Analytical Biochemistry 545 (2018) 49–53

Contents lists available at ScienceDirect

Analytical Biochemistry

journal homepage: www.elsevier.com/locate/yabio

Novel volumetric adsorptive microsampling technique for determination of perfluorinated compounds in blood

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ARTICLE INFO

Keywords:
Volumetric absorptive microsampling
Perfluoroalkyl acids
Environmental pollutant
Biomonitoring
LC–MS/MS

ABSTRACT

Microsampling is an attractive option for significantly reducing the volume of blood taken for chemical analysis allowing for blood samples taken as a 'finger-prick' with a lancet. A novel, volumetric adsorptive microsampling (VAMS™) device, which reproducibly collects a small volume of 10 µL whole blood in a hematocrit-independent manner, is evaluated in a human biomonitoring setting, and has been utilized for analysis of several perfluoroalkyl acids (PFAA). The results show that the VAMS technique is applicable for PFAA analysis, method has good linearity, repeatability, accuracy and is sufficiently sensitive for samples from general populations. The stability of PFAAs with VAMS devices is shown to be acceptable, which supports the sampling and transportation strategy of several study designs. Furthermore, as well as allowing for a quick and efficient extraction and analysis flow path, the VAMS microsampler is an easy to use device in a real-world sample collection scenario.

Introduction

Perfluoroalkyl acids (PFAAs), a subgroup of per- and polyfluorinated substances (PFAS), are persistent in the environment, bioaccumulative, and capable of interfering with biological systems at different levels. PFAS characteristics and exposure routes are reviewed in the literature [1,2]. One of the known detrimental effects of PFAAs is that they are able to modulate the functions of the human immune system [3–5]. In addition, PFAAs have been associated with elevated total and LDL cholesterol, increased breast cancer risk and disruption of thyroid hormones [6–9].

Challenges regarding sample pre-treatment and storage in PFAA analysis are described in the literature [10]. Currently, the most used instrumentation for determination of PFAAs is hyphenated liquid chromatography -triple quadrupole mass spectrometry (LC-MS/MS), and a variety of methods has been published for the instrumentation [11–13]. To minimize the instrumental sources of analytes and inter-run variability of the analytes, it is preferable to use a trap column between the LC pump and injector switching valve [14]. Human blood and serum are complex matrices which may give rise to co-eluting compounds and thereby suppression in the mass spectrometer (MS) and decreased sensitivity [15]. However, an automated column switching system has been presented, which minimize the sample pretreatment to protein precipitation with methanol [16]. Nevertheless, sample throughput may be limited by lengthy extraction times.

Since epidemiological research on the health effects of the environmental pollutants often employs biobank samples, only a limited volume of stored blood or serum can be allocated for the analysis. However, microsampling using VAMS devices offer a promising alternative for such studies. Current analytical methods for PFAAs analysis in blood and serum are based on liquid-liquid or SPE extraction, where a sample volume needed for analysis is usually hundreds of microliters [11,16,17]. Besides low sample volume of 10 µL, organic extractions from VAMS devices often negates the need for separate sample cleanup techniques which helps to optimize the sample cleanup step. Furthermore, the VAMS technique represents precise, accurate and hematocrit-independent sample collection, which solves one of the issues commonly observed with dried blood spot collection [18–20]. More

Abbreviations: LC-MS/MS, liquid chromatography-triple quadrupole mass spectrometry; LOQ, limit of quantitation; MPFAA, isotope labelled perfluoro alkyl acid; MPFDA, isotope labelled perfluorodecanoic acid; MPFDoA, isotope labelled perfluorododecanoic acid; MPFHpA, isotope labelled perfluoroheptanoic acid; MPFHxA, isotope labelled perfluorohexanoic acid; MPFHxS, isotope labelled perfluorohexanesulfonic acid; MPFNA, isotope labelled perfluorononanoic acid; MPFOA, isotope labelled perfluorooctanoic acid; MPFOS, isotope labelled perfluorooctaneosulfonic acid; MPFUnA, isotope labelled perfluoroundecanoic acid; NBCS, new born calf serum; PFAA, perfluoro alkyl acid; PFDA, perfluorodecanoic acid; PFDoA, perfluorododecanoic acid; PFHpA, perfluoroheptanoic acid; PFHpS, perfluoroheptanesulfonic acid; PFHxA, perfluorohexanoic acid; PFHxS, perfluorohexanesulfonic acid; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctaneosulfonic acid; PFUnA, perfluoroundecanoic acid; PFTeA, perfluorotetradecanoic acid; PFTrA, perfluorotridecanoic acid; SRM, selected reaction monitoring; RSD, relative standard deviation; RT, room temperature; VAMS™, volumetric adsorptive microsampling

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https://doi.org/10.1016/j.ab.2018.01.015
Received 30 November 2017; Received in revised form 16 January 2018; Accepted 18 January 2018

information about the VAMS device and collection procedure is available elsewhere [21].

To date, VAMS technique has been utilized in drug analysis with variety of biological matrices, such as blood, urine, plasma and oral fluid [22–24]. To best of our knowledge, this is the first study where VAMS technique is utilized for environmental pollutants. In this paper we describe a sensitive and validated VAMS–LC–MS/MS method for analysis of 12 perfluoroalkyl acids (PFAA) in human blood. In addition, the effect of different VAMS storage conditions on PFAA analysis was investigated.

Materials and methods

Samples

Blood samples using VAMS device were collected from 12 volunteers (aged 2–63 years). All the volunteers belong to Finnish general population with no known occupational exposure for any PFAAs.

Reagents and materials

Methanol was purchased from J.T. Baker (Deventer, the Netherlands), newborn calf serum (NBCS) from Gibco/Invitrogen Corporation (Auckland, New Zeeland), and N-methylpiperidine from Sigma-Aldrich (St. Louis, MO, USA). All the PFAAs, i.e. perfluorohexanoic acid (PFHxA), -heptanoic acid (PFHpA), -octanoic acid (PFOA), -nonanoic acid (PFNA), -decanoic acid (PFDA), -undecanoic acid (PFUnA), -dodecanoic acid (PFDoA), -tridecanoic acid (PFTrA), -tetradecanoic acid (PFTeA), -hexanesulfonic acid (PFHxS), -heptanesulfonic acid (PFHpS) and -octaneosulfonic acid (PFOS) were acquired from Wellington Laboratories Inc. (Guelph, Ontario, Canada). Isotope labelled PFAAs (abb. MPFAA) were used as internal standards. The individual isotope labelled PFAAs were MPHxA (1,2–$^{13}C_2$), MPFHpA (1,2,3,4–$^{13}C_4$), MPFOA (1,2,3,4–$^{13}C_4$), MPFNA (1,2,3,4,5–$^{13}C_5$), MPFUnA (1,2,3,4,5,6,7–$^{13}C_7$), MPFDoA (1,2–$^{13}C_2$), MPFHxS ($^{18}O_2$) and MPFOS (1,2,3,4–$^{13}C_4$), and they were also obtained from Wellington Laboratories Inc, whereas MPFDA (1,2,3,4,5,6,7,8,9–$^{13}C_9$) was from Cambridge Isotope Laboratories (Andover, MA, USA).

Instrumentation

Samples were collected with VAMS device (Neoteryx, CA, USA). In sample preparation step the samples were sonicated with Bransonic 3210 (Danbury, CT, USA) and centrifuged with Baxter Scientific Heraeus Biofuge 13 (Hampton, NH, USA). Perfluoroalkyl acids were separated and quantified with Thermo Scientific UltiMate 3000 Rapid Separation LC system (Germering, Germany) connected to Thermo Finnigan TSQ Quantum Discovery MAX triple quadrupole mass spectrometer (Waltham, MA, USA). Chromatographic peak integration was undertaken with the help of the software Xcalibur, and the final analyte concentrations were calculated in Microsoft Excel.

Preparation of samples

Human fingertip blood and test tube serum samples were collected with VAMS devices. After the collection of 10 µL the sample was dried at ambient temperature for 2 h, and VAMS device was stored at room temperature for 1 day before PFAA extraction. For extraction, the tip of the VAMS device was transferred to Eppendorf tube and 100 µL of 50% aqueous methanol containing all the internal standards was added. After sonication for 10 min with Branson 3210, the sample was centrifuged with Baxter Scientific Heraeus Biofuge 13 at 12900 g for 10 min. Liquid extract was transferred to polypropylene vial and stored at −20 °C until LC-MS/MS measurement. To obtain a calibration curve matrix-matched samples were prepared by using NBCS (serum with no detectable level of the analysed PFAAs) as the sample. The calibration samples were collected with VAMS device and prepared in the same way as the real blood samples above. Different calibration levels of PFAAs were prepared by spiking the respective native PFAA concentration in the aqueous methanol extraction solution to achieve the final concentration range of 0.15–20 ng/mL. The calibration samples with the same analyte concentrations were prepared separately for each sample batch (n = 3).

Table 1
PFAAs in blood samples using VAMS.

analyte[a]	Correlation coefficient for calibration curve (R^2)[b]	LOQ (ng/mL)	Samples above LOQ[c]	Range (ng/mL)
carboxylates				
PFHxA*	0.9994–0.9998	0.30	–	ND
PFHpA*	0.9995–0.9998	0.20	–	ND
PFOA*	0.9991–0.9995	0.20	12/12	0.21–1.4
PFNA*	0.9997–0.9999	0.15	8/12	LOQ - 0.86
PFDA*	0.9995–0.9998	0.15	1/12	LOQ - 0.52
PFUnDA*	0.9997–0.9999	0.20	1/12	LOQ - 0.63
PFDoDA*	0.9992–0.9995	0.30	–	ND
PFTrDA	0.9991–0.9994	0.30	–	ND
PFTeDA	0.9989–0.9992	0.50	–	ND
sulfonates				
PFHxS*	0.9995–0.9998	0.20	9/12	LOQ–0.72
PFHpS	0.9996–0.9999	0.20	–	ND
PFOS*	0.9980–0.9984	0.20	12/12	0.98–6.1

*Corresponding isotope labelled internal standard was used in analytical method.
ND, not detected in any of the blood samples.
[a] Acronyms are based on [1].
[b] Values are range of the results from triplicate analysis.
[c] 0 samples is marked as "-".

LC-MS/MS measurement and quantification of perfluoroalkyl acids

Twelve PFAAs (see Table 1) were analysed using liquid chromatography negative ion electrospray tandem mass spectrometry. A 10 µL injection of the extract was separated on a 30 mm × 2.1 mm, 3.5 µm Waters XBridge C18 column. To eliminate possible contamination of PFAAs from the inner LC parts, a Waters XBridge C18 trap column (50 mm × 2.1 mm, 5 µm) was installed between the pump and the injector switching valve. Methanol (10%) in water with 0.001% N-methylpiperidine was used as eluent A and 100% methanol with 0.01% N-methylpiperidine as eluent B. Chromatograms were recorded by selected reaction monitoring (SRM) with a specific transition per analyte. The details of LC-MS/MS parameters have been presented earlier [25]. For quantitation a matrix-matched calibration curve with concentrations range from 0.15 to 20 ng/mL ($R^2 \geq 0.998$ for each compound, Table 1) was used. Chromatographic peak integration was undertaken with the help of the software Xcalibur, and the final analyte concentrations were calculated in Microsoft Excel. PFAA levels in the blank samples, which were treated in the same way as the blood samples, were below the LOQ, therefore a blank subtraction from the sample concentration had no effect on the results. Monitoring a recovery of internal standards was considered unnecessary. Current pretreatment method uses only one simple extraction step without any steps that may affect the recovery (concentration, purification, precipitation etc.).

Method descriptives

For the method, a linearity was determined with matrix-matched standards in the concentration ranges from 0.15 ng/mL to 20 ng/mL. Preparation of the matrix-matched calibration samples is described above in Section 2.4. The calibration samples with the same analyte concentrations were prepared separately for each sample batch (n = 3). Linearity was plotted as relative peak areas (analyte/internal standard)