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A rapid method for the determination of perfluoroalkyl substances including structural isomers of perfluorooctane sulfonic acid in human serum using 96-well plates and column-switching ultra-high performance liquid chromatography tandem mass spectrometry

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ABSTRACT

To facilitate high-throughput analysis suitable for large epidemiological studies we developed an automated column-switching ultra-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) method for determination of perfluorocarboxylic acids (PFCAs; C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, and C_{13}), perfluoroalkyl sulfonic acids (PFSAs; C_4, C_6, C_8, and C_{10}), perfluorooctane sulfonamide (PFOSA), and five groups of structural perfluorooctane sulfonic acid (PFOS) isomers in human serum or plasma. The analytical procedure involves rapid protein precipitation using 96-well plates followed by an automated sample clean-up using an on-line trap column removing many potentially interfering sample components while through the mobile phase gradient the target analytes are eluted onto the analytical column for further separation and subsequent mass detection. The method was linear ($R^2 < 0.995$) at concentrations ranging from 0.01 to 60 ng mL^{-1} with method detection limits ranging between 0.01 and 0.17 ng mL^{-1} depending on the analyte. The developed method was precise, with repeatability ($n = 7$) and reproducibility ($n = 103$) coefficients of variation between 2% and 20% for most compounds including PFOS (2% and 8%) and its structural isomers (2–6% and 4–8%). The method was in conformity with a standard reference material. The column-switching HPLC–MS/MS method has been successfully applied for the determination of perfluoroalkyl substances including structural PFOS isomers in human plasma from an epidemiological study.

1. Introduction

Perfluoroalkyl substances (PFAS) are widely distributed in the environment due to extensive usage in a wide variety of commercial applications [1]. The exposure in humans originates primarily through ingestion of contaminated food as well as drinking water [2]. Perfluorinated carboxylic acids (PFCAs) and perfluorinated sulfonic acids (PFSAs) such as perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorooctane sulfonic acid (PFOS), and perfluorohexane sulfonic acid (PFHxS) are typically found in the highest concentrations in the general population [3]. Recently epidemiological studies have indicated that even the background exposure to industrial chemicals, such as the PFAS, might be related to negative health effects in the general population [4,5].

The exposure of PFAS in humans is generally measured by extracting the analytes from serum or plasma by the use of a number of different analytical procedures. These analytical procedures are, in general, based on liquid–liquid extraction (LLE), solid-phase extraction (SPE), and ion-paring extraction (IPE) together with clean-up using various particle size exclusion filters, centrifugation, and graphitized carbon [6–9]. In recent years, much interest has been focused on the analysis of structural PFOS isomers [10,11]. This is primarily because the structural isomer pattern in humans may be useful for understanding the routes and sources of PFOS exposure [12,13]. Mono- and di-substituted branched perfluoromethyl isomers originate from the electrochemical fluorination (ECF) process used to manufacture PFOS-based chemicals while the telomerization process almost exclusively results in linear

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PFOS [14]. Apart from being structurally different, the linear and branched isomers have been shown to differ from each other also in physico-chemical properties [15,16]. This suggests that different isomeric profiles in humans might result in different clinical effects as well.

Many of the previously described methods used for the analysis of fluorinated compounds have been based on manual extraction and clean-up procedures typically associated with low throughput. The operational disadvantages posed by manual procedures have during the last years been circumvented by the introduction of automated methods for analysis of PFAS. These automated solutions are predominantly based on on-line and column-switching extraction or automated on-line extraction [6,7]. The major advantages of such systems lie in the increased sample throughput capabilities as well as the improved method precision typically related with automated sample handling. Also, on-line clean-up generally leads to lower detection limits due to the larger injection volumes that can be used. A high-throughput procedure which employs protein precipitation and extraction using 96-well plates together with LC–MS/MS was presented by Flaherty et al. [17]. Initially this method was developed and validated for analysis of only PFOA but was later used for analysis of PFOA and nine additional PFAAs in an epidemiological study involving 69,030 human serum samples from the U.S. [18].

To be able to investigate the associations between a broad range of PFAS (PFCAs, PFASs, and PFOSA) including structural PFOS isomers and various health effects in the general population there is a need for comprehensive and sensitive well validated high-throughput procedures. The primary aims of the here presented study are to: (a) develop a sensitive high-throughput procedure for analysis of a broad range of fluorinated compounds including five groups of structural PFOS isomers, and (b), to validate and apply the procedure to human plasma samples from an epidemiological study.

2. Materials and methods

2.1. Chemicals and standards

Ammonium acetate (NH_4Ac, >99%, p.a. for HPLC), was purchased from Fluka (Steinheim, Germany). Methanol and water (LC–MS grade) were purchased from Fisher Scientific (Leicestershire, UK). Acetonitrile (Optima® LC–MS grade) and formic acid (98–100%) were purchased from Sigma Aldrich (Steinheim, Germany). Newborn bovine serum (New Zeeland) was purchased from Sigma–Aldrich (Steinheim, Germany) and stored frozen (≤20 °C) until analysis. For quality assurance (QA), the standard reference material serum SRM 1957 was purchased from the National Institute of Standards and Technology (NIST) at the US Department of Commerce (WA, USA), this SRM was stored frozen (≤20 °C) until analysis. The quality control (QC) reference sample consisted of a pooled human plasma collected in 2003 and supplied by Örebro University Hospital (Örebro, Sweden) and stored frozen (≤20 °C) until analysis.

A list of all analytes and standards with their abbreviations are given in Table S-1 in the Supplementary information. ^{13}C-labeled internal standards (IS), ^{13}C-labeled performance standards, native calibration standards (PFCAs, PFSAs, and PFOSA), and a mixture of linear and branched isomers of PFOS (brPFOSK) were purchased from Wellington Laboratories (Guelph, Ontario, Canada). One native performance standard, 7H-dodecafluoroheptanoic acid, was purchased from ABCR (Karlsruhe, Germany). All standards were prepared in methanol and stored refrigerated (4 °C).

2.2. Collection of samples

Plasma samples for an epidemiological study were collected between April 2001 and June 2004 from 1016 (50.2% women) 70 years-old participants of The Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) from the city of Uppsala, Sweden, as previously described [19]. All plasma samples were collected in the morning after an over-night fast and the samples were stored at ≤20 °C until analysis. The study was approved by the Ethics Committee of the University of Uppsala and the participants gave written informed consent.

2.3. Sample preparation

Each 96-well plate was considered as one run and consisted of one matrix matched calibration curve, four NIST SRM 1957, and seven batches of samples, which in turn consisted of one method blank, one QC reference sample, and ten authentic samples. The sample preparation procedure was performed as follows; all glassware and analytical syringes used were thoroughly rinsed with methanol. Frozen serum samples were allowed to thaw at room temperature overnight and then vortex mixed for 10 s before being sampled for the sample preparation procedure. Internal standards (10 μL of 0.2 ng mL^{-1} in methanol) and 150 μL serum or plasma were added to a 25 mg Ostro Protein Precipitation & Phospholipid Removal 96-well plate (Waters Corporation, Milford, USA) pre-conditioned with 450 μL acetonitrile. A 450 μL aliquot of acetonitrile (containing 1% formic acid) was added to all wells and mixed thoroughly with the sample by aspirating three times using an automated pipette. Samples were extracted using a 10″ vacuum manifold for approximately 5–7 min. Aliquots of 600 μL of the eluate from each collection plate insert were then transferred to glass LC-vials and evaporated down to 250 μL using nitrogen. The purified extracts were spiked with performance standards (10 μL of 0.2 ng mL^{-1} in methanol) and diluted with 750 μL of 2 mM NH_4Ac in water. All samples and standards were ultrasonicated for 10 min prior to instrumental analysis.

Once all parameters were established in the method development (Table S-2), these conditions were applied to in-house QC reference human plasma samples and NIST SRM samples. Validation was performed in accordance to the official guidelines by the International Union of Pure and Applied Chemistry (IUPAC) [20].

2.4. Matrix matched calibration

Matrix matched calibration standards were made using newborn bovine serum from New Zeeland. The standards were prepared by spiking 150 μL newborn bovine serum with the native standard mixture resulting in an 8-point matrix matched curve ranging from 0.01 to 60 ng mL^{-1} including the matrix blank. The matrix matched standards were further treated in the same way as authentic samples (see Section 2.3).

2.5. Instrumental analysis

Analyses were performed on Acquity UPLC coupled to a Quattro Premier XE HPLC–MS/MS system (Waters Corporation, Milford, USA) with an atmospheric electrospray interface operating in negative ion mode. Aliquots of 250 μL of the samples were injected onto a Xbridge C18 2.1 mm × 20 mm, 2.5 μm (Waters Corporation, Milford, USA) enrichment column connected to a Acquity UPLC BEH C18 2.1 mm × 100 mm, 1.7 μm (Waters Corporation, Milford, USA) analytical column operated by a six-port column switch valve (Waters Corporation, Milford, USA). A trap column (PFC Isolator column, Waters Corporation, Milford, USA) was installed between the pump and injector and used to retain fluorinated compounds