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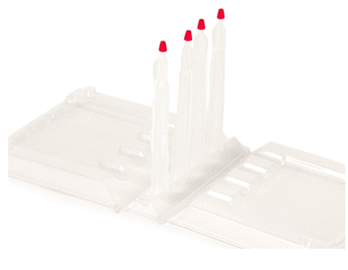
Novel Microsampling Technique with Mitra in Analysis of Perfluoroalkyl Acids in Blood

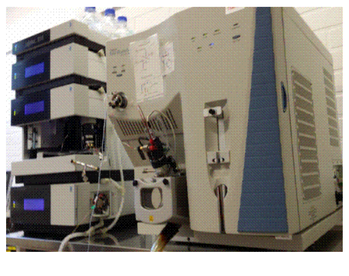
INTRODUCTION

Microsampling is an attractive option for significantly reducing the volume of blood taken for analysis allowing for blood samples taken as a 'finger-prick' with a lancet. A novel, patent-pending microsampling device Mitra(TM) reproducibly collects a small volume (10 µL) of whole blood in a haematocrit-independent manner. This technique overcomes the major challenge and limitation of conventional dried blood spot cards that have historically been used for microsampling. Perfluoroalkyl acids (PFAAs) are persistent in the environment, bioaccumulative, and capable of interfering with biological systems at different levels. One of the known detrimental effects of PFAAs is that they are able to modulate the functions of the human immune system[1-4]. In addition, PFAAs have been associated with elevated total and LDL cholesterol, increased breast cancer risk and disruption of thyroid hormones[5-9]. In this study the Mitra microsampler has been evaluated in an environmental monitoring setting, and has been utilized for analysis of PFAAs.

METHODOLOGY

Microsampling with Mitra
(10 µL whole blood)
↓
Extraction with aqueous methanol
↓
Sonication
↓
Centrifugation
↓
LC-MS/MS analysis[10]





RESULTS AND DISCUSSION

Table 1. Analysis of human blood samples (n=12)

analyte	samples above the LOQ	concentration in blood (ng/mL)	repeatibility of the method (RSD%)*
PFOS	12/12	0.99 – 6.1	1.6 – 11
PFOA	12/12	0.21 – 1.4	1.4 – 9.4
PFHxS	9/12	0.26 – 0.73	0.7 – 9.6
PFNA	7/12	0.21 – 0.86	7.1 – 12
PFDA	1/12	0.50	5.6
PFUnA	1/12	0.58	6.4

* Data obtained from triplicate samples

PFAA concentrations in human blood samples (8 male, 4 female) are shown in Table 1. Age of the study subjects varied from 3 to 63 years. Limit of quantitations (LOQs) for individual PFAAs in blood were from 0.2 to 0.5 ng/mL. Besides the main compounds PFOS and PFOA four other PFAAs were abled to quantify in human blood. The method showed a good repeatibility, and is sufficiently sensitive for samples from general populations.

Table 2. Analysis of in-house control serum sample (n=5)

analyte	calibration curve linearity (R)	accuracy (%)	repeatibility of the method (RSD%)
PFOS	0.998	103 ± 7	6.6
PFOA	0.999	98 ± 3	3.2
PFHxS	0.999	97 ± 3	3.1
PFHxA	0.999	103 ± 2	2.3
PFHpA	0.999	101 ± 5	5.1
PFNA	0.999	101 ± 4	4.0
PFDA	0.999	93 ± 5	5.6
PFUnA	0.999	101 ± 3	2.8
PFDoA	0.999	100 ± 2	6.6

Table 3. Analysis of interlaboratory* serum sample (n=3)

analyte	analyzed concentration (ng/mL)	acceptable range (ng/mL)
PFOS	6.3	2.2 – 12
PFOA	29	18 – 35
PFHxS	5.9	3.2 – 8.0
PFHxA	0.71	0.39 – 0.91
PFNA	3.1	1.9 – 4.6
PFUnA	1.5	0.98 – 2.3

* AMAP Ring Test for Persistant Organic Pollutants in Human Serum

Linearity, accuracy and repeatibility of the method was determined with in-house control serum sample. As shown in Table 2 all the above parameters were at level of excellence. To evaluate the method reproducibility AMAP interlaboratory serum sample was analysed. All the analysed PFAA concentrations were highly acceptable (Table 3). The accuracy of PFAAs from AMAP sample was in the range of 88 – 110% of the assigned values.

As well as allowing for a quick and efficient extraction flow path, Mitra microsampler is easy to use device in a real-world sample collection scenario. Furthermore, Mitra is a valid tool for studies where only a limited volume of sample can be allocated for the analysis.

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