Separation and determination of perfluorinated carboxylic acids using liquid chromatography with fluorescence detection

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INTRODUCTION

Perfluorinated carboxylic acids (PFCAs) constitute an important group of anthropogenic perfluoroalkyl substances that have been detected in the environment and in various organisms including humans. Widespread presence of PFCAs in the environment requires reliable and possibly inexpensive methods for their determination.

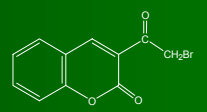
The most commonly used methods for determination of PFCAs are based on high-performance liquid-chromatography (HPLC) combined with mass spectrometry (MS) detection, employed both for environmental and biological samples. In spite of outstanding selectivity and sensitivity, these methods require expensive MS instrumentation with skilled operators, suffer from blank problems; often additional sample clean-up and/or preconcentration of analytes is necessary. Recently, we have developed methods based on capillary electrophoresis separations with either direct or indirect UV detection, exhibiting good selectivity for PFCA analysis ranging from C6 to C12 [1]; also CE chips have been used for determination of PFCAs with conductivity detection [2].

This work was focused on optimization of reversed-phase-HPLC method for determination of PFCAs ranging from C2 to C12 carbon atoms in the PFCA molecule, using fluorimetric detection. As the PFCAs do not exhibit native fluorescence, they have been derivatized with commercially available fluorogenic reagent 3-(bromoacetyl)cumarin (BAC), compared to earlier work with fluorophore synthesized in laboratory for that purpose [3]. Several factors affecting yield of derivatization such as concentration of BAC, temperature, time of reaction, and effect of different solvents have been optimized.

INSTRUMENTATION

The chromatographic measurements were carried out with Merck HPLC setup that consisted of a gradient pump model L-7100, a fluorescence detector model L-7485, a column thermostat L-7350, an autosampler L-7250, system controller D-7000 and PC operating with software D-7000. Separations were carried out using ODS column: Luna 150 x 4.6 mm, 4 µm (Phenomenex, Torrance, CA, USA).

Structure of fluorophore used



3-bromoacetylcoumarin (BAC)

Procedure of derivatization

To 1 ml of analyte solution in acetonitrile added
1 ml of 2 g L^{-1} of fluorophore BAC solution in acetone

⇓

20 min incubation at 70 °C

⇓

Cooling to room temperature

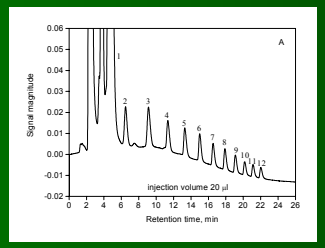
⇓

Injection into HPLC setup

HPLC PROCEDURE

The gradient elution was employed in optimized conditions at flow-rate of 1 ml/min. Gradient program: A - 60% ACN in water, B - ACN. 0 - 6 min: 100% A; 6 - 17 min 85% B, 17- 22 min 95% B, 22 - 26 min 100% B. Detection was carried out at excitation wavelength 306 nm and emission wavelength 419 nm.

Example chromatograms



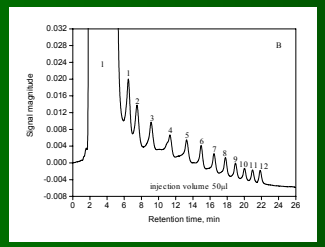


Fig.5. Chromatograms obtained for mixture of PFCAs standards at concentration of 2.5 ppm (A) and 100 ppb (B) each derivatized with BAC at optimized conditions.
1 – excess of BAC, 2 to 12 – signals for PFCAs with number of carbon atoms in PFCA molecule corresponding to peak numbering (e.g. 8 = C8-PFCA or PFOA).

Figures of merit of developed method

Calibration based on peak area

Analyte	Relative sensitivity (%) compared to that for C8-PFCA	Correlation coefficient[a)], r^2
C2-PFCA	123	0.9977
C3-PFCA	123	0.9984
C4-PFCA	113	0.9996
C5-PFCA	110	0.9966
C6-PFCA	106	0.9990
C7-PFCA	103	0.9987
C8-PFCA	**100**	0.9990
C9-PFCA	90	0.9993
C10-PFCA	72	0.9993
C11-PFCA	65	0.9995
C12-PFCA	54	0.9990

[a)] Calibration curves obtained in the 25 – 250 ppb range

Efficiency of separation and limits of detection

Analyte	Number of theoretical plates	Limit of detection (S/N=3)*, ppb for V_s= 50 µl
C2-PFCA	2700	0.69
C3-PFCA	3700	1.1
C4-PFCA	3300	1.4
C5-PFCA	4400	1.3
C6-PFCA	7400	1.4
C7-PFCA	9000	1.5
C8-PFCA	10400	1.5
C9-PFCA	11800	1.6
C10-PFCA	25800	1.8
C11-PFCA	28250	1.8
C12-PFCA	15700	2.3

*) S – signal magnitude taken as peak height
N – amplitude of base-line noise

Optimization of spectral conditions

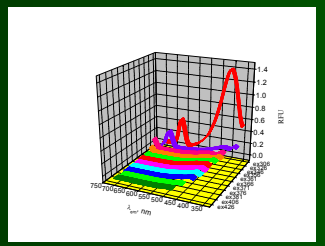


Fig.1. Emission spectrum recorded for 0.5 ppm BAC fluorophore at different excitation wavelengths.

Effect of temperature and time

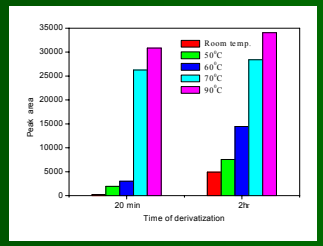


Fig.2. Effect of temperature and time of derivatization on yield of derivatization of C8-PFCA with BAC.

Effect of fluorophore concentration

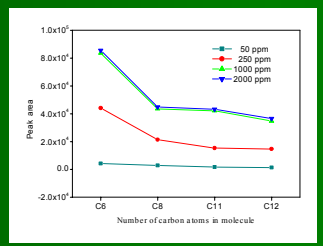
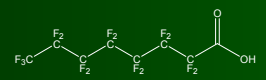
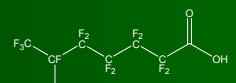


Fig.3. Effect of fluorophore BAC concentration used in reaction mixture on signal magnitude for derivatization of PFCAs at PFCAs concentration of 2 ppm.

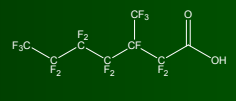
Determination of isomers



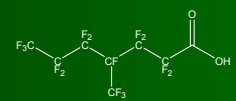
Perfluorooctanoic acid



6 – (trifluoromethyl) perfluoroheptanoic acid



3 – (trifluoromethyl) perfluoroheptanoic acid



4 – (trifluoromethyl) perfluoroheptanoic acid

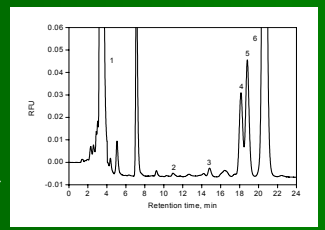


Fig. 4. Chromatogram of C8-PFCA isomers mixture obtained with fluorimetric detection after derivatization using 2000 ppm BAC in the reaction mixture and reaction time 20 min. Peak assignment: 1 – BAC, 2 - C6-PFCA, 3 – C7-PFCA, 4 – 4-(trifluoromethyl)-perfluoroheptanoic acid, 5 – 3-(trifluoromethyl)perfluoroheptanoic acid and 6-(trifluoromethyl)perfluoroheptanoic acid,
6 – C8-PFCA

RESULTS AND CONCLUSIONS

The main concept and aim of this work was to develop a method which can be, to some extent, an alternative to commonly used LC/MS/MS methods for determination of PFCAs, a method that would be both simpler instrumentally and more accessible to a wide scope of users, *e.g.* average environmental and water quality control laboratories.

In comparison to a similar earlier work [3], instead of laboratory synthesized fluorophore, a commercially available reagent (BAC) was employed. In spite of a very small differences in the structure of the fluorophore the maxium intensity of fluorescence was observed at a 60 nm shorter excitation wavelength that for the fluorophore employed earlier.

The essence for this work is the optimization of derivatization of analytes with BAC. As it is shown in Fig. 2 for C8-PFCA after 20 min derivatization at 90˚C already 92% of yield of derivatization was observed, compared to a 2hr process. In practice, 70°C is rather recommended to avoid products of hydrolysis of fluorophore itself and this temperature was employed with 20 min derivatization time. Fig. 3 illustrates that 2 g/l of BAC used in derivatization reaction is sufficient.

In order to obtain a complete separation of C2 to C12 PFCA in a single run, a gradient elution with ACN-Water and ACN is recommended (see HPLC procedure). The optimization of derivatization reaction was carried out in acetonitrile, as it is preferable solvent for elution of analytes retained on SPE columns for preconcentration and/or cleanup.

The advantage of developed procedure is also relatively small difference between response factors obtained for various PFCAs. Relative sensitivities compared to that of C8-PFCA (assumed as 100%) were in the range from 54 % for C12-PFCA to 123 % for the C2-PFCA.

Limits of detection (LOD) primarily depend on the injected sample volume and for a 50µl injections are in the range from 0.69 to 2.3 ppb. Further improvement of the LODs that will address the determination of PFCAs at environmental levels will be achieved by appropriate analyte concentration using solid-phase extraction. As it is shown in Fig. 4. the developed method is also capable of satisfactory chromatographic resolution of C8-PFCA isomers at optimized chromatographic conditions.

References

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2. M. Masár, L. Wójcik, D. Kaniansky, M. Trojanowicz, Zone electrophoresis separation of perfluorocarboxylic acids on a chip with conductivity detection, *J. Sep. Sci.*, In press.
3. T. Ohya, N. Kudo, E. Suzuki, Y. Kawashima, Determination of perfluorinated carboxylic acids in biological samples by high-performance liquid chromatography, *J. Chromatogr. B*, 720 (1998) 1-7.