A SIMPLE AND SENSITIVE METHOD FOR DETERMINATION OF A WIDE RANGE OF POLYFLUORINATED COMPOUNDS IN SERUM

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Introduction

Several polyfluorinated compounds (PFCs) are presently known to be ubiquitous environmental pollutants[1]. Perfluorooctanesulfonate serum concentrations can be used as a marker for total exposure of PFOS in biomonitoring studies[2]. Methods used for determinations of PFCs have been reviewed by de Voogt and Saez[3], Van Leeuwen and de Boer[4] and Villagrasa et al[5]. LC-MS is presently suggested to be the state-of-the-art method for determination of ionic PFCs[3]. Several different LC-MS and LC-MS/MS methods have been published including methods using both atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI)[5]. PFCs have commonly been extracted from the serum by liquid-liquid partioning using an ion-pairing agent or solid phase extraction off-line[6]. In recent years, a few column switching LC methods have been presented[7-9]. Important advantages with these methods are reduced manual handling, low sample volumes required, as well as absence of evaporation steps, leading to sensitive, inexpensive and reliable methods with minimal contamination problems.

The aim of this study was to develop and validate a simple, fast, sensitive and reliable method for the determination of a broad range of PFCs in serum, by modifying a standard LC-MS/MS instrument for column switching.

Materials and methods

Chemicals

An overview of the analytes and internal standards with their abbreviations, are given in Table 1. All standard solutions were purchased from Wellington Laboratories (Guelph, Ontario, Canada). Methanol and acetonitrile (HPLC grade) were obtained from LabScan (Dublin, Ireland). 2-propanol (HPLC grade) was purchased from Alfa Aesar (Karlsruhe, Germany). Ammonium hydroxide (puriss PA grade) was from Fluka (Steinheim, Germany). Formic acid (GPR Rectapur grade) was obtained form VWR BDH Prolabo (Fontenay-sous-Bois, France) and acetic acid (PA grade) was purchased from Merck (Darmstadt, Germany). Argon of purity 99.9999% was obtained from AGA (Oslo, Norway) and nitrogen gas was supplied by a Maxigas Nitrogen generator obtained form Dominick Hunter Ltd. (Gateshead, UK). Type I water was supplied from a TKA GenPure water purification system (TKA Water Purification Systems GmbH, Niederelbert, Germany). For method validation, serum from newborn calves from Invitrogen (Oslo, Norway) was used.

Preparation of calibration solutions and samples

The calibration solutions were prepared in serum from newborn calves as follows: 150 µl serum was transferred to a 2 ml centrifugation tube, added 30 µl internal standard, 30-120 µl of analytes and 0-90 µl of methanol to make up a total volume of 150 µl metanol, for precipitation of proteins, and then mixed using a whirl mixer. The calibration solutions covered a concentration range from 0.05 to 75 ng PFC/ml serum for all analytes. For preparation of unknown samples 150 µl serum was pipetted to a 2ml centrifugation tube and added 30 µl (600 pg) internal standard, 120 µl methanol and then mixed using a whirl mixer. Both calibration solutions and unknown samples were then centrifugated at 10,000 rpm for 15 minutes. The supernatant, approximately 200 µl, was transferred to a glass autosampler vial, added 500 µl 0.1 M formic acid and mixed on a whirl mixer. The extractes of a total volume of about 700 µl were then ready for injection into the column switching LC-MS/MS system.

Table 1. List of analytes and internal standards, their abbreviations, mass transissions and fragmentation voltages as well as estimated LOD/LOQ.

Compound	Abbreviation	Precursor ion/ product ion m/z	Fragmentation voltage V	LOD/LOQ ng/ml serum
Perfluorobutyric acid	PFBA	213/169	8	0.003 / 0.008
Perfluoropentanoic acid	PFPeA	263/219	8	0.008 / 0.03
Perfluorohexanoix acid	PFHxA	313/269	8	0.007 / 0.02
Perfluoroheptanoic acid	PFHpA	363/319	8	0.009 / 0.03
Perfluorooctanoic acid	PFOA	413/369	8	0.006 / 0.02
Perfluorononanoic acid	PFNA	463/419	8	0.004 / 0.01
Perfluorodecanoic acid	PFDA	513/469	10	0.002 / 0.005
Perfluoroundecanoic acid	PFUnDA	563/519	8	0.006 / 0.02
Perfluorododecanoic acid	PFDoDA	613/569	10	0.007 / 0.02
Perfluorotridecanoic acid	PFTrDA	663/619	15	0.004 / 0.01
Perfluorotetradecanoic acid	PFTeDA	713/669	15	0.05 / 0.2
Perfluorobutane sulfonic acid*	PFBS	299/299	20	0.009 / 0.03
Perfluoro hexane sulfonic acid*	PFHxS	399/399	20	0.007 / 0.02
Perfluoro heptane sulfonic acid*	PFHpS	449/449	20	0.01 / 0.04
Perfluoro octane sulfonic acid*	PFOS	499/499	30	0.003 / 0.009
Perfluoro decane sulfonic acid*	PFDS	599/599	30	0.05 / 0.2
Perfluorooctane sulfonamide*	PFOSA	498/498	20	0.02 / 0.05
N-methylperfluorooctane sulfonamide*	N-MeFOSA	512/512	15	0.009 / 0.03
N-ethylperfluorooctane sulfonamide*	N-EtFOSA	526/526	15	0.009 / 0.03
Perfluoro-n-[1,2,3,4-13C4]butanoic acid	MPFBA	217/172	8	
Perfluoro-n-[1,2-13C2]hexanoic acid	MPFHxA	315/270	8	
Perfluoro-n-[1,2,3,4-13C4]octanoic acid	MPFOA	417/372	8	
Perfluoro-n-[1,2,3,4,5-13C5]nonanoic acid	MPFNA	468/423	8	
Perfluoro-n-[1,2-13C2]decanoic acid	MPFDA	515/470	8	
Perfluoro-n-[1,2-13C2]dodecanoic acid	MPFDoDA	615/570	8	
Sodium perfluoro-1-hexane [$^{18}O_2$] sulfonate*	MPFHxS	403/403	15	
Sodium perfluoro-1-[1,2,3,4-13C4]octanesulfonate*	MPFOS	503/503	15	
n-methyl-d3-perfluoro-1-octanesulfonamide*	d-N-MeFOSA	515/515	10	
n-ethyl-d5-perfluoro-1-octanesulfonamide*	d-N-EtFOSA	531/531	10	

For analytes marked with an astrix (*) the pseudo-MRM approach was used.

Instrumentation and determination

The column switching LC system consisted of a HTC PAL autosampler (CTC analysis AG, Zwingen, Switzerland) with one six port switching valve and one ten port switching valve attached (CTC), and two Surveyor LC pumps (Thermo Finnigan, San Jose, CA, USA). To eliminate possible contamination with PFCs from Teflon parts in the LC pumps a Hypercarb (10 mm x 4 mm x 5 µm particles, Thermo Finnigan) guard column was installed between each of the LC pumps and the switching valves. A Betasil C8 (10 mm x 4 mm x 5 µm particles, Thermo Finnigan) was used as SPE column and a Betasil C8 (50 mm x 2.1 mm x 3 µm particles, Thermo Finnigan) as analytical column. As detector a TSQ Quantum (ThermoFinnigan) triple quadrupole mass spectrometer was used.

Analyses were performed by injecting a 400 µl aliquote of the sample into a loop of the six port valve. The sample was transferred to the SPE column by 0.1 M formic acid in water, where the analytes were retained. The pH of the SPE column was increased using 0.2% NH_4OH in water. The analytes were then eluted from the SPE column by backflushing onto the analytical column using a gradient of methanol, acetonitrile and water. The mass spectrometer was operated in negative ESI mode with a spray voltage of 3 kV and a skimmer offset of 5 V. A capillary temperature of 300 ºC, sheat gas pressure of 60 arbitary units (AU) and an auxillary gas pressure of 5

AU was used. The argon gas pressure was set to 0.2 Pa. The optimum dwell time was 0.05 s. An extensive wash of the LC system to avoid carry over of analytes and to prevent increasing back pressure was done by applying different combinations of methanol, acetonitrile, water, 0.2% NH_4OH in water and 50% 2-propanol in water. For all perfluorocarboxylic acids multiple reaction monitoring (MRM) was used. For the perfluorosulfonates and the perfluorosulfonamides pseudo MRM (see below) was used. The mass transitions and fragmentation voltages are given in Table 1.

Results and discussion

Method development

Multiple reaction monitoring (MRM) is known to be a very sensitive and selective MS/MS technique. However the response for the perfluorosulfonates and the perfluorosulfonamides was found to be much lower than for the perfluorocarboxylates. This was investigated further by comparing results of MRM with results of selected ion monitoring (SIM). For all perfluorosulfonates (PFBS, PFHxS, PFHpS, PFOS and PFDS), as well as the sulfonamides PFOSA, MeFOSA and EtFOSA, the response was high using SIM, but low using MRM, probably because of limited fragmentation. However, application of the pseudo MRM approach resulted in considerably increased response and was chosen as detection mode for these compounds. In this technique, the precursor ion is measured after application of a fragmentation voltage leading to fragmentation of interfering species but not the analyte, resulting in less background noise than SIM for the perfluorosulfonates and perfluorosulfonamides.

Sensitivity and linearity

The limit of detection (LOD, s/n=3) and limit of quantification (LOQ, s/n=10)) of the method was estimated by extrapolation of the result obtained by injecting matrix-matched calibration solutions (Table 1). The LODs were in the range 0.002 to 0.05 ng PFC/ml serum which is considerably lower than reported by others for similar methods for PFCs in serum, e.g., Kuklenyik et al[8] (0.05-0.8 ng PFC/ml serum) and Kärrman et al[10] (0.1-2 ng PFC/ml serum). To investigate the linearity of the method, calibration solutions were prepared in the concentration range of 0.05 to 50 ng/ml (n=35). Linear calibration curves were established using weighting (1/concentration). High linearity with correlation coefficients in the range 0.993-0.999 was found, however for EtFOSA, PfteDA, MeFOSA, PFTrDa, PFDS, PFDoDA, PFUnDA, PFOS, PFDA quadratic curve fit resulted in more accurate results and has been used for these compounds (figure 1).

Repeatability and accuracy

The repeatability and accuracy of the method was examined in the range of 0.05 to 50 ng PFC/ml serum using serum of newborn calves spiked at six different levels (n=3).The repeatability given as RSD of determined concentrations were within 10% in the range 1 to 50 ng PFC/ml serum. For the two lowest spiking levels the RSDs were higher, but were still considered satisfactory as these concentrations are close to LOQ. The accuracy of all analytes were between 85 and 121% in the range 0.2 to 50 ng PFC/ml serum, and most results were within 90-110%, excluding the results for PFDS at 0.2 ng/ml serum (Figure 1). At 0.05 ng/ml serum the accuracy was more variable, but acceptable this close to the LOQ.

Application

To investigate the applicability of the validated method ten samples of serum from persons with a high intake of Norwegian freshwater fish have been analysed. In addition to the PFCs most commonly reported in the literature (PFHxS, PFOS, PFOA, PFNA, PFDA, PFUnDA, PFDoDA and PFOSA), traces of PFHpA, PFTrDA, PFBS and PFHpS were detected. One sample contained PFTeDA as well. This demonstrates the method's high sensitivity and ability to detect a broad range of analytes which might be of high importance, e.g., when investigating routes of exposure and sources.

To summarize, the presented method is fast and simple and requires only 150 µl serum, thus making it suitable for large scale epidemiological studies. High sensitivity, accuracy and repeatability were demonstrated in a relatively wide concentration range. The method is easy to set up on standard LC-MS/MS equipment and is thus applicable for a wide range of laboratories.

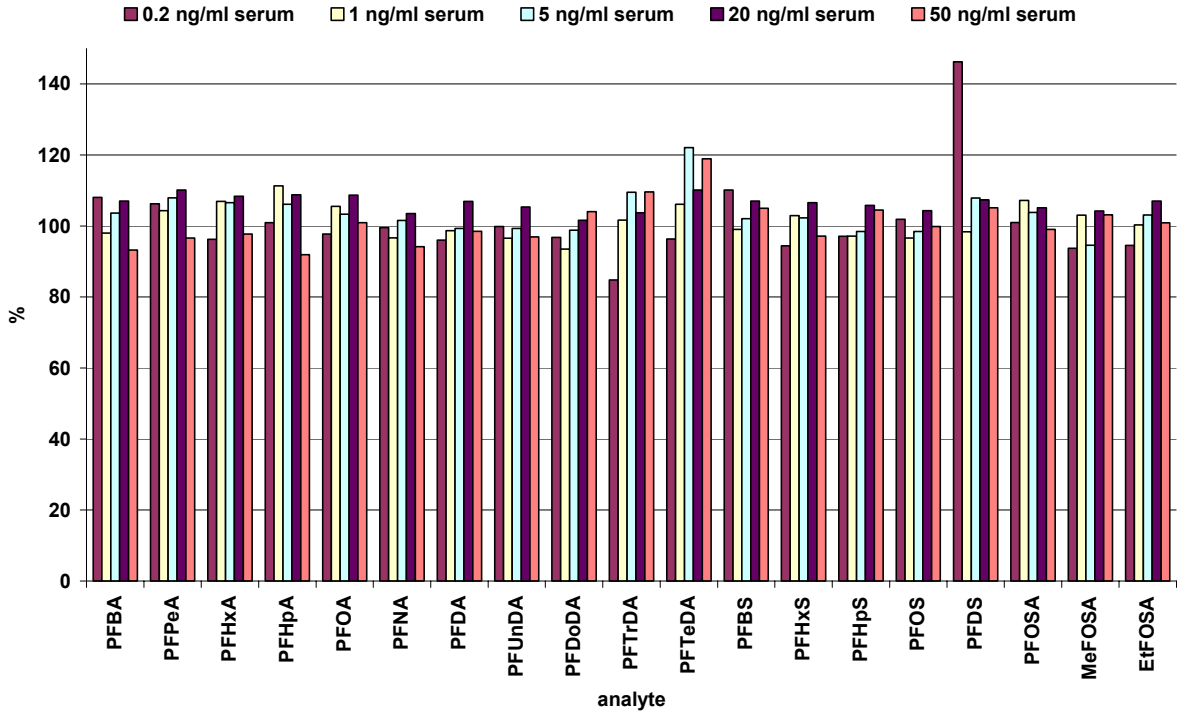

Figure 1. Accuracy at different spiking levels in serum from newborn calves (%).

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References

1. Houde, M., Martin, J. W., Letcher, R. J., Solomon, K. R., Muir, D. C. G. *Environ Sci Technol* 2006; 40: 3463.
2. Butenhoff, J. L., Olsen, G. W., Pfahles-Hutchens, A. *Environ Health Perspect* 2006; 114: 1776.
3. de Voogt, P., Saez, M. *Trends Anal Chem* 2006; 25: 326.
4. van Leeuwen, S. P. J., de Boer, J. *J Chromatogr A* 2007; 1153: 172.
5. Villagrasa, M., de Alda, M. L., Barcelo, D. *Anal Bioanal Chem* 2006; 386: 953.
6. Holm, A., Wilson, S. R., Molander, P., Lundanes, E., Greibrokk, T. *J Sep Sci* 2004; 27: 1071.
7. Kuklenyik, Z., Needham, L. L., Calafat, A. M. *Anal Chem* 2005; 77: 6085.
8. Inoue, K., Okada, F., Ito, R., Kawaguchi, M., Okanouchi, N., Nakazawa, H. *J Chromatogr B* 2004; 810: 49.
9. Karrman, A., van Bavel, B., Jarnberg, U., Hardell, L., Lindstrom, G. *Anal Chem* 2005; 77: 864.