DEVELOPMENT OF A METHOD FOR THE ANALYSIS OF PERFLUOROALKYLATED COMPOUNDS IN WHOLE BLOOD

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Introduction

The commercialisation of interfaced high performance liquid chromatography-mass spectrometry (HPLC-MS) facilitated selective and sensitive analysis of perfluoroalkylated (PFA) acids, a group of compounds frequently used for example as industrial surfactants and which are very persistent and biologically active, in a more convenient way than before. Since then a number of reports on PFA compounds found in humans and wildlife have been published (1,2,3,4,5). The most used technique for the analysis of perfluoroalkylated compounds has been ion-pair extraction followed by high performance liquid chromatography (HPLC) and negative electrospray tandem mass spectrometry (MS/MS). Tetrabutylammonium ion as the counter ion in the ion-pair extraction has been used together with GC-analysis (6), LC-fluorescence (7) and LC-MS/MS (8). Recently, solid phase extraction (SPE) has been used instead of ion-pair extraction for the extraction of human serum (9). Previously reported studies on human exposure have mainly been on serum, probably because there are indications that PFA acids bind to plasma proteins (10,11). We here present a fast and simple method that involves SPE and which is suitable for extracting whole blood samples. Further more, 13 PFAs (listed in *Table 1*) were included in the method, which uses HPLC and single quadropol mass spectrometry.

Materials and methods

In order to achieve a robust method, which completely extracts perfluoroalkylated compounds from human whole blood, denaturation of plasma proteins was introduced prior to extraction with SPE and final determination using HPLC interfaced to a single quadropol mass spectrometer. In addition, to avoid clogging the SPE sorbent, precipitation of the red blood cells was desirable. Three potential internal standards were also evaluated; perfluoroheptanoic acid (PFHpA),

1H,1H,2H,2H-perfluorooctane sulfonic acid (THPFOS) and 7H-perfluoroheptanoic acid. Three different denaturating agents (acetonitrile, trichloroacetic acid (TCA), formic acid) were evaluated, as well as different types of SPE sorbent materials.

Sample preparation. The frozen blood or plasma sample was allowed to thaw in room temperature and 0.75 ml was then transferred to a 15 ml polypropylene centrifuge tube, pre- washed with methanol (HPLC-grade, Labscan). An aliquot of 7.5 µl internal standard solution was added. After thorough mixing, the denaturation agent was added and the solution was sonicated for 15 minutes and centrifugated at 10000 x g for 30 minutes. The solution was extracted on a solid phase extraction column, preconditioned with methanol and water (ultra pure, laboratory produced). After washing the column with 40 v/v% methanol in water the column was put under vacuum suction until dryness. Elution was performed with 0.5 ml methanol. All solutions including the sample solution were allowed to run through the column material without vacuum. The extract was filtered through 0.2 µm polypropylene filter into a polypropylene vial.

Separation and quantification. The extracts were injected into an Agilent 1100 LC system with a Supelco Discovery HS C18 (50*2.1 mm, 3µm) column (Sigma-Aldrich) and a guard column of the same material. The instrument was equipped with a thermostated column department that was kept at 40°C. A water/methanol mobile phase gradient with 2mM ammonium acetate (99%, pa for HPLC, Fluka) was delivered with a flow rate of 0.3 ml/min. The gradient started at 35% methanol followed by a 20 min ramp to 90% methanol, a 10 min hold followed by a 10 min washing sequence with 100% methanol, and then reverting to initial conditions allowing 7 min stabilisation time. Detection was performed with an Agilent 1100 MSD mass spectrometer equipped with an atmospheric electrospray interface operating at negative ion mode with the following settings: nebulizer gas temperature 350°C, nebulizer gas pressure 20 psi, drying gas flow 13 ml/min and capillary voltage 3500V. Single ion monitoring was used measuring the [M-H] ion for sulfonic acids and [M-COOH] ion for carboxylic acids. Quantification was performed using relative response factors calculated from external calibration curves.

Results and discussion

Internal Standard: The internal standard (IS) approach was preferred over standard addition since our evaluation showed that the relative response factor for target analytes were equal with and without extraction with blood matrix as shown for PFOS in *Figure 1*. The relative response factors calculated using 7HPFHpA as

IS were higher in extracted whole blood compared with unextracted methanol while the corresponding response factors for THPFOS and PFHpA were the same. THPFOS and PFHpA were both used as IS for the quantification of a larger number of blood samples. THPFOS had the disadvantage to be a contaminant in some brands of PTFE septa for vial seals. Furthermore, the reproducibility of concentrations calculated with THPFOS was poorer than for PFHpA. A number of samples were analysed without PFHpA and THPFOS. No THPFOS could be detected but minor concentrations of PFHpA were found, lower than 0.5 % of the concentration PFHpA added as an internal standard.

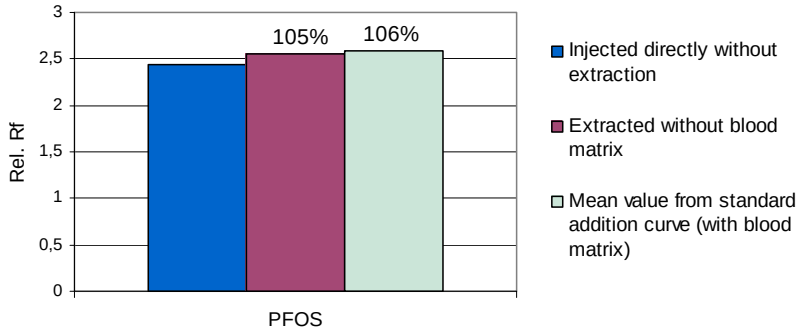

Figure 1. No matrix effect could be demonstrated using relative response factor and PFHpA as IS. Relative response factors of PFOS injected in methanol (unextracted) and extracted without and with whole blood matrix can be seen in the figure. Values above bars are % of the spiked v.s. the unextracted PFOS relative response factor.

Denaturation agent. Three different chemicals were evaluated for the denaturation of plasma proteins, acetonitrile , TCA and formic acid. Acetonitrile and TCA both effectively in precipitated red blood cells but at the cost of lower recovery compared to formic acid. Formic acid was found suitable for whole blood preparation, providing acceptable precipitation and good recovery (*Table 1*).

SPE sorbent material. In order to retain the PFAs, a nonpolar interaction between the PFAs and the sorbent functional group was used. A number of silica based apolar sorbents of different strength together with some moderate to weak apolar sorbents and 2 polymeric apolar sorbents, all from the same manufacturer, were studied. Presented in figure 2 are recoveries of 13 PFA compounds extracted on different sorbent materials. The polymeric sorbents (ENV, PPL) and some of the weak and moderately apolar sorbents (C18OH, CN) show low recoveris of PFAs. The carbon chain length (C2 or C18) of the sorbent functional group did not result in much different recovery except for the shorter PFAs (C4-C7). Three differerent C18 sorbents gave different results, for whole blood a C18 with 120 µm particle

size (C18 HF) showed better recovery compared to 40 µm (C18) while a C18 with 40 µm particle size and 500Å (C18 EWP) was not suitable at all.

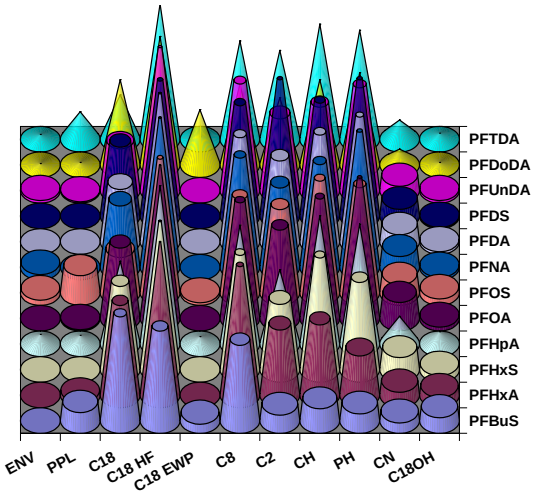

Figure 2. Recoveries of 12 PFA compounds extracted with 11 different SPE sorbents. The x-axis describes the different sorbent functional groups, except for ENV and PPL that are polymeric sorbents (styrene divinylbenzene).

Method performance. Formic acid, C18 HF and PFHpA as an internal standard were chosen for the final method. Recovery with triplicate determinations at three levels together with LOD and LOQ in human whole blood from Sweden can be seen in table 1. Recovery determination was performed by standard addition to a whole blood sample from Sweden, LOD is defined as the signal to noise ratio of 3 and quantified against external calibration curves. The LOQ was determined in similar way, but defined as the concentration at which repeated injections (n=6) results in RSD ≤ 20% but with a minimum signal to noise ratio of 5. The overall performance of the method was excellent with high recoveries and repeatability and low detection limits. The only exception is for PFBuS for which the method is clearly not fully optimized.

Acknowledgment
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Table 1. Recoveries, LOD and LOQ of 12 PFA compounds in whole blood with SPE and LC/ES-MS.

	Recovery % 1-8 pg/µl	Recovery % 5-13 pg/µl	Recovery % 10-27 pg/µl	LOD pg/µl	LOQ pg/µl
PFBuS $C_4F_9SO_3^-$	26±3	27±2	27±1	2	3
PFHxA $C_5F_{11}COO^-$	98±24	90±7	92±1	0.3	0.5
PFHxS $C_6F_{13}SO_3^-$	112±5	110±0.5	108±2	0.1	0.3
PFOA $C_7F_{15}COO^-$	79±5	97±2	96±1	0.5	0.8
PFOS $C_8F_{17}SO_3^-$	99±3	98±4	103±7	0.1	0.4
PFNA $C_8F_{17}COO^-$	81±10	84±4	90±4	0.1	0.3
PFDA $C_9F_{19}COO^-$	75±1	80±7	85±2	0.1	0.3
PFDS $C_{10}F_{21}SO_3^-$	69±5	83±6	87±11	0.5	0.8
PFOSA $C_8F_{17}SO_2NH_2$	81±10	na	na	0.1	0.3
PFUnDA $C_{10}F_{21}COO^-$	82±6	79±7	84±7	0.1	0.3
PFDoDA $C_{11}F_{23}COO^-$	64±14	na	na	0.3	0.9
PFTDA $C_{13}F_{27}COO^-$	75±24	na	na	0.1	0.5

n.a.= not analysed

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