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Development of a procedure for the determination of perfluorocarboxylic acids in sediments by pressurised fluid extraction, headspace solid-phase microextraction followed by gas chromatographic–mass spectrometric determination

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Abstract

A procedure for the determination of perfluorocarboxylic acids (i.e. $PFC_{7-10}A$) in sediment by pressurized fluid extraction (PFE), derivatization, headspace solid-phase microextraction and GC–MS determination in the negative ion chemical ionisation mode was developed. The PFE extraction variables such as solvent composition, number and time per extraction cycle, and extraction temperature were optimised. In the optimum extraction conditions, recoveries exceeding 95% with a limit of detection and RSDs of 0.5–0.8 ng g^{-1} and 15.5–16.8%, respectively, were obtained. The developed analytical procedure was applied to harbour sediments where PFC_8A and $PFC_{10}A$ were detected for the first time at low ppb concentrations (i.e. 8–11 ng g^{-1}).

Keywords: Sediment; PFCA; PFE; Derivatization; HS-SPME; GC–NCI-MS

1. Introduction

Perfluorinated organic compounds have unique physical, chemical, and biological properties, closely related to their high-energy carbon fluorine bond [1]. Perfluorinated surfactants belong to the class of perfluorinated organic compounds which have arisen awareness because some of them are globally distributed, environmentally persistent, bioaccumulative and potentially harmful [1–4]. Perfluorinated surfactants have been used in different commercial and industrial applications [2–5] as paints, lubricants, PTFE synthesis, polishers, food packaging and fire-fighting foams [6]. Among the anionic perfluorinated surfactants, the perfluorocarboxylic acids (PFCAs) have been detected in a variety of aqueous matrices [7,10–12], biota [7–9] and human blood [13]. PFCAs have been determined in water samples by gas chromatography coupled to mass spectrometry (GC–MS) as alkylester derivatives [10,11] or underivatized by liquid chromatography coupled to tandem mass spectrometry (LC/MS/MS) [7,10,12]. However, very recently, several research teams have stated the analytical challenges involved in the perfluoroalkyl research [14].

The need for analytical methods to determine perfluorinated surfactants in environmental matrices of relevance is highlighted as a requirement for addressing questions about their occurrence, behaviour, and impact of PFCAs in the environment [4,6]. In this regard, despite the clear advantages of solid-phase microextraction (SPME) in sample preparation [15], its application to PFCA determination in environmental matrices is limited to aqueous matrices [11].

In this work, we are focusing on the development of an analytical procedure for the PFCA determination in marine sediments for the first time since they can be a sink for PFCAs [16]. In fact, PFCAs are completely ionised (PFC_8A, $pKa = 0.5$) in seawater and can originate insoluble species in

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Table 1
GC–NCI-MS selected working conditions

Carrier gas	Helium[a]	Reagent gas	Ammonia[b]
Injector temperauture	300 °C	Temperature ion source	175 °C
Oven temperature program	50 °C (2 min) to 250 °C (3 min) at 8 °C min^{-1}	Reagent gas pressure	0.019 Pa
Transfer line temperature	240 °C	Ion repeller voltage	1.0 V
Splitless time	3 min	Scan acquisition	175–750 *m/z*
Solvent delay	11 min	Scans s^{-1}	1.32

[a] Purity 99.999% at 1 mL min^{-1} constant flow.
[b] Purity 99.995%.

presence of cationic species which are constituents of seawater. Therefore, pressurised fluid extraction (PFE) and SPME were chosen as they are faster than conventional extraction techniques and they permit to minimise the use of solvents. Their determination was carried out by GC–MS in the negative ion chemical ionisation (NCI) mode due to its sensitivity and selectivity for the PFCA alkyl ester derivatives [11]. Therefore, the PFE variables such as solvent composition, number of cycles, time per cycle, and extraction temperature were optimised. The developed analytical procedure was applied to the determination of PFCAs in harbour sediments.

2. Experimental

2.1. Chemicals and reagents

The following chemicals were all obtained from Sigma–Aldrich (Steinheim, Germany): tridecafluoroheptanoic (PFC_7A, 99%), pentadecafluorooctanoic (PFC_8A, 96%), nonadecafluorodecanoic ($PFC_{10}A$, 98%) and perfluorododecanoic acids ($PFC_{12}A$, 95%). Boron trifluoride [∼10% (∼1.3 M) in MeOH] was form Fluka (Steinheim, Germany) methanol, dichloromethane (DCM) and acetone Suprasolv were purchased from Merck (Darmstadt, Germany). Saturated NaCl solution was prepared using Milli Q water and sodium chloride obtained from Carlo Erba (Milano, Italy). Stock and working solutions were all stored at 4 °C. Sea sand thin grain QP from Panreac (Barcelona, Spain) was employed as PFE extraction cell filler. It was heated overnight at 400 °C before its employment. Glassware was washed with the detergent not containing PFCAs and then rinsed well with deionised water, MeOH, *n*-hexane and then dried at 80 °C in an oven. Commercially available dimethylpolysiloxane (PDMS 100 μm) fibres and SPME holder were provided by Supelco (Bellefonte, PA, USA).

2.2. Sediment samples

Surface sediment samples ($n = 7$) were collected in several sampling campaigns during 2002 and 2003 with a van Veen grab in different sites of Barcelona commercial harbour and marinas, located in the western Mediterranean (Catalonian coast, NE of Spain). Before the extraction, all samples were freeze-dried, homogenised, sieved through a 120 μm and stored at −20 °C in the darkness. The analytical procedure was optimised using previously tested sediment samples that did not contain PFCAs, and spiked with 10 ng g^{-1} in methanol [17]. Extraction was performed after an equilibration time of the spiked samples overnight.

2.3. Apparatus

An Applied Separations PSE (Allentown, PA, USA) equipped with 11 mL extraction cell was used for the sediment samples extraction. GC–NCI-MS analysis was carried out using an Agilent-6890 Plus GC system coupled to an Agilent 5973N MS system (Palo Alto, CA, USA). The selected column was a ZB-624 column (6% cyanopropylphenyl-94%-dimethylpolysiloxane, 60 m × 0.25 mm × 1.4 μm film thickness) obtained from Phenomenex (Torrence, CA, USA). The instrumental conditions used for the GC–MS determination are summarised in Table 1.

2.4. Extraction procedure

Approximately 3 g of freeze-dried, homogenised and sieved sediment were transferred to the extraction cell and then filled with previous decontaminated sand. The sample was then extracted with a solvent mixture and extracts were collected in 50 mL vessels. Different extraction variables, such as solvent composition, extraction time per cycle, number of static extraction cycles and extraction temperature were optimised. Extracts were dried, first by rotary evaporation at 40 °C and then transferred to a 20 mL headspace vial and dried under a gentle N_2 stream until complete dryness. After the surrogate ($PFC_{12}A$) addition, a precleaned metallic clip, working as a magnetic stirrer, was also added before vials were sealed with a PTFE septum. Although $PFC_{12}A$ might occur in several environmental matrices, it was not identified in the ones analysed in this study. In this way, several sediments were analysed with and without adding $PFC_{12}A$ in order to confirm its absence. $PFC_{12}A$ has been used in this study as internal standard (IS) and also as surrogate for the derivatisation step. The use of a new metallic clip for each extraction avoided the memory effects, which have been observed while using the conventional PTFE based magnetic stirrers. Once it was sealed, a negative pressure was applied into the vial by a gas tight syringe. Then, 1.0 mL of the boron trifluoride derivatising reagent was added by injec-

tion through the septum and the vial was heated at 70 °C for 1 h. After the derivatisation reaction took place, 15 mL of NaCl saturated water was added, also by injection through the septum. Then, the vial was immersed in a thermostated water bath at 30 °C and magnetically stirred at 1100 rpm as described previously [11]. A PDMS fibre was exposed in the headspace mode during 30 min. Once this extraction period was finished, the fibre was immediately inserted into the GC injector at 300 °C during 3 min.

2.5. Quantification

The determination was carried out by GC–MS in the NCI mode using ammonia as reagent gas. Quantitation of PFCAs was based on the sum of the ion currents corresponding to $m/z = [M]^-$, $[M - HF]^-$, $[M - OC_4H_9F]^-$, $[M - O_2C_5H_9F]^-$ and $[M - O_2C_5H_9F_3]$. PFCAs calibration curves were computed as a ratio between the PFCA standard area to $PFC_{12}A$ surrogate. The correlation between PFC concentration was determined by assuming a linear regression with typical r^2 values of 0.992–0.997. Procedural blanks were under the method detection limits.

3. Results and discussion

3.1. Optimisation of the PFE procedure

PFE was selected to extract PFCAs because it has already been successfully applied to the extraction of anionic surfactants from sediment [15] and it is competitive versus conventional solvent extraction techniques in terms of extraction time and solvent consumption leading to similar recoveries. However, because the specific physicochemical properties of the PFCA perfluorinated alkyl chain, the relevant extraction variables such as solvent composition, extraction temperature, number of cycles and extraction time per cycle were optimised (Sections 3.1.1–3.1.3).

3.1.1. Solvent composition optimisation

The extraction strategy was to optimise the solvent polarity aiming to increase the PFCA extraction recovery from sediment and to minimise the coextraction of interferences. Therefore, a variety of medium to polar extracting agents were sequentially evaluated (Fig. 1). Once the composition of the extraction agent was selected, other extraction variables were considered in order to minimise the number of cycles, solvent usage and extraction time (Fig. 2).

First PFE experiments were performed with three extraction cycles of 10 min at 100 °C and 140 bars. With these preselected conditions according to previous publications, several solvents such as hexane, DCM, ethyl acetate, acetone and methanol as well as different binary solvent mixtures were evaluated. Extraction efficiency for all the analytes increased with the solvent polarity. The less polar solvent mixture, hexane/DCM 1:1, yielded to a very poor extraction recovery,

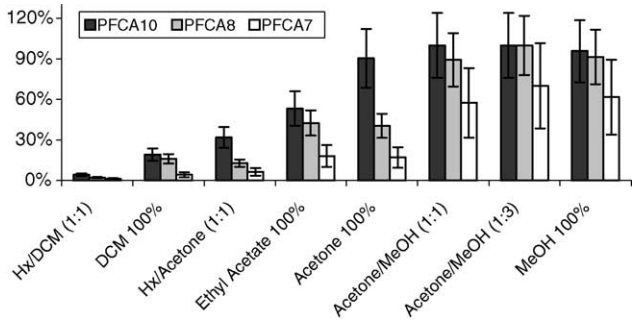

Fig. 1. Optimisation of PFE solvent mixture for the extraction of PFCAs from sediment. Extraction conditions were three cycles of 10 min at 100 °C and 14 MPa.

increasing from 100% DCM, hexane/acetone 1:1 and 100%. ethyl acetate. Extraction with 100% acetone yielded higher $PFC_{10}A$ recovery but no significant increase was obtained for the lower homologues (i.e. PFC_8A and PFC_7A) when ethyl acetate and acetone were used. Finally, the extraction efficiency was clearly enhanced when methanol was used as extractant, especially for PFC_8A and PFC_7A for whose results the recovery was more than twice the recovery obtained by using acetone as extracting solvent. The observed behaviour for the different compounds is in agreement with their polarity; in this way PFC_7A, the most polar compound, showed the lowest recovery. In summary, the higher the methanol content, the better was the recovery. Although the differences in the recoveries obtained with acetone/methanol 1:1, acetone/methanol 1:3 and methanol 100% were not significant, acetone/methanol 1:3 was selected as the most appropriated solvent mixture compromising PFCA extraction efficiency and selectivity from sediments.

3.1.2. Extraction time

The extraction time per cycle was evaluated from 2 to 15 min, with acetone/methanol 1:3, doing three extraction cycles at 100 °C and 14 MPa. According to the results obtained (not shown), the target analytes present a fast desorption and solubilization in the selected solvent mixture, and therefore, an extraction cycle time longer than 2 min does not lead to an improvement in the extraction efficiency. In

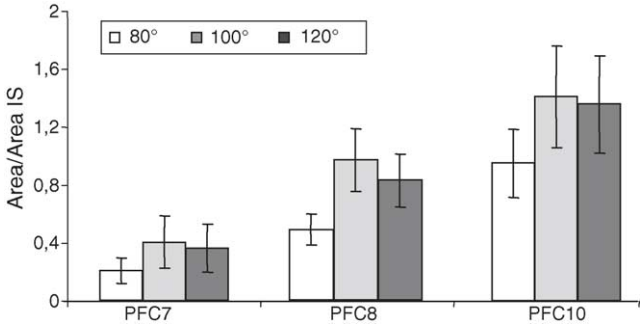

Fig. 2. Effect of the extraction temperature on the extracted amount of PFCAs from sediment. Extraction conditions were two PFE cycles of 2 min with acetone/MeOH 1:3.

other words, no significant effect in the extraction yields by increasing the extraction time per cycle was obtained, and on the other hand, longer extraction times could lead to the coextraction of other interfering compounds from the sample. Thus, only 2 min is enough to achieve a suitable extraction for all the analytes and this time was used in the subsequent experiments.

3.1.3. Number of static extraction cycles

Experiments including two, three and four extraction cycles 2 min long, with acetone/methanol 1:3, at 100 °C and 140 bars, were performed in order to maximise the extracted amount of analytes. Results (not shown) clearly indicate that the extraction efficiency remains constant independently of the number of cycles. In this way, two extraction cycles are enough to yield a quantitative extraction due to the high affinity of the target analytes for the selected solvent mixture. Furthermore, despite the obtained extracts were more complex while increasing the cycles number, neither a decrease of the extraction yield nor the presence of interferences was observed. Therefore, the next experiments were carried out with only two extraction cycles.

3.1.4. Extraction temperature

Temperatures ranging from 80 to 120 °C were investigated in order to evaluate the extraction temperature effect. Theoretically, better recoveries would be expected when temperature is increased [18] but even if the results obtained show that the recovery slightly improves by increasing temperature from 80 to 100 °C no significant improvement is obtained between 100 and 120 °C (Fig. 2). Consequently, 100 °C was selected as an adequate extraction temperature. In this work, pressure was not considered as a variable because it is high enough to keep as liquid all the solvents used and 14 MPa was kept for all the extractions.

3.2. Derivatisation and headspace SPME extraction

Because of the complexity of the PFE extract recovered from sediment since polar solvents are needed (Section 3.1.1), a cleanup step is mandatory before the PFCA determination by any chromatographic technique. Nevertheless, the amphiphilic character of the PFCAs makes the cleanup procedures difficult to achieve. Therefore, an analytical procedure based on derivatisation and headspace SPME determination was applied. In fact, the lack of volatility of the PFCAs allows a solvent evaporation to dryness without losses of analytes removing, in this way, the coextracted volatile compounds. Then, a methylation reaction was applied to the dried extract followed by headspace SPME (see Section 2.4 for more details). Therefore, only the volatile methyl esters can be extracted in the headspace with the SPME fibre. Indeed, in order to decrease the derivatised analytes solubility in the water phase and therefore increase the Henry constant of the PFCA methyl esters, a salting out effect with NaCl was carried out [15]. Finally, polydimethylsiloxane fibre (PDMS) was selected according to the hydrophobicity of the PFCA methyl esters and the extraction variables previously optimised [15].

Table 2
Optimal PFE conditions obtained for the extraction of PFCAs from marine sediments

Amount of solid sample	3 g
Extraction pressure	14 MPa
Extraction temperature	100 °C
Solvent composition	Acetone/MeOH 1:3
Static cycle time	2 min
Static extraction cycles	Two cycles
Solvent flush	10 s
Purge time	2 min

3.3. Figures of merit of the analytical procedure

Optimum PFE conditions are summarised in Table 2. Then, once the PFE procedure was optimised, the quality parameters of the developed analytical procedure were assessed by using GC–NCI-MS. Table 3 shows the accuracy, precision and detection and quantification limits obtained for the target analytes. The method reproducibility was determined by performing extractions of five sediment samples spiked at the low ppb level (15–18 ng g^{-1}). The relative standard deviations (%RSDs) obtained for the $PFC_{8–10}A$ were from 15.5 to 16.8%. These RSD values are acceptable taking into account the matrix complexity, the low spiking level and the several analytical steps involved in the extraction procedure developed. The accuracy defined as the bias of the measurement (ratio between measured and actual value) was calculated from the spiked since no reference materials are available and shown in Table 3. Values were over 95% showing the accuracy of the analytical procedure.

Finally, the limits of detection calculated from the signal to noise ratio (S/N) observed in samples (S/N = 3) as well as quantification limits (S/N = 10) were in the low ppb level (Table 3), which are acceptable for the monitoring of environmental samples taking into account that PFCAs can be

Table 3
Accuracy, precision, limits of detection (LOD) and quantification (LOQ) for the PFCA determination in sediment

PFCAs	Spiked level (ng g^{-1})	Measured (ng g^{-1})	Accuracy (%)[a]	RSD (%) (n = 6)	LOD (ng g^{-1})	LOQ (ng g^{-1})
PFC_8A	15.0	14.8	98.7	16.8	0.8	2.3
$PFC_{10}A$	18.0	18.5	102.8	15.5	0.5	1.6

[a] Calculated from the spiked and the measured values.

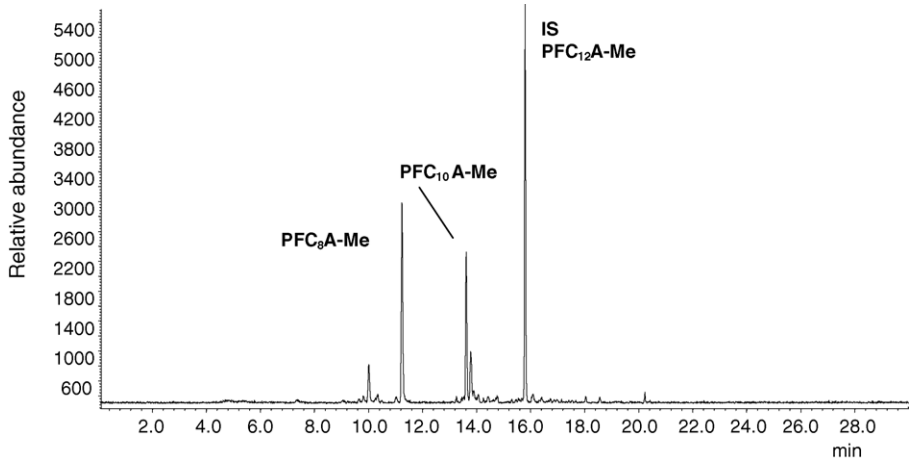

Fig. 3. GC–NCI-MS selected ion chromatogram for a real sediment sample extracted according with the optimal PFE conditions (two cycles of 2 min, 100 °C, acetone/MeOH 1:3).

accumulated in sediment due to their low solubility in seawater.

3.4. Application of the developed methodology

Sediment samples ($n = 7$) were collected from different areas of the Barcelona commercial harbour, where some PFCAs were already identified in seawater samples. Fig. 3 shows the profile obtained for a real sediment sample extracted using the optimal conditions, where PFC_8A and $PFC_{10}A$ were detected and quantified, being detected in 50% of samples analysed in the range from 10.4 to 12.4 ng g^{-1}. The PFCA distribution found in sediments (Fig. 4) reflects the patterns of fire fighting foams usage containing PFCAs in the commercial harbour where flammable items are handled. Indeed, they were not detected (<1.3–2.6 ng g^{-1}) in sampling sites located in marinas.

4. Conclusions

A method based on PFE and SPME has been developed to the determination of PFCAs in marine sediments. The SPME procedure is based on a previous procedure developed for

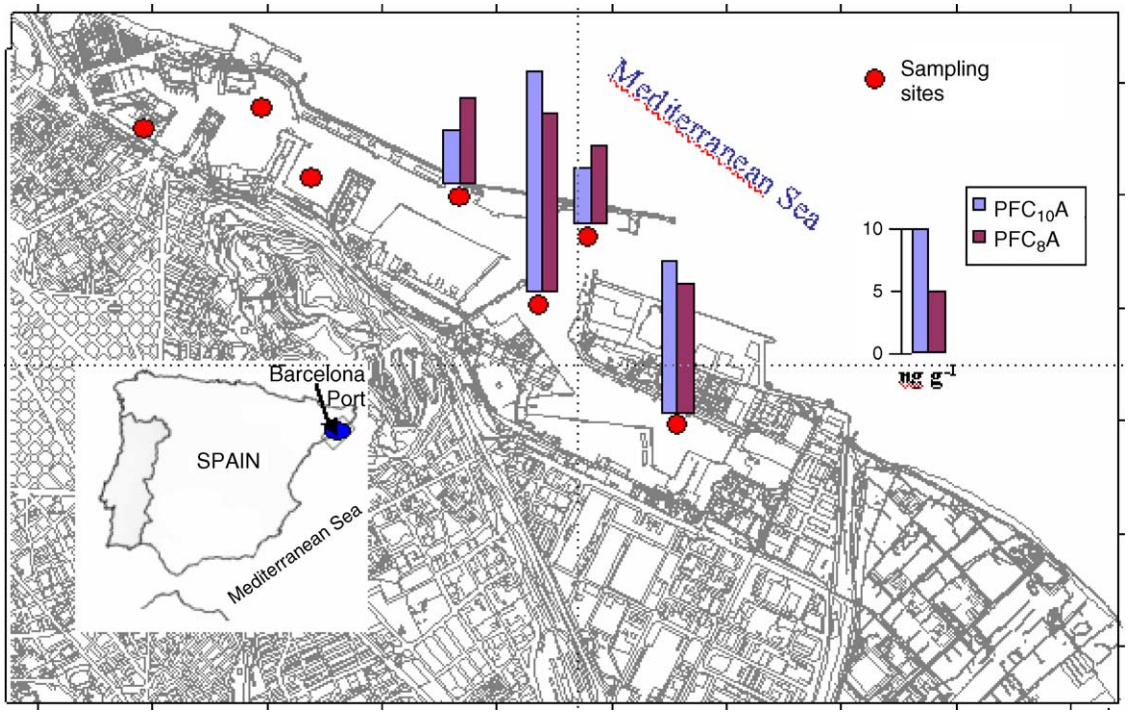

Fig. 4. Surface distribution of PFCAs in the Barcelona harbour sediments.

PFCAs in aqueous matrices [11] and in this work we have focused on the key variables that might affect the PFE from sediment. The optimised PFE technique enables the extraction of PFCAs from sediment with an accuracy exceeding the 95%. The solvent mixture is shown as the most important factor affecting to the extraction efficiency, while the number of cycles or the extraction time per cycle are found to be not relevant parameters on the target analytes extraction from sediments. PFE in combination to SPME and GC–NCI-MS detection provides a suitable methodology for the PFCA determination from marine sediments with detection limits in the low ng g^{-1} level. The proposed methodology was successfully applied to contaminated harbour sediments, showing the occurrence of both PFC_8A and $PFC_{10}A$ at concentrations from below the detection limit to moderate concentrations (12 ng g^{-1}).

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References

[1] J.P. Giesy, K. Kannan, Environ. Sci. Technol. 36 (2002) 146A.
[2] R. Renner, Environ. Sci. Technol. 35 (2001) 154A.
[3] K. Ohmori, N. Kudo, K. Katayama, Y. Kawashima, Toxicology 184 (2003) 135.
[4] F.M. Hekster, R.W.P.M. Laane, P. de Voogt, Rev. Environ. Contam. Toxicol. 179 (2003) 99.
[5] E. Kissa, Fluorinated Surfactants: Synthesis, Properties and Applications, Dekker, New York, 1994.
[6] C.A. Moody, J.A. Field, Environ. Sci. Technol. 34 (2000) 3864.
[7] C.A. Moody, J.W. Martin, W.C. Kwan, D.C.G. Muir, S.A. Mabury, Environ. Sci. Technol. 36 (2002) 545.
[8] K.J. Hansen, L.A. Clemen, M.E. Ellefson, H.O. Johnson, Environ. Sci. Technol. 35 (2001) 766.
[9] K. Kannan, H.J. Choi, N. Iseki, K. Senthilkumar, D.H. Kim, S. Masunaga, J.P. Giesy, Chemosphere 49 (2002) 225.
[10] C.A. Moody, J.A. Field, Environ. Sci. Technol. 33 (1999) 2800.
[11] R. Alzaga, J.M. Bayona, J. Chromatogr. A 1042 (2004) 155.
[12] N. Yamashita, K.C. Kannan, S. Taniyasu, Y. Horii, T. Okazawa, G. Petrick, T. Gamo, Environ. Sci. Technol. 38 (2004) 5522.
[13] K.C. Kannan, S. Corsolini, J. Falandysz, G. Fillmann, K.C.S. Kumar, B.G. Loganathan, M.A. Mohd, J. Olivero, N.V. Wouwe, K.M. Aldous, Environ. Sci. Technol. 38 (2004) 4489.
[14] J.W. Martin, et al., Environ. Sci. Technol. 38 (2004) 248A.
[15] J. Pawliszyn, Applications of Solid Phase Microextraction, RSC Chromatography Monographs, Cambridge, 1999.
[16] J.W. Martín, D.M. Whittle, D.C.G. Muir, S.A. Mabury, Environ. Sci. Technol. 38 (2004) 5379.
[17] G.L. Northcott, K.C. Jones, Environ. Toxicol. Chem. 19 (2000) 2418.
[18] B.E. Richter, B.A. Jones, J.L. Ezzell, N.L. Porter, N. Avdalovic, C. Pohl, Anal. Chem. 68 (1996) 1033.