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Liquid chromatography/mass spectrometry analysis of perfluoroalkyl carboxylic acids and perfluorooctanesulfonate in bivalve shells: Extraction method optimization

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ABSTRACT

Different extraction methods, including extraction by organic solvents with and without acetic acid digestion, and mixed inorganic acid digestion coupled with solid phase extraction (SPE), were developed for the analysis of perfluorinated carboxylic acids (PFCAs) and perfluorooctanesulfonate (PFOS) in bivalve shells. The extracts were separated, identified and quantified by liquid chromatography–electrospray ionization–tandem mass spectrometry (LC–ESI–MS/MS). The method utilizing mixed acid digestion coupled with SPE performed more efficiently than other extraction methods. Matrix recoveries of the optimized methods ranged from 92% to 104%, with limits of detection of 0.05–0.43 ng/g. The optimized method was successfully applied to the analysis of PFCAs and PFOS in shell samples of two bivalves from Bohai Bay, China. PFCAs and PFOS concentrations in the shells ranged from 0.3 ng/g to 4.1 ng/g, 1–50 times lower than those in the soft tissues of bivalves for most target analytes. No relationship between PFCAs and PFOS in shells and in soft tissues was found; this is explained by the different contaminant uptake mechanism of shells and soft tissues.

1. Introduction

Perfluorinated compounds (PFCs) occur in numerous consumer products as active ingredients and impurities, and as degradation products of derivatives, e.g. in oil, water and stain repellents for paper, leather, textiles, and fire retardant foams [1]. These chemicals are released into the environment during production and application and also after waste disposal [2,3]. The carbon–fluorine bond is the most stable single bond in organic chemistry, and PFCs are thermally and chemically more stable than the analogue hydrocarbons. Since the global occurrence of PFCs was first reported in 2001 [4], a number of studies have been conducted from analytical and approaches [5–9]. Perfluorinated carboxylic acids (PFCAs) and perfluorinated alkylsulfonates (PFASs), such as perfluorooctanoic acid (PFOA) and perfluorooctanesulfonate (PFOS), are among the most important groups of PFCs. They have been reported to be of toxicological concern, and globally distributed [2,10–14].

Studies show that PFCAs and PFASs, unlike traditional persistent organic pollutants (POPs), are proteinophilic [5,6]. Therefore, these chemicals prefer to accumulate in protein-rich tissues, such as livers and bloods, rather than in fat tissues [5]. Some extraction methods have been developed and applied to analyze PFCs in the soft tissues of biological samples, including liquid–liquid extraction (LLE) [15,16], ion pair extraction (IPE) [17–20], liquid–solid extraction (LSE) [15,16,21–23], and solid phase extraction (SPE) [24–28]. Few studies have addressed methods for analyzing the distribution of PFCs in "hard tissue", i.e. biomineral matrices (bone, tooth, shell, egeshell, otolith, etc.), despite the non-negligible amount of organic matrices, including proteins that do exist in these hard tissues.

As a feed additive, shell powders can enter the bodies of pets, and are also like to enter the human food supply when used to feed livestocks or poultries [29]. Sometimes, shell powders are also directly consumed by humans when used as food additives or a source of medicinal calcium. Methods used for biological soft tissue or soil/sediment extraction might not be suitable for extracting PFCs from shells because the contaminants gradually enter the crystal structure of shells through the biomineralisation process. However, no studies on method for analyzing the contamination levels of PFCs in shells have been conducted until now.

In this study, different extraction methods, including organic solvent extraction, acetic acid digestion coupled with solvent extraction, and mixed inorganic acid digestion coupled with SPE, were tested for the analysis of PFCAs (C4–C12) and PFOS in shells, and the extraction parameters were optimized. The optimum method was applied to detect PFCAs and PFOS in the shells of two

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Table 1
Details of samples analyzed in this study.

Sample name	%OM[a] (dry-mass basis)	Shell weight per valve (g, dry-weight)	Soft tissue weight per bivalve (g, dry-weight)
Shell powder	0.73 ± 0.02	–	–
Clam (*Clinocardium californiense*)[b]	0.73 ± 0.06	11.32 ± 1.03	1.85 ± 0.18
Razor clam (*Solen strictus*)[b]	1.32 ± 0.05	3.08 ± 0.35	0.94 ± 0.18

[a] Organic matter content (%OM) was detected by the method of potassium dichromate oxidation titration.
[b] Number of bivavle samples was 6.

bivalves from Bohai Bay, China, and the target compound concentrations in the soft tissues of the bivalves were also determined for comparative purpose.

2. Experimental

2.1. Chemicals

Standards of PFBA (99.5%, Sigma–Aldrich Chemical, St Louis, MO, USA), PFPeA (95%, Tokyo Kasei, Tokyo, Japan), PFHxA (98%, Matrix Scientific, Columbia, SC, USA), PFHpA (98%, Matrix Scientific, Columbia, SC, USA), PFOA (98%, Strem Chemicals, Bischheim, France), PFNA (98%, Fluorochem, Derbyshire, UK), PFDA (98%, Fluorochem, Derbyshire, UK), PFUnA (96%, Matrix Scientific, Columbia, SC, USA), PFDoA (95%, Sigma–Aldrich Chemical, St Louis, MO, USA), and PFOS (99%, Sigma–Aldrich Chemical, St Louis, MO, USA) were used in this study. Besides, $^{13}C_8$-labeled PFOA (Cambridge Isotope Laboratories, Andover, MA, USA) and $^{13}C_4$-labeled sodium PFOS (Wellington Laboratories, Guelph, Ontario, Canada) were used as the internal standards in analysis. Methyl tert-butyl ether (MTBE), acetone, methanol, acetic acid (CH_3COOH) and ammonium acetate (CH_3COONH_4) were of HPLC reagent grade and purchased from Tianjin Guangfu Fine Chemical Research Institute.

2.2. Shell samples preparation

Explorative evaluations were performed using a commercial natural shell powder purchased from a shell flour mill, which uses oyster and clam shells as raw materials. After method development was completed, the method was used to determine PFCAs and PFSA concentrations in the shells of two bivalves: clams (*Clinocardium californiense*) and razor clams (*Solen strictus*), which were caught live from Bohai Bay, China (see Figs. S1 and S2 in the supplementary materials). In order to compare the distribution of PFCs in hard and soft tissues of the bivalves, the concentrations of PFCAs and PFOS in the soft tissues of the clams and razor clams were also determined.

Six clams and six razor clams were size-selected for sample-to-sample homogeneity. Their shells were opened by knife, and the animals were excised completely. The shells of clam and razor clam were washed to remove adhering organisms and rest of sediments on the outer part of the valves. The pairs of valve shells were weighted and then ground to powder respectively, using a stainless steel grinder. The soft tissues of the animals were cleaned up to remove silts, and they were freeze-dried. Details of the samples analyzed in this study are tabulated in Table 1.

2.3. Extraction tubes and beakers cleaning

Polypropylene (PP) centrifuge tubes (15 ml and 50 ml) with screw caps (CNW Technologies GmbH, Düsseldorf, German) and quartz beakers (250 ml) were used in the extractions. Preliminary experiments indicated that the dissolution of PFC compounds from these vessels was neglected. Prior to use, solvent-soluble contaminants in the tubes were removed by adding MTBE or methanol to the tubes and rotating them on a rotator for 24 h. The beakers were cleaned by soaking in 50% HNO_3 solution for 8 h before use.

2.4. Sample extraction methods

2.4.1. Extraction by organic solvents with and without acetic acid digestion

Studies have shown MTBE, acetone, and methanol to be efficient for the extraction of PFCs from biota and sediment samples [9]. To evaluate the capability of different solvents for extracting PFCAs and PFSAs from shells, 5 g of commercial natural shell powder was extracted with 10 ml of the organic solvent (MTBE, acetone, or methanol) in an 15 ml PP centrifuge tube at 30 °C for 12 h in a constant-temperature shaker. Subsequently, the extract was centrifuged at 4000 rev/min for 15 min and the supernatant was replaced with 5 ml of fresh solvent. The extract was combined with the first one after another 12 h of extraction. The combined extract was spiked with 5 ng of ^{13}C PFOA and 5 ng of ^{13}C PFOS before it was evaporated to dryness under a gentle nitrogen stream and dissolved in 1 ml of methanol. Before analysis, the samples were filtered through a 0.2-μm nylon filter and transferred to a 2 ml HPLC autosampler vial.

In subsequent experiments, MTBE was used as the extraction solvent. In order to improve extraction, acetic acid was added to the shell powder samples prior to MTBE extraction to destroy the carbonate structure of the shell. Five grams of shell powder in triplicate was placed in 50 ml PP centrifuge tubes, with 5 ml, 10 ml, or 20 ml of acetic acid added, respectively. They were mixed for 30 min in a shaker. Then 10 ml of MTBE was added and extraction was conducted as described above. The extracts spiked with 5 ng of ^{13}C PFOA and 5 ng of ^{13}C PFOS were evaporated to dryness under a gentle nitrogen stream, because acetic acid and MTBE are mutual soluble and volatile. Then 1 ml of methanol was used to dissolve the analytes before analysis.

2.4.2. Extraction by SPE after acid digestion

A mixture of HCl (10 ml, 20%) and HNO_3 (5 ml, 50%) was added to 5.0 g of shell powder in a 250 ml quartz beaker, and which was then shaken. The beaker was ultrasonicated at 30 °C for 30 min. Then, Milli-Q water was added to dilute the acid extract to 100 ml, and the solution pH was adjusted to 6 with NaOH solution. Before SPE extraction, the samples were filtered using a 0.45-μm nylon filter and spiked with 5 ng of ^{13}C PFOA and 5 ng of ^{13}C PFOS. The samples were then passed through Oasis WAX (150 mg, 6 cm^3) cartridges (Waters Corporation, Milfor, MS, USA) pre-conditioned with 4 ml of methanol (containing 0.1% ammonium hydroxide) and 4 ml of Milli-Q water [30]. A flow rate of 1 drop/s was maintained through the loading. The cartridges were then washed with 25 mM sodium acetate buffer solution (pH 4) and dried completely under vacuum. The target compounds were eluted in 4 mL of methanol (containing 0.1% ammonium hydroxide) into a 15 mL PP centrifuge tube and concentrated under nitrogen to a final volume of 1 mL. These extracts were filtered using a 0.2-μm nylon filter into an autosampler vial with a polypropylene cap.

After the mixed acid digestion, residues of the shell powders were collected and freeze-dried, and then extracted with 2 ml of MTBE at 30 °C for 12 h. The extracts were evaporated and dissolved in 1 ml of methanol before analysis.

Table 2
Analyte formulas, molecular weights, acronyms, ions monitored for LC–MS/MS, and conditions of cone voltages and collision energies.

Analyte formula	Molecular weight	Acronym (short formula)	Precursor ion (m/z)	Product ion (m/z)	Cone voltage (V)	Collision energy (V)
$CF_3(CF_2)_2COOH$	214	PFBA	213	169	12	10
$CF_3(CF_2)_3COOH$	264	PFPeA	263	219	10	11
$CF_3(CF_2)_4COOH$	314	PFHxA	313	269	12	11
$CF_3(CF_2)_5COOH$	364	PFHpA	362.8	319	13	11
$CF_3(CF_2)_6COOH$	414	PFOA	412.8	369	14	11
$CF_3(CF_2)_7COOH$	464	PFNA	463	419	14	13
$CF_3(CF_2)_8COOH$	514	PFDA	513	469	16	13
$CF_3(CF_2)_9COOH$	564	PFUnA	563	519	18	12
$CF_3(CF_2)_{10}COOH$	614	PFDoA	613	569	18	13
$CF_3(CF_2)_7SO_3H$	500	PFOS	499	80	55	45
$^{13}CF_3(^{13}CF_2)_6{}^{13}COOH$	422	$^{13}C_8$-PFOA	421	376	14	11
$CF_3(CF_2)_3$ ($^{13}CF_2)_4SO_3Na$	526	$^{13}C_4$-PFOS	503	80	55	45

The shell powders of clams and razor clams were also extracted using this method, with 50 μL of 100 ng/ml ^{13}C PFOA and ^{13}C PFOS mixture solution spiked into 5 g of shell powders prior to digestion.

2.4.3. Soft tissue extraction

The PFCs in the soft tissues of the bivalves were analyzed using MTBE extraction coupled with WAX-SPE, a modified version of the method reported by Yeung et al. [28]. In brief, 1 g of freeze-dried soft tissues was homogenized with MTBE, ultrasonicated for extraction, and then centrifuged. The supernatant was evaporated, dissolved with methanol, and diluted with Milli-Q water to a final volume of about 100 ml for WAX SPE cleanup. More details and discussion of recovery of the soft tissue extraction are described in the supplementary materials. It should be noted that, all PFCs concentrations in shell or soft tissue samples are in dry weight in this study.

2.5. LC/MS analytical methods

The extracts were analyzed on a model Alliance 2695 Separations Module equipped with a Quattro Micro™ atmospheric pressure ionization (API) tandem quadrupole mass spectrometer (MS1 quadrupole–MS2 quadrupole; Waters Company, USA). For separation, an X-Terra MS C_{18} column (2.1 mm i.d. × 150 mm, 5 μm, Waters, Ireland) was used. Optimum separation was achieved with a binary mobile phase at a flow rate of 250 μL/min. The two mobile phases were 2.5 mM ammonium acetate methanol solution (A) and 2.5 mM ammonium acetate water solution (B), with an initial gradient of 10% A. At 0.8 min the gradient increased to 60% A, and then continuously increased to 100% at 12.8 min before reverting to original conditions at 14.3 min. Column temperature was maintained at 40 °C. The mass chromatogram of PFCAs is shown in Fig. 1.

The HPLC system was interfaced to the API tandem quadrupole mass spectrometer operated in electrospray negative mode. Instrumental parameters were optimized to transmit the $[M-H]^-$ ion for all analytes. The ionization source working parameters were as follows: capillary and sample cone voltages of 3.25 kV and 10–18 V (Table 2); source and desolvation temperatures of 100 and 300 °C; and cone and desolvating gas flows (nitrogen) of 20 and 600 l/h. The collision gas was argon, and the collision energies are listed in Table 2.

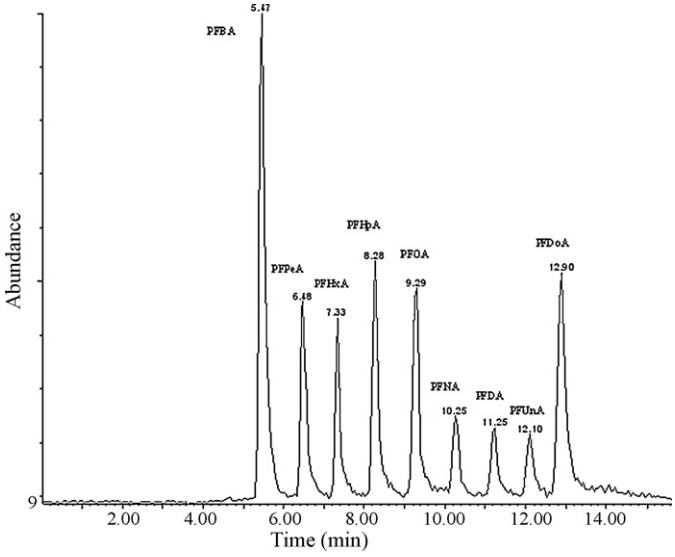

Fig. 1. Mass chromatograms of PFCAs in standard mixture (10 ng/ml).

Multiple responses monitoring (MRM) analysis was used to verify analyte identity. For each analyte, quantification was based on the response of a single product ion (Table 2). Internal calibration was used to quantify analytes. $^{13}C_8$-PFOA and $^{13}C_4$-PFOS were used as the internal standards for PFCAs and PFOS, respectively. The

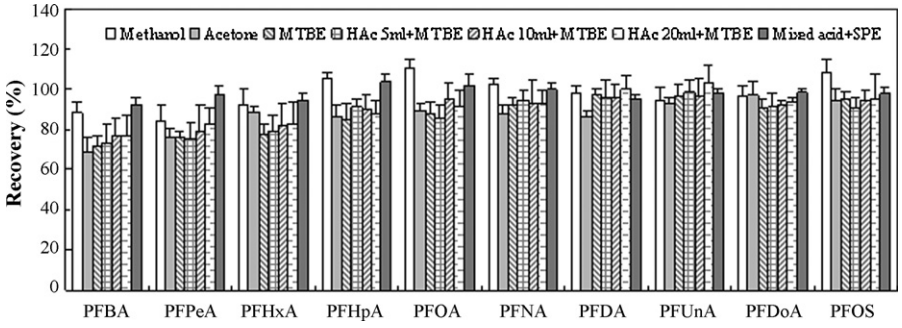

Fig. 2. Recovies of PFCAs and PFOS in shell powders analyzed by organic solvents extraction, acetic acid (HAc) digestion coupled with MTBE extraction, and mixed inorganic acid degestion coupled with SPE.

analytical procedure was carried out in triplicates to evaluate the precision.

3. Results and discussion

3.1. Impurities in the solvents

No significant amount of the target contaminants was found in most chemicals used in this study (water, MTBE, methanol, acetic acid, HNO_3, HCl, NaOH, ammonium hydroxide, sodium acetate, and Milli-Q water). However, 3.09 ng/ml PFOA and 0.74 ng/ml PFBA were detected in acetone. Therefore, acetone was distilled with a quartz distiller before use, which purified PFOA and PFBA to <0.02 ng/ml. The material of the WAX SPE column was confirmed to be free of PFCAs and PFOS using a control elution experiment.

3.2. Extraction using organic solvents

The results of shell powder extraction by three organic solvents, MTBE, acetone, and methanol, are given in Table 3. For the three organic solvents, MTBE was the most efficient in extracting PFCAs and PFOS from shell powder. Six PFCAs were detected in the MTBE extracts, while only PFOA was detected when methanol was used. Acetone proved unsuitable for extracting PFCAs and PFOS from the shell powder, because no target contaminants at concentrations above the LOD were detected in the acetone extracts.

The typical biomineral calcium carbonate structure of the shells was thought to interfere with the extraction of chemicals bound to the organic matrix in the shell microstructure. Therefore, acetic acid was added to the shell powder to destroy the mineral structure before MTBE extraction. According to Eq. (1) (below), 6 g of acetic acid (about 5.72 ml at 25 °C) is needed to react with 5.0 g $CaCO_3$. Therefore, the use of 5, 10, and 20 ml of acetic acid was tested to identify the effect of acetic acid on extraction. The results show that the adding of acetic acid significantly improves extraction efficiency for most target analytes (Table 3). For example, the concentrations of PFOA and PFOS detected by MTBE extraction increased from 1.04 to 2.14 ng/g and <0.43 to 1.06 ng/g, respectively, when 20 ml of acetic acid was added before MTBE extraction. However, it seems that the calcium carbonate structure cannot be destroyed completely, even by 20 ml of acetic acid, because the increase in residue weight is relatively small (Table 3). According to Eq. (1), a complete reaction of acetic acid with 5.0 g of $CaCO_3$ should result in 7.9 g of $(CH_3COO)_2Ca$. This suggests that only a small fraction of the $CaCO_3$ reacted with the acetic acid before MTBE extraction

$$2CH_3COOH + CaCO_3 \rightarrow (CH_3COO)_2Ca \downarrow + H_2O + CO_2\uparrow \quad (1)$$

3.3. Mixed acid digestion coupled with SPE

The amount of mixed nitric/hydrochloric acid slightly exceeded the amount necessary to react with 5.0 g of $CaCO_3$, so no insoluble calcium salt remained. After dilution and SPE extraction, the detected concentrations of PFCAs (C4–C12) were 0.89, 0.98, 1.66, 2.30, 3.35, 2.39, 0.17, 0.82, and 0.91 ng/g respectively, while the detected concentration of PFOS was 1.71 ng/g (Table 3). The residue was extracted with 2 ml of MTBE, but no target analytes were detected in it. The method of "inorganic acid + SPE" appeared to be more efficient than organic solvent extraction, especially for the PFCAs with long perfluoroalkyl chain (C7–C12), and PFOS. This might be due to the completely destruction of the biomineral structure of the shells by the inorganic acids (HNO_3 and HCl), which released those chemicals bound to the organic matrix in the carbonate structure.

Table 3
Quantification of PFCAs and PFOS in commercial shell powder (ng/g) by different extraction methods. Statistics for three complete parallel extractions with three repeated measures for each method[a].

Extraction method	Residue weight (g)	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoA	PFOS
MTBE (10 ml + 5 ml)	4.95	0.25 ± 0.11	0.22 ± 0.02	0.61 ± 0.33	0.53 ± 0.25	1.04 ± 0.37	<LOD	<LOD	<LOD	0.18 ± 0.08	<LOD
Acetone (10 ml + 5 ml)	4.95	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
Methanol (10 ml + 5 ml)	4.76	<LOD	<LOD	<LOD	<LOD	0.79 ± 0.19	<LOD	<LOD	<LOD	<LOD	<LOD
Acetic acid (5 ml) + MTBE (10 ml + 5 ml)	5.47	0.63 ± 0.03	1.35 ± 0.03	1.12 ± 0.02	<LOD	1.34 ± 0.01	0.92 ± 0.05	<LOD	<LOD	<LOD	<LOD
Acetic acid (10 ml) + MTBE (10 ml + 5 ml)	5.73	0.67 ± 0.20	1.15 ± 0.08	1.04 ± 0.06	0.28 ± 0.04	1.29 ± 0.08	0.64 ± 0.02	<LOD	<LOD	<LOD	0.49 ± 0.04
Acetic acid (20 ml) + MTBE (10 ml + 5 ml)	5.82	0.74 ± 0.03	1.34 ± 0.03	1.30 ± 0.22	1.59 ± 0.12	2.14 ± 0.39	1.42 ± 0.07	<LOD	<LOD	0.11 ± 0.01	1.06 ± 0.05
Mix acid [b] + SPE	0.53	0.89 ± 0.09	0.98 ± 0.01	1.66 ± 0.33	2.30 ± 0.95	3.35 ± 0.37	2.39 ± 1.25	<LOD	0.82 ± 0.68	0.91 ± 0.72	1.71 ± 0.18
MTBE (2 ml) [c]		<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

[a] Concentrations of target analytes in both shell and soft tissue samples were not corrected for the recovery percentages.
[b] Mixed acid contains HNO_3 (5 ml, 50%) and HCl (10 ml, 20%).
[c] For residues of mixed acid digestion.

3.4. Recoveries of different extraction methods for spiked samples

For each extraction method, triplicate samples of shell powder (5 g) were spiked with 100 μL of 100 ng/ml mixture solution (containing PFCAs and PFOS), and recoveries of two-step extraction for various PFCAs and PFOS were determined (Fig. 2). The recoveries indicated that methanol, acetone, and MTBE extraction method for long chain PFCAs (C10–C12), were similar ranging from 86% to 97%. For PFOS and some PFCAs (>C7) added into the shell powders, methanol, acetone, and MTBE extraction were comparable. Methanol extraction gave better recoveries for some short chain PFCs (C4–C9), with a recovery range of 85–110%. Acetone and MTBE resulted in recoveries ranging from 68% to 92% for these analytes. Lower recoveries of FPBA, PFPeA, and PFHxA (C4-C6) might be due to the evaporation during the drying step. The relatively high recoveries mean that the added chemicals cannot bind to the matrix of shell structure, and can be extracted efficiently by these methods. Pre-adding of acetic acid seems not to affect the recoveries of the spiked target analytes.

For the "mixed acid + SPE" method, the recoveries of all the target compounds were in the range of 92–104%, with a better RSD lower than 6% ($n = 3$). Compared with the organic solvents extraction, the increased recoveries of short chain PFCs (C4–C9) might be attributed to the much shorter evaporation stage in this method. For long chain analytes (C10–C12), the recoveries of the two methods are comparable.

3.5. Detection limits

Limits of detection (LODs) of target analytes based on three of signal-to-noise (S/N) ratio are 0.07 (PFBA), 0.06 (PFPA), 0.05 (PFHxA), 0.05 (PFHpA), 0.08 (PFOA), 0.21 (PFNA), 0.23 (PFDA), 0.35 (PFUnA), 0.07 (PFDoA) and 0.43 (PFOS) ng/g, when 5 g samples of shell powder were extracted by mixed acid digestion coupled with SPE. Limits of quantification (LOQs), defined as S/N ratio of 10 are 0.24 (PFBA), 0.21 (PFPA), 0.17 (PFHxA), 0.18 (PFHpA), 0.27 (PFOA), 0.70 (PFNA), 0.77 (PFDA), 1.17 (PFUnA), 0.23 (PFDoA) and 1.43 (PFOS) ng/g, respectively.

3.6. Application to environmental samples

The method of mixed acid digestion followed by SPE was used to analyze PFCAs and PFOS in clam (*C. californiense*) and razor clam (*S. strictus*) shells from Bohai Bay, China. From the mass chromatogram of shells of a razor clam (Fig. S3 in the supplementary materials), it can be seen that the interference of the shell matrix was limited when the "acid digestion + SPE" method was used. It indicated a relatively high selectivity of this method. Table 4 shows the contamination levels of PFCAs and PFOS in the shells of these two kinds of bivalve. For clams and razor clams, the concentration ranges of the target PFCs were 0.5–2.3 and 0.3–4.0 ng/g, respectively. Among the PFCAs, the highest concentration found was PFPeA (C5), followed by PFBE (C4), PFHxA (C6), and PFHpA (C7). Hence, as the alternatives of PFOA (C8), greater importance should be attached to the contamination of PFCAs with shorter perfluoroalkyl chain.

Furthermore, PFCAs with perfluoroalkyl chain length shorter than 10 were detected in the soft tissue samples of the two bivalves in concentrations ranging from 4.5 to 53.3 ng/g dry-weight (Table 4). These concentration levels were comparable to those reported in aquatic wildlife in adjacent waters [27,31]. The typical PFCs, PFOA and FPOS, were detected in both soft tissues of both bivalves at moderate concentration levels (4.5–5.0 ng/g for PFOA and 3.6–4.6 ng/g for PFOS) compared to other analytes. Contrary to our expectations, concentrations of PFCAs with long perfluoroalkyl chains (PFDA, PFUnA, and PFDoA) were low in all soft tissue samples. However, this composition distribution agrees with the

Table 4
Quantification of PFCAs and PFOS in shells and soft tissues of clam and razor clam (ng/g, dry-weight). Statistics for six parallel extractions with three repeated measures for each kind of bivalve.

Sample name	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFNA	PFDA	PFUnA	PFDoA	PFOS
Shells of clam	1.85 ± 0.02	2.39 ± 0.47	1.71 ± 0.15	1.84 ± 0.10	0.57 ± 0.08	1.17 ± 0.14	1.20 ± 0.20	1.15 ± 0.21	1.05 ± 0.15	0.71 ± 0.17
Shells of razor clam	1.40 ± 0.16	4.11 ± 0.16	0.96 ± 0.20	0.57 ± 0.04	1.79 ± 0.11	1.09 ± 0.36	0.82 ± 0.05	0.92 ± 0.15	0.34 ± 0.06	0.62 ± 0.31
Soft tissues of clam	9.68 ± 6.00	10.84 ± 1.90	32.43 ± 3.22	10.61 ± 0.37	4.50 ± 0.13	7.80 ± 2.37	<LOD	<LOD	<LOD	4.55 ± 1.42
Soft tissues of razor clam	7.85 ± 0.70	5.98 ± 3.23	53.28 ± 21.70	4.52 ± 3.80	5.03 ± 3.27	4.54 ± 1.47	<LOD	<LOD	<LOD	3.62 ± 1.17

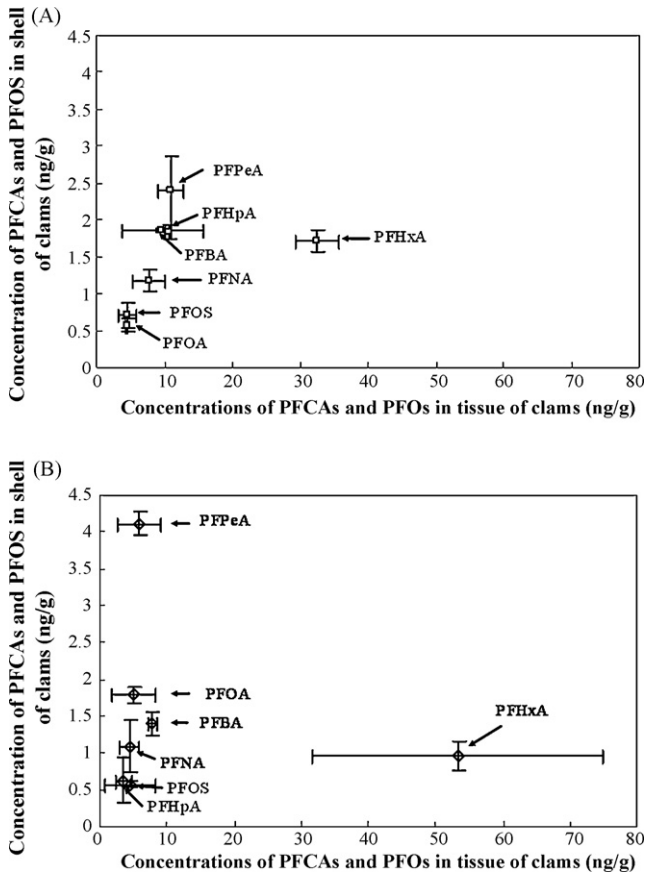

Fig. 3. Detected concentrations of PFACs and PFOS in shell and soft tissue of (A) clam (*Clinocardium californiense)* and (B) razor clam (*Solen strictus). Note*: Concentration units in the figure are in dry weight. The concentrations of PFDA, PFUnA, and PFDoA are not shown in the figures; because they are lower than LOD in the soft tissues of bivalves.

results of our recent investigation of effluents from wastewater treatment plants in Tianjin, a city in Bohai Bay [32]. Except for PFHxA, PFCA concentrations in the shell samples were 1–10 times lower than those of the soft tissues (Table 4). Unlike soft tissues, shells uptake PFCs by adsorption or passive deposition of the target chemicals to the shell organic matrix followed by a biomineralisation process. Therefore, we hypothesize that the contaminants in shells are difficult to release, even if the concentration of contaminants in environments decreased. In the soft tissues of clams and razor clams, PFHxA showed the greatest concentrations among the PFCAs. However, no corresponding high concentration of PFHxA was found in the shells. Therefore, we hypothesize that the emergent exposure to PFHxA happened near the sampling time, or some secondary pollution occurred in the sampling or transport procedure. No linear relationship between concentrations of PFCAs in shells and soft tissues was found (Fig. 3), even ignoring PFHxA. This also proves that while contaminant concentrations in the soft tissue of aquatic wildlife may reflect the current contamination levels in the environments [33–35], shells may be a "record" of contaminants to which a bivalve was exposed during its growth period [36,37]. Furthermore, compared to the PFCs concentrations in the soft tissues, the standard deviations (SDs) of concentrations detected in the shell samples were much smaller (Table 4 and Fig. 3), suggesting that influencing factors, such as individual difference and secondary pollution during sampling and transporting, have a much smaller effect on PFCs uptake by shells. Bivalve shells may therefore be a better biomonitor for monitoring the PFC contamination of aquatic environments.

4. Conclusions

Shells have a special biomineral structure of calcium carbonate crystal mixed with an organic matrix that absorbs and sequestrates contaminants during biomineralisation. Few studies have conducted quantitative analysis of organic contaminants in bivalve shells. PFCAs and PFOS in shells can be extracted efficiently using the "acid digestion coupled with WAX-SPE" method developed in this study. Almost all target analytes were detected in a commercial shell powder and in the shells of two bivalves from Bohai Bay, China. These contaminants may enter the human food supply because shell powders are used as additives in feed, food, or medicine.

The soft tissues of bivalves (soft tissue) are usually used as biomonitors of many contaminants. We found that though the concentrations of most PFCAs and PFOS in shells were lower than in soft tissue, the amount of contaminants in shells and soft tissues of bivalves are comparable, due to the larger weight of shells than soft tissues. Like soft tissue, shells could also be considered as biomonitoring matrix for some organic contaminants, such as PFCs, because (i) smaller individual differences were found in PFCs concentrations among shell samples compared with soft tissue samples; (ii) secondary pollution is less likely to affect shell samples during sampling and transportation; and (iii) the shells was hypothesized to "record" past exposure to contaminants, since the contaminants deposited in the biomineral structures are not subject to release.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chroma.2009.11.074.

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