Talanta 111 (2013) 170–177

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Trace analysis of mono-, di-, tri-substituted polyfluoroalkyl phosphates and perfluorinated phosphonic acids in sewage sludge by high performance liquid chromatography tandem mass spectrometry

Runzeng Liu, Ting Ruan, Thanh Wang, Shanjun Song, Miao Yu, Yan Gao, Junjuan Shao, Guibin Jiang*

State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China

ARTICLE INFO

Article history:
Received 19 December 2012
Received in revised form
25 February 2013
Accepted 27 February 2013
Available online 7 March 2013

Keywords:
Polyfluoroalkyl phosphates
Perfluorinated phosphonic acids
Ultrasonic extraction
Solid phase extraction
Sewage sludge

ABSTRACT

A new method using ultrasonic extraction and solid phase extraction (SPE) clean-up pretreatments was developed for the analysis of mono-, di- and tri-substituted polyfluoroalkyl phosphates (abbreviated as mono-PAPs, di-PAPs and tri-PAPs) and perfluorinated phosphonic acids (PFPAs) in sludge from wastewater treatment plants (WWTPs). For the ultrasonic extraction of three mono-PAPs, three di-PAPs and three PFPAs in sludge samples, a mixture of tetrahydrofuran/acetic acid (1:1, v/v) was found to be the most suitable extraction solvent. The subsequently optimized clean-up and enrichment procedures were carried out with weak anion exchange (WAX) cartridges in-line coupled with graphitized carbon black (ENVI-Carb) tubes. Two tri-PAPs were ultrasonically extracted by acetonitrile/tetrahydrofuran (1:1, v/v) and cleaned by mixed-mode anion exchange (MAX) in-line coupled with ENVI-Carb cartridges. The analytes were analyzed by optimized high performance liquid chromatography tandem mass spectrometry (HPLC–MS/MS) method either in negative or positive ionization mode. The method quantification limits (MQLs) of the 11 analytes in sludge ranged from 0.6 to 5.1 ng/g, meanwhile the total recoveries of the pretreatment varied from 24% (6:2 mono-PAP) to 107% (PFDPA). The method was successfully applied to analyze 16 sewage sludge samples collected from seven provinces in China, and two mono-PAPs were identified with concentrations ranging from $<$MQLs to 10.7 ng/g.

1. Introduction

Due to good surface tension properties and thermal stability, poly- and perfluorinated alkyl substances (PFASs) are widely used in a variety of consumer products and industrial applications such as fire fighting foams [1], insecticides [2], textile coatings, paper packaging materials [3] and as polymerization aids in fluoropolymer manufacturing processes [4]. The ubiquitous presence of PFASs in water, air, sediment, wildlife and human [5–11] has triggered an increasing health concern on these chemicals, especially perfluoroalkyl sulfonic acids (PFSAs) and perfluoroalkyl carboxylic acids (PFCAs), due to their bioaccumulative properties [12], persistence, long-range transport potential [13] and toxic effects [14,15]. Besides major contribution from direct source emissions, many polyfluorinated precursors such as fluorotelomer alcohols, perfluorinated sulfonamides, perfluorooctane sulfonyl fluoride and polyfluorinated iodine alkanes could be transformed into related perfluorinated acids and also contribute to the global distribution of PFSAs and PFCAs [16–22].

Polyfluoroalkyl phosphates (PAPs) belong to a group of hydrophobic phosphates that are mainly mono-, di- and tri-substituted by partially fluorinated alkyl chains (abbreviated as mono-PAPs, di-PAPs and tri-PAPs). Commercial products usually consist of different fluoroalkyl chain length mixtures (typically C8-C12) and were reported to be primarily used in food-contact paper industries in recent years to replace the previously phased-out leveling and wetting agents [23–25]. Under certain conditions, PAPs could migrate into food from its contact paper and thus might be another potential exposure pathway of PFASs to humans [24]. Perfluorinated phosphonic acids (PFPAs) are another group of fluoroalkyl mono-substituted phosphonate derivative but are structurally different in that the perfluorinated carbon tail is directly attached to the phosphonate unit. The PFPAs are commonly used as pesticides, lubricant and anti-foaming agents in textile industry [26]. Di-PAPs and perfluorooctyl phosphonic acid (PFOPA) have been reported to be present in the environment such as drinking water and sewage sludge, and even in human serum [27,28]. Furthermore, recent studies have shown that PAPs and PFPAs could be bio-transformed into relevant PFCAs in activated sludge and in biota such as rat and juvenile rainbow trout [29–31].

* Corresponding author. Tel.: +86 10 6284 9334; fax: +86 10 6284 9179.
E-mail address: gbjiang@rcees.ac.cn (G. Jiang).

0039-9140/$ - see front matter © 2013 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.talanta.2013.02.063

Similar to other hydrophobic substances, most PAPs and PFPAs could be associated with suspended particulate matters and sedimentary sludge [32], and reliable analytical methods to monitor their environmental concentrations and fate in solid matrices are therefore important. However, trace analytical methods for the determination of PAPs and PFPAs in the environment are still limited, especially for complex environmental matrices. D'eon et al. were the first to use an ion-pairing based liquid–liquid extraction method to extract di-PAPs and PFCAs from sludge and paper fiber samples with di-PAP recoveries ranging from 38% to 53% [27]. However, the analyte instrument analysis of the method might be vulnerable to matrix interferents due to the lack of clean-up procedures in the sample pretreatment processes. Solid phase extraction (SPE) method is commonly used pretreatment strategy for the analysis of various kinds of PFASs homologs from different complex environmental matrixes such as soil, sediment and sludge [33,34]. And optimized solvent–mixture extraction prior to the SPE protocol were also reported to be able to further expand the SPE application scope to maximize extract efficiencies of different PFCA and PFPA homologs from complex food and sludge matrices [28,35]. To the best of our knowledge, there are currently few reports on simultaneous extraction of different kinds of PAPs and PFPAs in sewage sludge by optimizing the solvent extraction procedures.

In this study, a simple and efficient method was developed for the trace analysis of eight PAPs and three PFPAs from sludge samples by comparing a variety of solvent extraction strategies. Weak anion exchange (WAX), mixed-mode anion exchange (MAX), weak cation exchange (WCX) and graphitized carbon black (ENVI-Carb) SPE adsorbents were further optimized in the subsequent enrichment and clean-up procedures. Finally this method was applied to the analysis of PAPs and PFPAs in 16 sludge samples collected from different WWTPs in China.

2. Experimental

2.1. Chemicals and reagents

Analyte name, structure and other relevant data are shown in Table 1. All mono-, di-, tri-substituted and mass-labeled PAPs (6:2 mono-PAP, 8:2 mono-PAP, 10:2 mono-PAP, 6:2 di-PAP, 8:2 di-PAP, 10:2 di-PAP, 6:2 tri-PAP, 8:2 tri-PAP and 6:2 di-PAP-d4) were obtained from Chiron AS (Trondheim, Norway). PFPAs standards (PFHxPA, PFOPA, PFDPA) and the internal standard Cl-PFHxPA were supplied by Wellington Laboratories Inc. (Guelph, Ontario, Canada). The purity for all the target analytes were $>99\%$ except for di-PAPs ($>94\%$). Stock standard solutions (50 μg/mL) were individually prepared in methanol and intermediate solutions were weekly prepared from the stock standard solutions by appropriate methanol dilution. Oasis WAX (6 cm^3, 150 mg), MAX (6 cm^3, 150 mg) and WCX (6 cm^3, 150 mg) SPE cartridges were purchased from Waters (Milford, MA, USA), and ENVI-Carb (6 cm^3, 250 mg) cartridges were from Supelco (Bellefonte, PA, USA). All solvents were of HPLC grade. Tetrahydrofuran (THF), methanol (MeOH) and acetonitrile (ACN) were purchased from J.T. Baker (Phillipsburg, NJ, USA). Ultrapure water (18.3 MΩ cm) was generated by a Milli-Q purification system (Millipore, Billerica, MA, USA). Acetic acid (HAc) was from Mallinckrodt (Phillipsburg, NJ, USA). Formic acid and ammonia solutions (NH_4OH, 28–30%) were from Dikma (Richmond Hill, ON, Canada) and Alfa Aesar (Ward Hill, MA, USA), respectively.

2.2. Sample collection and preparation

Sixteen sludge samples were collected from different WWTPs in seven provinces in China in February 2011. Approximately 500 g of freshly digested sludge was collected at the dewatering process, packed in aluminum foil, sealed in polypropylene bag, and immediately express-delivered to our lab. All sludge samples were freeze-dried, homogenized, sieved through a stainless steel 100-mesh sieve and then stored in -20 °C until analysis. Detailed information on the sampling location, treatment processing volume and WWTP treatment type is given in the supplementary data (Table S1).

2.3. Sample extraction

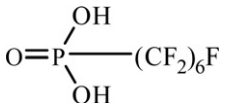
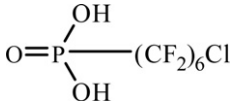
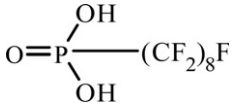
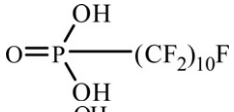
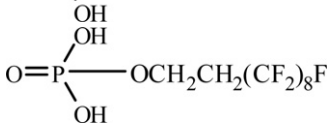
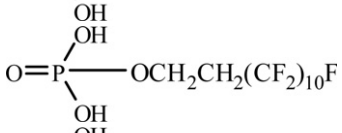
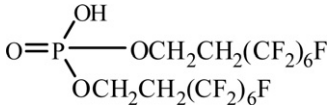
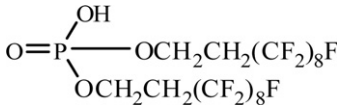
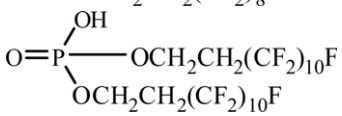
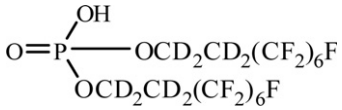
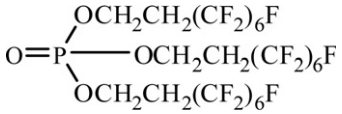
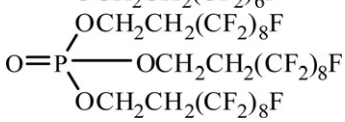
The 11 analytes were extracted by two different solvent extraction strategies as follows. For mono-, di-PAPs and PFPAs, an aliquot of 0.3 g sample was placed into a 15 mL polypropylene centrifuge tube and was extracted by 5 mL of THF/HAc (1:1, v/v) in a 50 °C ultrasonic water bath for 20 min. The HAc was a diluted ultrapure water solution with concentration of 1 mol/L (1 M). Supernatant extract was decanted after the centrifuge tube was centrifuged at 4500 rpm for 5 min. The extraction procedure was repeated for 3 times and the extract was combined and then diluted to 100 mL by ultrapure water for the subsequent sample clean-up process. For the analysis of tri-PAPs, the extraction procedure was quite similar except that ACN/THF (1:1, v/v) was used as extraction solvent. Disposable polypropylene tubes, bottles and pipettes were used thoroughly to prevent analyte adsorption and contamination from glassware.

2.4. Sample clean-up and enrichment

Different SPE adsorbents are often specifically chosen to enrich analytes and remove matrix interferents in sample extracts. In this study, WAX cartridge in-line coupled with ENVI-Carb was selected for the elimination of interferents as well as recovery optimization of the mono-, di-PAPs and PFPAs. Before use, the WAX cartridges were preconditioned by 5 mL MeOH containing 5% NH_4OH, 5 mL MeOH and 5 mL ultrapure water in sequence. Diluted extracts from the ultrasonic extraction procedure were passed through the cartridges at a flow rate of 1–2 drops/s. After loading, the cartridges were washed with 5 mL of HAc at pH=4 and 20 mL of ACN/THF (1:1, v/v) mixture to maximize matrix interferent elimination. The cartridges were then dried under vacuum for 10 min and centrifuged at 4500 rpm for 10 min. After that the WAX cartridges were coupled to the 5 mL MeOH preconditioned ENVI-Carb tubes via polyethylene (PE) adapter caps. Elution of 3×3 mL MTBE/MeOH (90:10, v/v) containing 5% NH_4OH was applied to ensure maximum recoveries of the target compounds. The eluate was concentrated under nitrogen flow to a final volume of 1 mL for instrument quantification analysis. Before injection, 6:2 di-PAP-d4 and Cl-PFHxPA were added into the final eluates as internal standards for di-PAPs and PFPAs, respectively.

For ultrasonic extracts of tri-PAPs, the subsequent clean-up and enrichment procedures were conducted with MAX in-line coupled with ENVI-Carb adsorbents. The MAX cartridges were preconditioned by 5 mL MeOH containing 2% formic acid, 5 mL MeOH and 5 mL ultrapure water. The diluted extracts were passed through the conditioned cartridges at a flow rate of 1–2 drops/s. After loading, the cartridges were washed with 5 mL of ultrapure water containing 5% NH_4OH and 30 mL of MeOH/H_2O (1:1, v/v) mixture. The cartridges were then dried, centrifuged, and coupled to the 5 mL MeOH preconditioned ENVI-Carb tubes via PE adapter caps. The target compounds were finally eluted with 3×3 mL of MTBE/MeOH (90:10, v/v) containing 2% formic acid, and concentrated under nitrogen flow to a final volume of 1 mL.

Table 1
Analyte name, structure and other relevant data.

Analyte	Acronym	CAS no.	Structure	MW[a] (*m*/*z*)
Perfluorohexyl phosphonic acid	PFHxPA	40143-76-8		400
1-Chloro-perfluorohexyl phosphonic acid acid	Cl-PFHxPA	N.A.[b]		416.5
Perfluorooctyl phosphonic acid	PFOPA	40143-78-0		500
Perfluorodecyl phosphonic acid	PFDPA	52299-26 -0		600
6:2 Mono-substituted polyfluoroalkyl phosphate	6:2 mono-PAP	57678-01-0		444
8:2 Mono-substituted polyfluoroalkyl phosphate	8:2 mono-PAP	57678-03-2		544
10:2 Mono-substituted polyfluoroalkyl phosphate	10:2 mono-PAP	57678-05-4		644
6:2 Di-substituted polyfluoroalkyl phosphate	6:2 di-PAP	57677-95-9		790
8:2 Di-substituted polyfluoroalkyl phosphate	8:2 di-PAP	678-41-1		990
10:2 Di-substituted polyfluoroalkyl phosphate	10:2 di-PAP	1895-26-7		1190
Deuterated 6:2 di-substituted polyfluoroalkyl phosphate	6:2 di-PAP-d4	N.A.[b]		794
6:2 Tri-substituted polyfluoroalkyl phosphate	6:2 tri-PAP	N.A.[b]		1136
8:2 Tri-substituted polyfluoroalkyl phosphate	8:2 tri-PAP	N.A.[b]		1436

[a] Average molecular weight.
[b] CAS number not available.

2.5. Instrumentation

An Alliance 2695 high performance liquid chromatography interfaced with a Quattro Premier XE triple-quadrupole mass spectrometer (HPLC–MS/MS, Waters, Milford, MA) was used for the determination of the PAPs and PFPAs. A Kinetex C18 column (3 μm, 50 × 4.6 mm, Phenomenex, Torrance, CA) was chosen for chromatographic separation. Column temperature was set at 40 °C. MeOH (A) and ultrapure water (B), both containing 0.5% formic acid, were chosen as mobile phases. Mono-, di-, tri-PAPs and PFPAs were analyzed in four different separate runs and the gradient elution conditions were similar with those published elsewhere with little modifications [36]. For mono-PAP analytes, the gradient was initiated at a composition of 40:60 (A:B, v/v). Gradient elution program was optimized with composition A linearly increased to 95% in 2.5 min and then held for 7.5 min.

After immediately returning to the initial composition of 40:60, the column was allowed to re-equilibrate for 4 min. For di-PAPs and PFPAs, the composition A increased from 40% to 95% in 3.5 min and held for 6.5 min. Additional 4 min was also used to re-equilibrate the column to the initial composition with a total run time of 14 min. For tri-PAPs, the composition A increased from 60% to 95% in 3.5 min and held for 7.5 min. After returning to the initial composition of 60:40, the column was conditioned for 4 min giving a total run time of 15 min. Flow rate for all of the separation procedures was set at 0.4 mL/min.

The mass spectrometer was operated in positive or negative electrospray ionization (ESI) mode (see Table S2). The source and desolvation temperature were set at 120 °C and 450 °C respectively. Desolvation gas flow was 800 L/h, cone gas flow was 50 L/h, while argon pressure in the collision cell was kept at 3.8×10^{-3} mbar for MS/MS measurement. The precursor ions were $[M-H]^-$ for mono- and di-PAPs, $[M+H]^+$ for tri-PAPs and $[M-2H]^-$ for PFPAs. The major product ions were $[PO_3]^-$ (m/z=79) for PFPAs, $[H_2PO_4]^-$ (m/z=97) for mono- and di-PAPs, and $[H_4PO_4]^+$ (m/z=99) for tri-PAPs. Detailed information on the optimized parameters and monitored ion transitions for each analyte is given in Table S2.

2.6. Quantification and method validation

For the positive identification of the PFPAs and PAPs, confirmation criteria published elsewhere was used [37,38]. In short, the analyte relative retention time should match that of the calibration standards at a tolerance level of 2%. A signal-to-noise ratio of 10:1 was set as a threshold for positive quantification. For PAPs, the ratio of the quantification to the confirmation ions in real samples should be within 20% of that in the standards. As only one product ion could be found for each PFPA analyte in the collision-induced dissociation process, the ratio of the quantification to the confirmation ions was not taken into account in the confirmation process. For analyte quantification, seven point calibration curves (1, 2, 5, 10, 20, 50, 100 ng/mL) were constructed in MeOH as standard solutions.

Procedures were carried out to validate the PAP and PFPA trace determination protocols. In this study, the concentrations of di-PAPs and PFPAs were calculated by internal calibration curve method with 6:2 di-PAP-d4 and Cl-PFHxPA used as internal standards for correcting the deviation caused by matrix effects for di-PAPs and PFPAs, respectively. As no commercial isotope-labeled standards were available for mono-PAPs and tri-PAPs, the quantification of mono-PAPs and tri-PAPs were assessed relative to a standard prepared in MeOH by external standard calibration curve method. To test linearity range of the standard calibration curve, seven concentration levels of standard mixtures (1–100 ng/mL) were injected in triplicate. Method quantification limits (MQLs) in sludge samples were calculated by analyzing 10 ng/g spiked sludge samples and on a minimal value of signal-to-noise of 10 (S/N=10), while equipment quantification limits (EQLs) were calculated on the basis of S/N=10 in the chromatograms of 1 ng/mL standards in MeOH. The matrix effects of the analytes were evaluated by comparing the 50 ng/mL spiked analytes in eluates of uncontaminated sludge extracts from SPE clean-up procedures with the same concentration in MeOH. Analyte recoveries and method accuracy were investigated by spiking standard solutions into uncontaminated sludge at the concentrations of 16.7 ng/g and 167 ng/g. When analyzing the collected sewage sludge samples, one procedural blank sample was added to every batch of eight samples. Besides, in order to check for potential instrumental contamination, MeOH was routinely injected into the instrument during sample processing sequences.

3. Results and discussion

3.1. Sample clean-up and concentration

Solid phase extraction is the most widely used technique for sample clean-up and enrichment of PFASs in environmental water samples and sludge extracts [28,33–35]. When selecting cartridges, SPE adsorption mechanism and analyte characteristics such as acid dissociation constant (pK_a) should be taken into account. The measured first dissociation constants ($\mathrm{Log}\ pK_{a1}$) were 2.1, 2.4 and 3.4 for PFHxPA, PFOPA and PFDPA [28], respectively, while the estimated values for all mono-PAPs was around 2.3 and 3.0 for di-PAPs by using the SPARC model [39]. WAX cartridge contains a polymeric reversed-phase, weak anion exchange mixed-mode sorbent which is suitable for acids with $\mathrm{Log}\ pK_a<5$, and thus might be appropriate for mono-, di-PAPs and PFPAs. However, other retention effects such as hydrophobic adsorption or cation exchange might be more important during the SPE pretreatment procedure for the neutral tri-PAP analytes. Thus, MAX and WCX adsorbents were also chosen and tested to check for the effects of possible adsorption mechanisms for the PAP and PFPA analytes. The SPE pretreatment procedures conducted on WCX cartridge were the same as that on MAX cartridge, which are listed in Section 2.4. A volume of 100 mL ultrapure water at the spiked concentration of 0.5 ng/mL was used for the cartridge optimization procedure. As expected, results in Table 2 showed that WAX provided the most satisfactory recoveries for most analytes with recoveries ranging from 52% to 119% and was then initially chosen for the simultaneous pretreatment of PAPs and PFPAs. And the recoveries in the present study (74–91% and 59–119% for PFPAs and PAPs) were comparable with reported recoveries of WAX cartridges for PFPAs (40–56% in spiked pure water samples) [40] and PAPs (65–110% in spiked drinking water samples) [37].

After cartridge selection, SPE washing condition was further optimized. MeOH (0, 10, 20 mL), MeOH/THF (1:1, v/v, 20 mL) and ACN/THF (1:1, v/v, 20 mL) were investigated as mentioned elsewhere in order to maximize interferent removal [28,35]. Our work showed that MeOH, MeOH/THF (1:1, v/v) or ACN/THF (1:1, v/v) wash could result in poor recoveries for tri-PAPs ($<10\%$ for both 6:2 tri-PAP and 8:2 tri-PAP), which suggested that WAX cartridge was not appropriate for tri-PAPs, and then MAX cartridge which provided tri-PAP recoveries varying from 71% to 76% was alternatively selected for tri-PAP pretreatment procedures. For other analytes, however, the organic solvents could eliminate much interferents and did not lead to obvious target losses (Fig. 1). As also shown in Fig. 1, it is interesting to find that most recoveries of the analytes raised along with the MeOH volume, indicating that the solvent wash could have a positive effect on

Table 2
Recoveries of PAPs and PFPAs spiked onto different SPE cartridges (n=3).

Compound	Recovery (mean ± SD, %)		
	WAX	MAX	WCX
6:2 Mono-PAP	103 ± 2	94 ± 1	8 ± 1
8:2 Mono-PAP	88 ± 7	88 ± 3	24 ± 2
10:2 Mono-PAP	59 ± 4	73 ± 2	38 ± 4
6:2 Di-PAP	108 ± 24	7 ± 1	78 ± 17
8:2 Di-PAP	119 ± 17	13 ± 1	65 ± 5
10:2 Di-PAP	73 ± 7	9 ± 1	46 ± 6
6:2 Tri-PAP	77 ± 5	76 ± 1	54 ± 12
8:2 Tri-PAP	52 ± 3	71 ± 4	46 ± 13
PFHxPA	91 ± 3	3 ± 1	2 ± 1
PFOPA	90 ± 1	3 ± 1	3 ± 2
PFDPA	74 ± 2	4 ± 1	12 ± 9

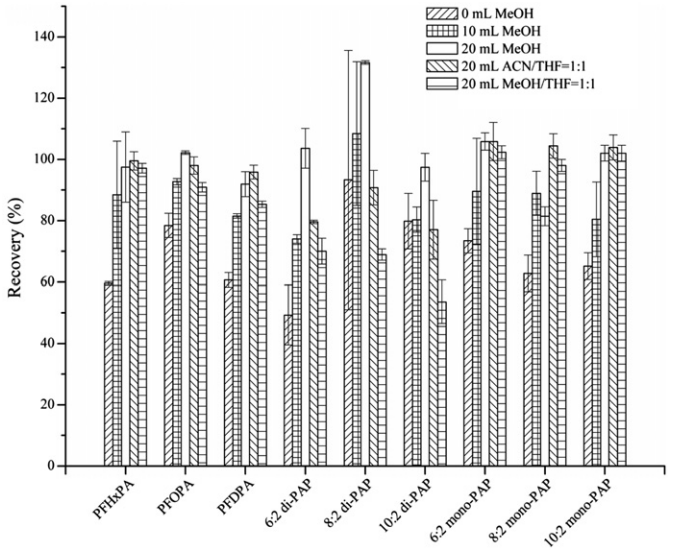


Fig. 1. Target recoveries obtained on WAX cartridges after different volume and kind of solvent wash ($n=3$).

the improvement of analyte recoveries. It might be possible that mono-, di-PAPs and PFPAs could be strongly bonded to WAX adsorbents by both ion-exchange and hydrophobic effect, and a primary organic solvent washing step could weaken the hydrophobic interactions between the analytes and the adsorbent, which eventually facilitates analyte elution. Similar phenomenon was also reported by another study that 25% of MeOH was added to water samples before loading onto the SPE tubes in order to weaken hydrophobic interactions and facilitate mono- and di-PAP elution [37]. Meanwhile, ACN/THF (1:1, v/v) has been reported to be more efficient than MeOH in removing dye or lipid interferents, due to its stronger hydrophobic effect [35]. In our work, the results showed that the ACN/THF (1:1, v/v, 20 mL) wash could provide comparable satisfactory recoveries (77–106%) with those of 20 mL MeOH (81–132%), and thus was chosen as the washing solvent for WAX cartridge.

For MAX cartridges, organic solvent mixed with ultrapure water was used as washing solvent because 100% organic solvent wash can lead to poor recoveries for tri-PAPs (recoveries $<10\%$). In this study, different volumes (10, 20 and 30 mL) of MeOH/H_2O (1:3, 1:1 and 3:1, v/v) were investigated. The results listed in Table S3 showed that MeOH/H_2O (1:1 or 1:3, v/v) could provide relatively satisfactory recoveries (78–92%), while MeOH/H_2O (3:1, v/v) wash could lead to a sharp decrease of the recoveries (3–34%). Thus, 30 mL MeOH/H_2O (1:1, v/v) was chosen as washing solvent for the MAX cartridge.

Recoveries of the target analytes were also investigated when the optimized SPE procedure was applied to extracts of 0.3 g sludge samples, each of which were spiked with 50 ng of all target analytes. The result in Fig. S2 showed that the optimized SPE procedure can provide parallel SPE recoveries for spiked sludge extracts (70–119%) compared with those of the spiked ultrapure water samples (77–106%). So the optimized SPE method based on spiked water samples is also adequate for sludge extract matrix.

3.2. Solvent extraction of sewage sludge

Efficient extraction of amphiphilic molecules such as PAPs and PFPAs requires the use of solvents with proper Hildebrand solubility parameter (δ_T), which provides a measure of the overall intermolecular forces resulting from the additive effect of dispersion (δd), dipole–dipole (δp) and hydrogen bonding (δh) forces ($\delta^2T=\delta^2d+\delta^2p+\delta^2h$) [35]. To ensure efficient extraction of PAPs and PFPAs from sludge samples, several individual solvents and solvent mixtures (THF, ACN, MeOH, MeOH/THF=1:1, ACN/THF=1:1) were tested. As both WAX and MAX cartridges were used for distinct analyte groups, different extraction strategies were applied. In this part, freeze-dried sludge samples were spiked with standards at a concentration of 167 ng/g before extraction by immersion of the sludge sample into analyte spiked acetone solvent and aged overnight till the acetone was completely evaporated. Fig. 2 showed that ACN/THF (1:1, v/v) was the best extraction solvent for 6:2 tri-PAP and 8:2 tri-PAP with recoveries of $64\pm4\%$ and $85\pm2\%$, respectively. MeOH/THF (1:1, v/v) provided somewhat lower recoveries at $55\pm4\%$ and $79\pm1\%$ for 6:2 tri-PAP and 8:2 tri-PAP, and the individual solvents MeOH, THF and ACN led to even lower recoveries (20–75%). It suggested that individual solvents could be difficult to provide sufficient polar and non-polar interactions with tri-PAPs to ensure efficient extraction from sludge samples [35].

For the mono-, di-PAP and PFPA extraction, THF, ACN, MeOH, MeOH/THF=1:1 and ACN/THF=1:1 provided unsatisfactory recoveries ($<12\%$ for mono-PAPs and PFPAs, data not shown). THF/H_2O mixture was usually used to extract PFPAs and PFCAs from sediment, sludge and biological samples due to its proper Hildebrand solubility parameter [28,35]. Satisfactory extraction efficiencies were also observed in our work when using THF/H_2O (1:1, v/v) as extraction solvent with PFPA recoveries ranged from 83% to 99%. However, the THF/H_2O (1:1, v/v) mixture could not be applied for PAP extraction due to low recoveries ranging from 5% to 34% for all of the PAP analytes. The low solvent extraction recoveries for the PAPs might be due to the large number of organic moieties present in sludge which could consequently form strong bonds with the target compounds, and thus prevent exhaustive extraction by the solvent. It has been found that acetic acid added into the extraction solvent can increase extraction of PFASs by weakening analyte–matrix interactions, and increase PFAS recoveries from sediment and sludge samples [9]. Thus, THF/HAc mixture was also applied for sludge sample extraction procedure in the present work. The results indicated in Table 3 showed that 1 M HAc adding in THF could dramatically increase the PAP and PFPA recoveries. THF/HAc (1:1, v/v) extraction provided the most satisfactory recoveries for all the analytes with PFPA recoveries from 88% to 107%, which were better than reported PFPA recoveries (75–82%) when HAc was not used [28].

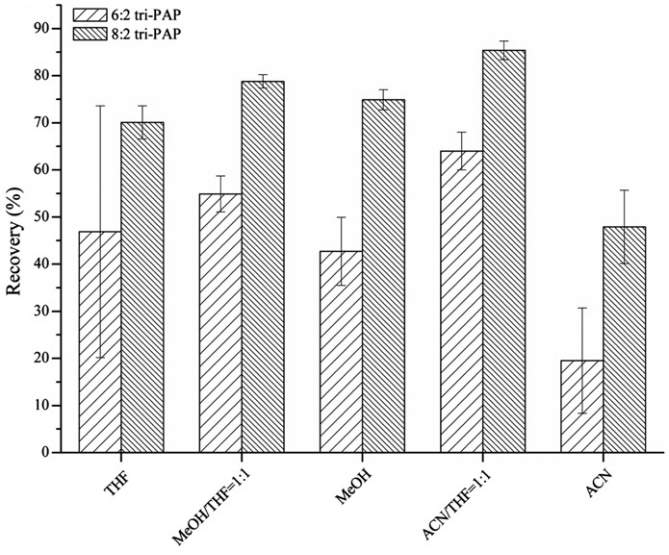


Fig. 2. Extraction recoveries of tri-PAPs by different extraction solvents ($n=3$).

The di-PAP recoveries were found from 54% to 100%, while the mono-PAP recoveries were relatively low, ranging from 24% to 40%. As shown in Table 1, it is possible that the low mono-PAP extraction recoveries might be due to the presence of –O–CH_2–CH_2– unit in the molecular structures, which could strongly bind to sludge sorbent molecules by hydrogen and covalent bonding or other interactions [41]. While, higher recoveries of di-PAPs (54–100%) and tri-PAPs (61–85%) compared with mono-PAPs (24–40%) might result from the additional numbers of substituted polyfluorinated hydrocarbon chains which increase the steric effect [29], and could thus restrict the contact between the –O–CH_2–CH_2– unit and the sorbent molecules in the sludge.

Table 3
Mono-PAP, di-PAP and PFPA extraction recoveries obtained by using THF/HAc mixture as extraction solvent ($n=3$).

Compound	Recovery (mean ± SD, %)		
	THF/HAc=1:3	THF/HAc=1:1	THF/HAc=3:1
6:2 Mono-PAP	13 ± 1	24 ± 1	4 ± 1
8:2 Mono-PAP	9 ± 3	37 ± 1	11 ± 1
10:2 Mono-PAP	13 ± 6	40 ± 1	20 ± 1
6:2 Di-PAP	74 ± 6	79 ± 5	78 ± 9
8:2 Di-PAP	22 ± 2	54 ± 2	53 ± 3
10:2 Di-PAP	10 ± 3	100 ± 5	90 ± 12
PFHxPA	72 ± 2	88 ± 8	56 ± 3
PFOPA	45 ± 3	99 ± 1	78 ± 5
PFDPA	24 ± 1	107 ± 3	91 ± 6

3.3. Matrix effects

Quantification analysis of polar PFASs in complex environmental samples by LC–MS/MS is often subjected to serious ionization suppression/enhancement by co-eluting compounds [42]. The matrix effect (ME) for each analyte was calculated by the percentage of signal intensity in sample matrix versus the signal of the same concentration in MeOH solvent. ME above 100% means ionization enhancement, while ME < 100% suggests ionization suppression. Results in Fig. 3(A) showed that no severe matrix effects were found for most analytes (except 8:2 mono-PAP and 10:2 mono-PAP) with ME varied between 81% and 117%. The high degree of matrix enhancement of 8:2 mono-PAP (130%) and 10:2 mono-PAP (170%) might be because of the mono-PAP co-eluting interferents were not efficiently removed in the clean-up procedure. ENVI-Carb was used to yielded a "matrix-effect free method" for PFCAs determination in environmental samples such as soils, sediments, and sludge samples [34], and was also

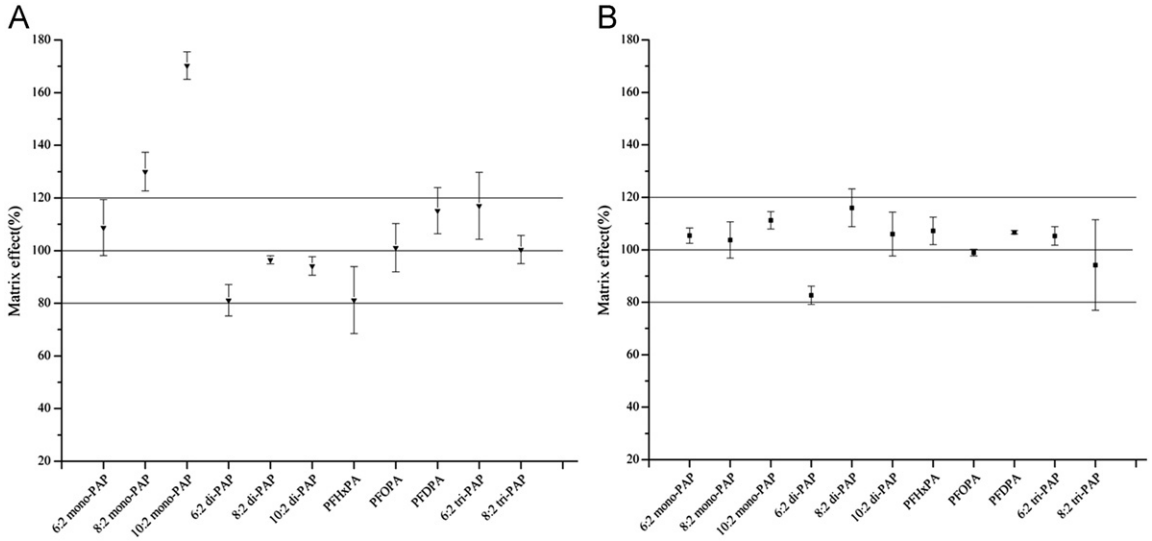


Fig. 3. Matrix effects of the target PAPs and PFPAs. (A) Shows the matrix effects when the sludge extracts were cleaned by WAX or MAX cartridges, while (B) shows matrix effects when ENVI-Carb tubes were also used in the clean-up procedure.

Table 4
Validation data and performance of the optimized method.

Compound	Limit of quantification		Linearity/R^2	Recovery[a] (%)		Precision[b] (RSD, %)	
	EQL (pg)	MQL (ng/g)		16.7 ng/g spiking	167 ng/g spiking	16.7 ng/g spiking	167 ng/g spiking
6:2 Mono-PAP	8.6	3.3	0.9997	29	24	5	3
8:2 Mono-PAP	8.1	3.1	0.9986	48	37	6	3
10:2 Mono-PAP	9.3	4.1	0.9986	69	40	6	2
6:2 Di-PAP	6.6	1.7	0.9990	41	79	6	6
8:2 Di-PAP	14.6	2.1	0.9987	78	54	14	4
10:2 Di-PAP	20.1	3.2	0.9988	93	100	3	3
6:2 Tri-PAP	1.3	0.7	0.9933	56	64	5	4
8:2 Tri-PAP	0.7	0.6	0.9997	50	85	3	2
PFHxPA	10.1	5.1	0.9986	30	88	9	9
PFOPA	3.3	1.3	0.9999	85	99	2	1
PFDPA	2.7	1.9	0.9999	102	107	6	3

[a] The mean recovery ($n=3$).
[b] The same data from recovery experiment.

reported in the clean-up procedure to decrease matrix effects in PFCA pretreatments for food samples [35]. The retention mechanism of ENVI-Carb is based primarily on the specific π–π interactions, and aromatic interferents could be specifically removed [34]. Thus, ENVI-Carb tubes were further coupled with the WAX cartridges in the clean-up procedure in order to decrease matrix effects of 8:2 and 10:2 mono-PAPs. Results in Fig. 3(B) showed that ENVI-Carb had a positive effect in efficient removal of the mono-PAP co-eluting interferents in the SPE clean-up procedure that the matrix enhancement of 8:2 mono-PAP and 10:2 mono-PAP decreased to 116% and 106% from $130 \pm 7\%$ and $170 \pm 5\%$, respectively. Even though severe matrix effects were not observed for tri-PAPs, repeated injections of dirty eluates (due to deficiency of MeOH aqueous solution to remove interferents) should be avoided. So ENVI-Carb tubes were also coupled with the MAX cartridges in the clean-up procedure. Moreover, Figs. S3 and S4 also showed that the recoveries of all target analytes by using WAX in-line coupled with ENVI-Carb cartridges (77–100%) or MAX in-line coupled with ENVI-Carb cartridges (68–83%) were comparable with those using WAX cartridges only (77–106%) or MAX cartridges only (87–88%), suggesting the coupled ENVI-Carb tubes had little influences on the analyte recoveries in the SPE pretreatment procedures.

Table 5
Concentrations (ng/g) of detected mono-PAPs in WWTP sludge.

Sample location		8:2 Mono-PAP	10:2 Mono-PAP
Shandong province	Plant 1	3.3	4.7
	Plant 2	1.6[a]	2.1[a]
	Plant 3	3.1	6.0
	Plant 4	1.6[a]	N.D.[b]
	Plant 5	1.6[a]	4.3
	Plant 6	4.0	2.1[a]
	Plant 7	1.6[a]	5.0
Shaanxi province	Plant 8	4.1	6.3
	Plant 9	3.3	7.0
Zhejiang province	Plant 10	1.6[a]	2.1[a]
	Plant 11	N.D.[b]	N.D.[b]
Henan province	Plant 12	4.7	10.7
	Plant 13	1.6[a]	4.3
Shanghai	Plant 14	1.6[a]	N.D.[b]
Guangdong	Plant 15	N.D.[b]	N.D.[b]
Hubei province	Plant 16	1.6[a]	4.3

[a] Sample peaks with $3 < S/N < 10$ were calculated as 1/2 MQL.
[b] Not detected.

3.4. Method performance

To evaluate the performance of the optimized method, linearity, sensitivity, recovery and accuracy were taken into account. Results from validation procedures of the analytical method are shown in Table 4. Least squared regression analysis showed good linearity ($R^2 > 0.993$) of the standard calibration curve within the range of 1–100 ng/mL for all analytes in MeOH. MQLs ranged from 0.6 (8:2 tri-PAP) to 5.1 (PFHxPA) ng/g when analyzing 0.3 g sludge sample, while EQLs were between 0.7 (8:2 tri-PAP) and 20.1 (10:2 di-PAP) pg. The total recoveries of all target analytes were evaluated at low (16.7 ng/g) and high (167 ng/g) spiking levels. Comparable recoveries were found at low (29–102%) and high spiking levels (24–107%) for most analytes, which indicated that the extraction method was appropriate for a wide concentration range. The low recovery of PFHxPA at 16.7 ng/g spiking level (30%) compared with the 167 ng/g spiking level (88%) might partly due to the high MQL of PFHxPA (5.1 ng/g). To the best of our knowledge, this is the first paper to present a method for extracting mono-PAPs and tri-PAPs from sludge. The PFPA and di-PAP recoveries (88–107% and 54–100%, respectively) are better than the previously reported ones (75–82% for PFPAs, 38–53% for di-PAPs) [27,28]. The relative standard deviations (RSD, $n=3$) at both spiking levels were all less than 15%, and for most of the analytes, the RSDs were less than 10%. These results indicated that our optimized extraction method could be applied to the analysis of PAPs and PFPAs in sludge samples.

3.5. Application in environmental samples

The optimized method was applied to 16 sludge samples collected from different WWTPs in seven provinces of China for a primary investigation on the occurrence of the PAPs and PFPAs

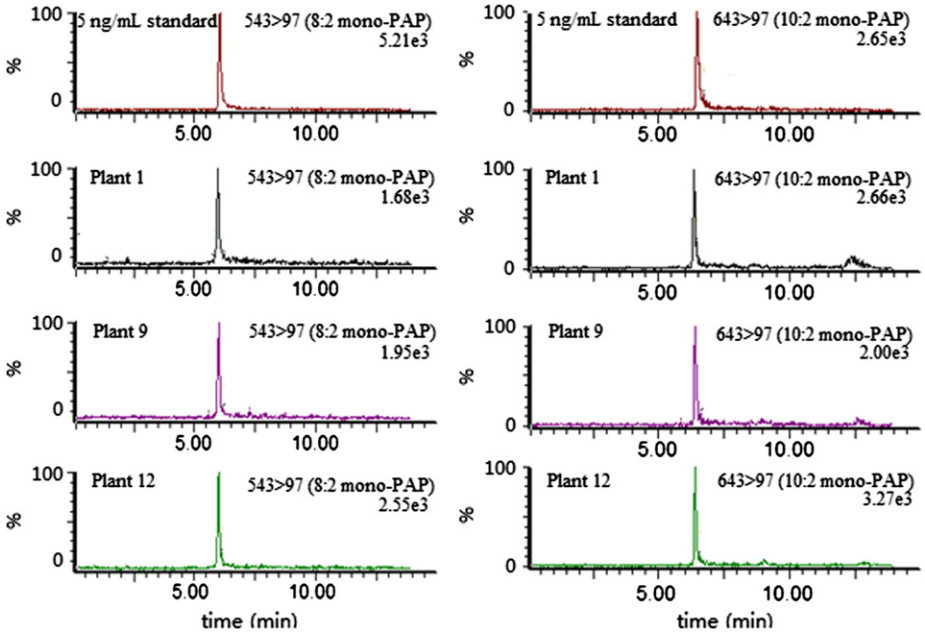


Fig. 4. LC–MS/MS MRM chromatograms of detected analytes. Left panels show 8:2 mono-PAP while right ones are for 10:2 mono-PAP. The top one is for 5 ng/mL standard while the bottom three are from sludge samples.

in the relevant areas. One procedural blank was included in each batch of eight samples to check for possible contaminations in the pretreatment processes. Before injection, 5 ng 6:2 di-PAP-d4 and Cl-PFHxPA were added to the sample extracts as the internal standards for di-PAPs and PFHxPA. No blank residue was found and the measured analyte concentrations in the samples were not spike recovery corrected. Only 8:2 mono-PAP and 10:2 mono-PAP could be detected in the samples with concentrations ranging from $<$MQL to 4.7 ng/g for 8:2 mono-PAP and from $<$MQL to 10.7 ng/g for 10:2 mono-PAP (Table 5). Representative chromatograms of the detected analytes are shown in Fig. 4. The detection of mono-PAPs in sewage sludge suggested their use in commercial products at the sampling locations. No di-PAPs were detected, which was different from previously reported high di-PAP concentrations (47–200 ng/g) in sewage sludge in Canada [27]. Meanwhile, none of tri-PAP and PFPA homologs were detected in the collected sludge samples. The difference in concentrations and composition profiles of target PAPs and PFPAs in sewage sludge among different studies could indicate different usage of PAPs and PFPAs among different regions. To our knowledge, this is the first study reporting the occurrence of 8:2 mono-PAP and 10:2 mono-PAP in sewage sludge in China.

4. Conclusions

A new pretreatment method was developed for the determination of eight PAPs and three PFPAs in sludge samples by optimization of the solvent extraction and clean-up strategies. ACN/HAc (1:1, v/v) was used to extract mono-, di-PAPs and PFPAs from sludge samples, and WAX cartridge in-line coupled with ENVI-Carb was selected for the subsequently clean-up and enrichment procedures. For tri-PAPs, ultrasonically extracted by ACN/THF (1:1, v/v) and cleaned by MAX in-line coupled with ENVI-Carb were chosen. The method was successfully applied to analyze 11 target compounds in 16 sludge samples from seven provinces of China. Only 8:2 mono-PAP and 10:2 mono-PAP were found in the sludge samples with a range from $<$MQLs to 10.7 ng/g. The developed method could be used to evaluate the presence and distribution of PAPs and PFPAs from potential point sources such as WWTPs, which would provide a better understanding of the environmental fates and transport of the phosphate-containing polyfluorinated chemicals.

Acknowledgments

This work was jointly supported by the National Natural Science Foundation (21207140, 20890110, 20621703, and 21177147) and the National Basic Research Program of China (2009CB421605). Further support was given by the External Cooperation Program of Chinese Academy of Sciences Grant no. GJHZ1202.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2013.02.063.

References

[1] C.A. Moody, J.A. Field, Environ. Sci. Technol. 34 (2000) 3864–3870.
[2] B.D. Key, R.D. Howell, C.S. Criddle, Environ. Sci. Technol. 31 (1997) 2445–2454.
[3] 3M, Fluorochemical Use, Distribution and Release Overview, U.S. Environmental Protection Agency Docket AR 226-0550, 1999.
[4] Fluoropolymer Manufacturers Group, Detecting and Quantifying Low Levels of Fluoropolymer Polymerization Aids—A Guidance Document, The Society of the Plastics Industry, Inc., Washington, DC, 2003.
[5] M. Shoeib, T. Harner, P. Vlahos, Environ. Sci. Technol. 40 (2006) 7577–7583.
[6] D. Cao, Z.D. Wang, C.G. Han, L. Cui, M. Hu, J.J. Wu, Y.X. Liu, Y.Q. Cai, H.L. Wang, Y.H. Kang, Talanta 85 (2011) 345–352.
[7] S. Taniyasu, K. Kannan, Y. Horii, N. Hanari, N. Yamashita, Environ. Sci. Technol. 37 (2003) 2634–2639.
[8] N. Yamashita, K. Kannan, S. Taniyasu, Y. Horii, G. Petrick, T. Gamo, Mar. Pollut. Bull. 51 (2005) 658–668.
[9] C.P. Higgins, J.A. Field, C.S. Criddle, R.G. Luthy, Environ. Sci. Technol. 39 (2005) 3946–3956.
[10] J.P. Giesy, K. Kannan, Environ. Sci. Technol. 35 (2001) 1339–1342.
[11] J.G. Li, F.F. Guo, Y.X. Wang, J.Y. Liu, Z.W. Cai, J.L. Zhang, Y.F. Zhao, Y.N. Wu, J. Chromatogr. A 1219 (2012) 54–60.
[12] J.M. Conder, R.A. Hoke, W.W. De, M.H. Russell, R.C. Buck, Environ. Sci. Technol. 42 (2008) 995–1003.
[13] M.F. Simcik, J. Environ. Monit. 7 (2005) 759–763.
[14] G.L. Kennedy Jr., J.L. Butenhoff, G.W. Olsen, J.C. O'Connor, A.M. Seacat, R.G. Perkins, L.B. Biegel, S.R. Murphy, D.G. Farrar, Crit. Rev. Toxicol. 34 (2004) 351–384.
[15] C. Lau, J.R. Thibodeaux, R.G. Hanson, J.M. Rogers, B.E. Grey, M.E. Stanton, J.L. Butenhoff, L.A. Stevenson, Toxicol. Sci. 74 (2003) 382–392.
[16] J.W. Martin, S.A. Mabury, P.J. O'Brien, Chem.–Biol. Interact. 155 (2005) 165–180.
[17] L. Xu, D.M. Krenitsky, A.M. Seacat, J.L. Butenhoff, M.W. Anders, Chem. Res. Toxicol. 17 (2004) 767–775.
[18] C.R. Sun, H.Z. Sun, Y.Q. Lai, J.J. Zhang, Z.W. Cai, Anal. Chem. 83 (2011) 5822–5826.
[19] G.W. Olsen, J.M. Burris, J.H. Mandel, L.R. Zobel, J. Occup. Environ. Med. 41 (1999) 799–806.
[20] T. Ruan, Y.W. Wang, Q.H. Zhang, L. Ding, P. Wang, G.B. Qu, C. Wang, T. Wang, G.B. Jiang, Environ. Sci. Technol. 44 (2010) 5755–5761.
[21] C.J. Young, M.D. Hurley, T.J. Wallington, S.A. Mabury, J. Phys. Chem. A 112 (2008) 13542–13558.
[22] J.M. Armitage, U. Schenker, M. Scheringer, J.W. Martin, M. Macleon, I.T. Cousins, Environ. Sci. Technol. 43 (2009) 9274–9280.
[23] U.S. FDA, Indirect Food Additives: Paper and Paperboard Components, Code of Federal Regulations, 21 CFR 176.170, U.S. Food and Drug Adminstration, U.S. Government Printing Office, Washington, DC, 2003.
[24] T.H. Begley, K. White, P. Honigfort, M.L. Twaroski, R. Neches, R.A. Walker, Food Addit. Contam. 22 (2005) 1023–1031.
[25] T.H. Begley, W. Hsu, G. Noonan, G. Diachenko, Food Addit. Contam. 25 (2008) 384–390.
[26] Perfluorinated Substances and Their Uses in Sweden, Report Nr 7/06, 2006. ISSN: 0284-1185.
[27] J.C. D'eon, P.W. Crozier, V.I. Furdui, E.J. Reiner, E.L. Libelo, S.A. Mabury, Environ. Sci. Technol. 43 (2009) 4589–4594.
[28] X. Esparza, E. Moyano, J. de Boer, M.T. Galceran, S.P.J. van Leeuwen, Talanta 86 (2011) 329–336.
[29] H. Lee, J. D'eon, S.A. Mabury, Environ. Sci. Technol. 44 (2010) 3305–3310.
[30] J.C. D'eon, S.A. Mabury, Environ. Health Perspect. 119 (2011) 344–350.
[31] H. Lee, A.O.D. Silva, S.A. Mabury, Environ. Sci. Technol. 46 (2012) 3489–3497.
[32] C.P. Higgins, R.G. Luthy, Environ. Sci. Technol. 40 (2006) 7251–7256.
[33] M. Llorca, M. Farré, Y. Picó, D. Barceló, J. Chromatogr. A 1218 (2011) 4840–4846.
[34] C.R. Powley, S.W. George, T.W. Ryan, R.C. Buck, Anal. Chem. 77 (2005) 6353–6358.
[35] A.B. Gómez, S. Rubio, S.v. Leeuwen, J. Chromatogr. A 1217 (2010) 5913–5921.
[36] H. Lee, S.A. Mabury, Environ. Sci. Technol. 45 (2011) 8067–8074.
[37] H.H. Ding, H. Hui, M. Yang, J.Y. Hu, J. Chromatogr. A 1227 (2012) 245–252.
[38] Commission Decision 2002/657/EC of 12 August 2002 Implementing Council Directive 96/23/EC Concerning the Performance of Analytical Methods and the Interpretation of Results, OJ No. L 221, 2002.
[39] 〈http://ibmlc2.chem.uga.edu/sparc/〉, 2012 (accessed 09.06.12).
[40] S. Ullah, T. Alsberg, U. Berger, J. Chromatogr. A 1218 (2011) 6388–6395.
[41] N. Wang, J.X. Liu, R.C. Buck, S.H. Korzeniowski, B.W. Wolstenholme, P.W. Folsom, L.M. Sulecki, Chemosphere 82 (2011) 853–858.
[42] J.W. Washington, J.J. Ellington, T.M. Jenkins, J.J. Evans, J. Chromatogr. A 1154 (2007) 111–120.