,16,18,23] are usually carried out prior SPE in order to prevent the clogging of pre/columns caused by the precipitation of blood proteins. Clean-up strategies include column washing [15,20,22], filtration [10,20], centrifugation [22], dispersive graphitized carbon with glacial acetic acid [3], and Wax SPE of extracts [19], being common the use of multiple-steps methods. Main drawbacks of these strategies are much hands-on time due to the need for repetitive washes [10], low recoveries for long-chain PFASs due to co-precipitation with serum protein [16,19], thorough washings of SPE cartridges after sample percolation [15,19], problems of contamination in the different procedural steps, lack of enrichment or even dilution of the samples (only online SPE techniques permit to concentrate them) or the need for additional column switching equipment investments [16,22,23]. In addition most of the developed methodologies require matrix-matched calibration for accurate quantitation of PFASs. Usual detection limits (LODs) are in the range 0.1–3 ng mL^{-1} [17] although a method allowing PFAS quantification at concentrations as low as 0.05 ng PFAS mL^{-1} serum has been reported [16]. According to the above statements, the development of new methods for exact quantitation of PFASs, which feature straightforward and reduced sample handling (thus reducing risk of contamination and loss of analytes) is necessary for large-scale human biomonitoring. With this aim, this article investigates the potential of amphiphile-based supramolecular solvents.

Supramolecular solvents (SUPRASs) are water-immiscible liquids made up of supramolecular assemblies dispersed in a continuous phase. The outstanding properties of SUPRASs for extractions derive from their special structure and the high concentration of the ordered aggregates that constitute them, which provide a huge amount of binding sites. SUPRASs have regions of different polarities that offer a variety of interactions (e.g. hydrophobic, hydrogen bonds, ion-dipole, and π-cation) for the extraction of analytes with a wide polarity range. These properties permit the development of simple and robust sample treatment methods for most of the solutes, especially for amphiphilic compounds which form mixed aggregates with the ordered structures in the solvents. To date, SUPRASs based on non-ionic [24], zwitterionic [25], cationic [26] and anionic [27] aqueous micelles, reverse micelles [28] and vesicles [29] have been successfully used for the extraction of pollutants from biological, food and environmental samples. A review covering progress on both theoretical and practical aspects related to the use of supramolecular solvents for analytical extractions has been recently reported [30]. Applications have mainly focus on the extraction of polycyclic aromatic hydrocarbons, pesticides, surfactants, pharmaceuticals, vitamins, drugs, dyes, hormones, bisphenol A, phenols and toxins mainly in liquids (e.g. [28,31–34]) and more recently in solid samples [35–37]. Supramolecular solvents are compatible with LC but they have to be removed before injection in gas chromatography or capillary electrophoresis.

Against conventional organic solvents, SUPRASs are specially suited for the extraction of amphiphilic contaminants such as PFASs since analyte-extractant mixed aggregates are formed through both interactions between fluorocarbon chains and polar groups. The use of SUPRASs for the extraction of these emerging contaminants is here presented for the first time. With this aim, a SUPRAS made up of hexanoic acid reverse micelles was here synthesized for the first time through a self-assembly process, and then characterized and assessed for the extraction/concentration of PFASs from serum samples prior to LC/triple quadrupole (QQQ) MS–MS determination. The selection of hexanoic acid (HA) was based on both the dispersion and hydrogen bonding interactions they can establish with PFASs and its short hydrocarbon chain length that allows an early elution from the chromatographic column thus preventing coelution with the PFASs usually found in blood (i.e. PFHxS, PFOS, PFOA and C_9–C_{14} perfluoroalkyl carboxylates). Carboxylic acids with chain lengths shorter than hexanoic acid were not employed because of their high water solubility and accordingly low yield of SUPRAS production under the proper self-assembly conditions. Parameters affecting extraction efficiency and concentration factors were optimized and the applicability of the proposed method to the determination of PFASs in different sera was assessed.

2. Experimental

2.1. Chemicals

The seven target PFASs studied were as follows: PFOA, perfluorononanoic acid (PFNA); perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA); perfluorotetradecanoic acid (PFTeDA), potassium perfluoro-1-hexanesulfonate (PFHxS) and PFOS. PFOA, PFNA, PFDA, PFHxS and PFOS were supplied by Wellington Laboratories and PFUnDA and PFTeDA were obtained from Aldrich (Schenelldorpf, Germany). Stable isotope analogues, $^{18}O_2$PFHxS (MPFHxS), $^{13}C_4$PFOA (MPFOA), $^{13}C_5$PFNA (MPFNA), $^{13}C_2$PFDA (MPFDA), $^{13}C_4$PFOS (MPFOS), $^{13}C_2$PFUnDA (MPFUnDA) and $^{13}C_2$-perfluododecanoic acid (MPFDoDA), supplied by Wellington Laboratories (Ontario, Canada), were used as internal standards (ISs) to control potential losses of PFASs during extraction and MS performance (e.g. ion suppression and enhancement).

Hexanoic acid, sodium taurodeoxycholate hydrate (TDCA) and ammonium acetate were purchased from Sigma–Aldrich (Steinheim, Germany) and LC-grade methanol, tetrahydrofuran (THF) and hydrochloric acid were obtained from Panreac (Madrid, Spain). Ultra-high-quality water was obtained from a Milli-Q water purification system (Millipore, Madrid, Spain). Stock standard solutions, each containing a mixture of target PFASs or method ISs at 100 ng mL^{-1}, were prepared in methanol and stored in closed polypropylene tubes at 4 °C. Standards were prepared by dilution of the stock solution with a 75:25 THF:water (v/v) mixture solution.

2.2. Apparatus and materials

The LC–MS system used was an AB Sciex 4000 Qtrap® mass spectrometer (Foster City, CA, USA), with a negative-ion TurboSpray interface coupled to an Agilent 1200 Series LC system (Palo Alto, CA, USA). The stationary phase was a SymmetryShield™ RP 18 column (particle size 3.5 μm, i.d. 2.1 mm, length 50 mm) from Waters (Milford, MA, USA). A SymmetryShield™ RP 8 guard column (particle size 3.5 μm, i.d. 3.9 mm, length 20 mm) was inserted before the analytical column. Coloumetric Karl Fischer titrator from Metrohm (Herisau, Suize) was used for determination of water content in the SUPRAS. Two mL-microtubes Safe-Lock from Eppendorf Iberica (Madrid, Spain), glass balls (3 mm diameter) from Albus (Córdoba, Spain), a Reax Heidolph vortex (Schwabach, Germany), with an attachment for 10 test tubes, a High Speed Brushless centrifuge MPW-350R (Warsaw, Poland), and a 50-μL microsyringe 750 NR from Hamilton (Bonaduz, Switzerland) were used for sample preparation and extraction. The volume obtained of SUPRASs under different experimental conditions was measured with a digital calliper from Medid Precision, S.A. (Barcelona, Spain).