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Determination of 4-*n*-octylphenol, 4-*n*-nonylphenol and bisphenol A in fish samples from lake and rivers within Hunan Province, China

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ABSTRACT

The presence of endocrine disrupting chemicals such as 4-*n*-octylphenol, 4-*n*-nonylphenol and bisphenol A in environmental samples and artificial products may cause health problems for human and organisms. This developed method was efficient for monitoring of those tracing emerging contaminates and the Plackett-Burman factorial design introduced in extraction step was timesaving. Matrix effect was also considered to ensure proper quantitation. Good linearity ($r^2 > 0.99$), low Method Detection Limits (MDL) and Method Quantitation Limits (MQL) range from 0.18 to 0.54 ng/g and 0.60 to 1.80 ng/g were obtained. Three level spiking experiment (4, 10, 15 ng/g) showed recoveries range from 74% to 113% for 4-*n*-octylphenol and 4-*n*-nonylphenol with RSD range from 2% to 11%. The method was finally applied to the analysis of real fish samples taken from Hunan Province, China, offering a reliable and effective means for monitoring of trace emerging contaminates in fishes largely consumed by residents.

1. Introduction

The occurrence of endocrine disrupting chemicals (EDCs) in the environment has received broad interest over the past decades. Pollutants alkylphenol have been increasingly detected in water, wastewater, soil, sediments and biota [1]. Alkylphenol polyethoxylates, 4-*n*-octylphenol (4-*n*-OP) and 4-*n*-nonylphenol (4-*n*-NP) are both used as raw materials or additives in manufacturing products, bearing the similar structures but differ at their chain length [2]. It was said that these degradative products like OP and NP are more toxic than the parent compound [3]. Bisphenol A (BPA), mainly used in the production of polycarbonate plastics and epoxy resins, is often found in food and beverage containers, some dental fillings, household dust, air and other materials [4,5]. All these three chemicals are known as endocrine disruptors which can cause hormonal dysfunctions in the endocrine system of organism even at very low concentration.

With the widespread use of alkylphenol-related products, large amounts of alkylphenols have been released into different environmental systems. Accordingly, large amount of OP, NP and BPA have been detected in surface water, ranging from 18 to 20 ng/L [6], from 117 to 865 ng/L and from 4 to 377 ng/L [7] respectively. EDCs have been detected in the crops irrigated with simulated reclaimed water containing NP and BPA, indicating the uptake of pollutants by crops [8]. As we all know, fish populations constitute an important part of aquatic ecosystems. Their accumulation of EDC via diffusion across the gills and skin are several times higher than the surrounding water [9], posing risks to ecosystems and human health. Up to 4.6, 18.9, 83.5 ng/g [10] of OP, NP, BPA were detected in muscle tissue (dry weight) of wild fish species from Dianchi Lake, China, and up to 9.67, 8.17, 5.87 μg/g [11] dry weight in various tissues of carp fish samples from Anzali wetland, Iran. Because of a growing consumption of fish worldwide [12], the occurrence and distribution of endocrine disrupters in edible fish species are therefore important for both ecology and human health, especially for Asian people who consumed large amounts of fish in their daily diet [13].

Traditional methods for fish tissue extraction involve Soxhlet extraction [11], pressurized liquid extraction [14] and microwave-assisted extraction [15]. However, these techniques are time-consuming and need large amount of solvents. QuEChERS method, originally developed for extraction of pesticides in fruit and vegetables, is now widely used in multi-matrices like soil [16], river sediments [17], olives [18], milk and honey [19], liver [20] and fish tissues [21] because of its easy operation and timesaving and other advantages as named. However, research of QuEChERS-related publication on analysis of 4-*n*-OP, 4-*n*-NP and BPA in fish tissues was very few. The aim of this study was to develop an efficient method for the analysis of alkylphenols in fish samples which combines the advantages of QuEChERS method and the simplicity of ultrasonic-assisted extraction. To remove the lipids of fish matrices, an

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efficient cleaning up procedure must be taken. The usual methods like gel permeation chromatography (GPC) [22], solid phase extraction (SPE) [23] cartridges are time-consuming and require additional equipment, so different d-SPE sorbent combinations were tested to obtain good recovery. As this phenolic analytes, namely, OP, NP, BPA, are low-volatile and polar, to improve the chromatographic performance and sensitivity, a derivatization step was done by silylation reaction which improves various gas chromatographic parameters such as accuracy, reproducibility, sensitivity and resolution [24]. The final determination was carried out by GC-MS.

The developed method was efficient and validated for the determination of target analytes of 4-*n*-OP, 4-*n*-NP and BPA in real fish samples taken from Hunan Province, China. Outcomes serve a great purpose for enabling their rapid, sensitive and selective determination and monitoring in environmental samples at trace levels.

2. Experimental

2.1. Chemicals and reagents

Standards of 4-*n*-octylphenol (≥99.5%), 4-*n*-nonylphenol (≥99.0%), bisphenol A (≥98.5%), bisphenol A-D16 (≥98%) were all purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany), of which bisphenol A-D16 was used as internal standard (I.S.). Individual stock solution was prepared in acetone at 1 mg/mL and stored in the dark at −20 °C, while internal standard was prepared in methanol at concentration of 100 mg/L and stored at the same condition.

HPLC-grade solvent acetonitrile, methanol, *n*-hexane were obtained from Sigma-Aldrich (St. Lois, MO, USA) and acetone was supplied by TEDIA (Fairfield, USA). Anhydrous magnesium sulfate (anhydrous $MgSO_4$) and sodium chloride (NaCl) were analytical grade from Aladdin Reagent Co. Ltd. (Shanghai, China) and Sinopharm Co. Ltd. respectively. To ensure efficient removal of phthalates and residual water, both were treated for at least 4 h at 450 °C in muffle furnace. The derivatization reagent bis(trimethylsilyl) trifluoroacetamide (BSTFA) containing 1% methyltrichlorosilane (TMCS) were supplied by SUPELCO (Bellefonte, USA). In the QuEChERS procedure tubes containing pre-weighed amounts of PSA, C18 were purchased from ANPEL Laboratory Technologies (Shanghai) Inc. The other d-SPE sorbents like Neutral aluminum oxide (Sinopharm Co. Ltd) and Cleanert-NH_2 silica gel powder (Agela Technologies) was also used.

2.2. Sample collection

Twenty-eight fish samples were collected from Hunan Province, China, covering 9 prefecture level cities (as shown in Fig. 1, illustrated with ArcGIS 10.2) between July and November 2015, all samples were wrapped with tin-foil paper and frozen at −25 °C. Samples were mainly demersal freshwater fishes from local places and consumed by most of the surrounding residents. That places are all along with Lake Dongting and four rivers (Xiang River, Zi River, Yuan River, Li River) which were largely used for aquaculture, farm irrigation, industry, drinking water as well as other purposes. Since the endocrine disrupting alkylphenols in aquatic ecosystems can accumulate up the food chain and ingested

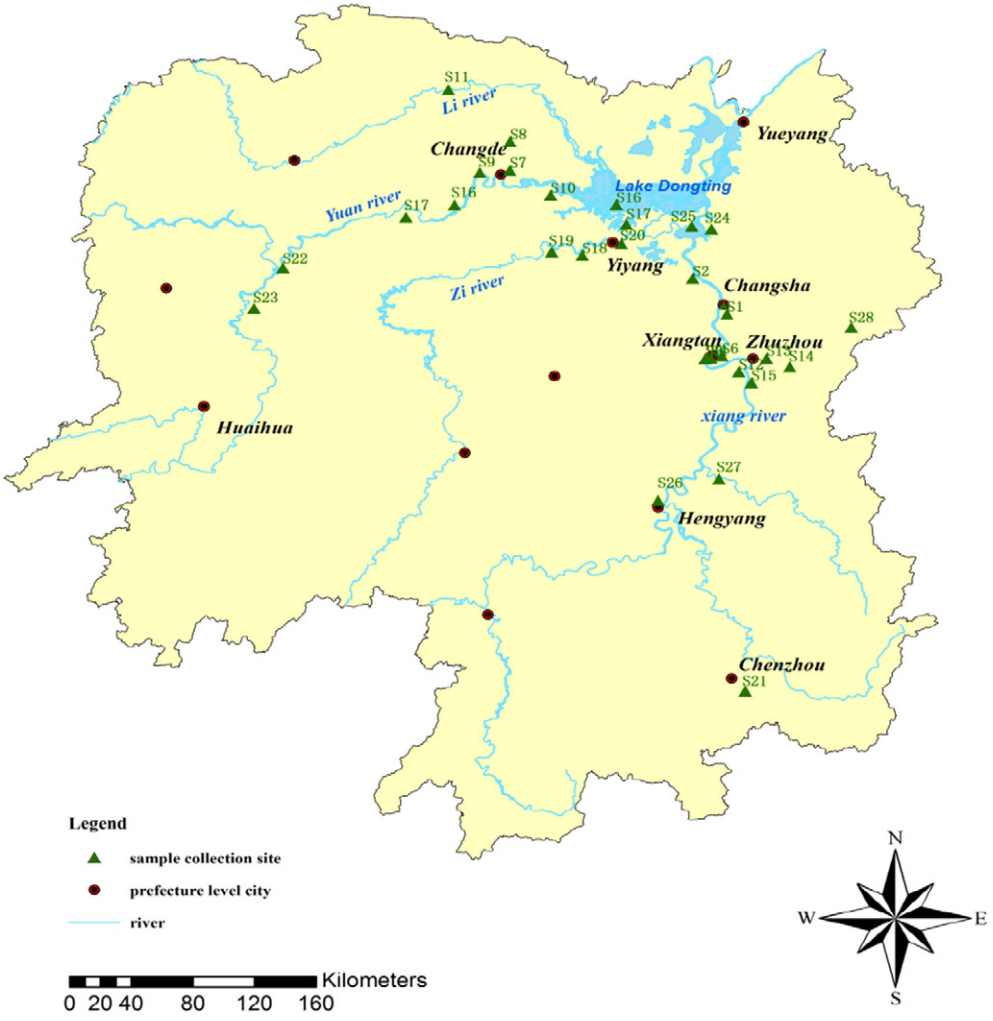

Fig. 1. Distribution of sampling sites in Hunan Province, China.

by people through food or water, it was necessary to monitor these trace pollutants in biota.

2.3. Sample preparation

Firstly, about 500 g fish's edible part was processed and homogenized in a meat grinder and the sample preparation was carried out in a 50 mL polypropylene tube (containing pre weighed 1 g NaCl). 5 g (wet weight) fish tissue was accurately weighted and ultrasonically extracted with 5 mL of acetonitrile for 10 min, then the tube was centrifuged for 4 min at 8000 rpm in order to obtain good separation of the organic phase, after that an aliquot of 3 mL of the supernatant (organic phase) was transferred to a 15 mL polypropylene tube containing 1 g of $MgSO_4$, then transferred to a tube containing 100 mg of PSA and 100 mg of C18 and 200 mg $MgSO_4$, vortexed for 1 min and centrifuged for 3 min at 8000 rpm, later 1 mL of the extract was transferred to an autosampler vial and 50 μL of the internal standard (50 μg/L) was added. To make sure the accuracy of measurement, procedural blank and solvent blank were done each batch.

The final extract was evaporated to dryness under a gentle flow of nitrogen, then an aliquot of 120 μL acetone was added to the vials as solvent medium followed by addition of 60 μL BSTFA(1%TMCS). The vials were sealed and heated in the water bath at 65 °C for 60 min. The derivatized extracts were allowed to cool to room temperature and were then dried under N_2. The dried derivatized residues were redissolved into 200 μL of *n*-hexane for analysis by GC-MS.

2.4. GC-MS analysis

Analysis was performed on Agilent 7890A-5975C GC-MS (Agilent Technologies, USA). A HP-5MS capillary column (30 m × 0.25 mm i.d. 0.25 μm film, J&W, CA, USA) was used. The GC temperature program was as follows: initially 80 °C for 1 min, a temperature ramp of 15 °C/min up to 160 °C, then 8 °C/min up to 230 °C and 5 °C/min up to 260 °C, finally a temperature ramp of 15 °C/min up to 290 °C and then holding this temperature for 2 min. The temperature of transfer line was set at 290 °C and the ion source temperature was 230 °C. Helium was used as carrier gas, and the flow rate was set constantly at 1 mL/min. Samples (1 μL) were injected with the injection temperature at 280 °C in the splitless mode. Mass spectrometry was performed with the electron impact (EI) mode at 70 eV.

Quantitation of the phenolic silyl derivatives was performed in the SIM mode and done by an internal standard method. The selected ions were those at *m/z* 179, and 278 for 4-*n*-OP; *m/z* 179 and 292 for 4-*n*-NP; *m/z* 357 and 372 for BPA and *m/z* 368 and 386 for internal standard.

3. Result and discussion

3.1. Screening of the significant variables by Plackett-Burman design

In this study, a two-level Plackett-Burman factorial design of 12 runs was applied to screen the significant variables affecting the efficiency of extraction. Variables such as extraction volume (V), weight of salt (m), ultrasonic time (t), ultrasonic temperature (T) and ultrasonic intensity (E) was considered according to the preliminary experiments and reported studies and one dummy factor (D) was introduced to find systematic error or unknown variables affecting the system [25]. The factorial design was evaluated using recovery rate of spiked target analytes at the concentration of 4 ng/g. The selected low-level (−) value and high level (+) values of extraction volume (V), weight of salt (m), ultrasonic time (t), ultrasonic temperature (T) and ultrasonic intensity (E) were (5, 10 mL), (1, 2 g), (10, 30 min), (20, 40 °C) and (80, 100 W) respectively. The extraction efficiency of target analytes and the PB design was shown in Table 1.

Standardized Pareto charts ($P < 0.05$) of the significant variables was illustrated in Fig. 2, the critical value of the three target analysts was 2.571 at 95% confidence level. The significant factor affecting the extraction efficiency of 4-*n*-octylphenol, 4-*n*-nonylphenol and bisphenol A from fish samples was extraction volume, while the non-significant variables of weight of salt (m), ultrasonic time (t), and ultrasonic intensity (E) were set as 1 g, 10 min and 80 W respectively for the next experiment because of its negative effect (gray bar) on extraction efficiency and ultrasonic temperature (T) was set as 40 °C because of its positive effect (white bar). The dummy factor was not significant which indicated that there was no systematic error [26] or unknown variables affecting the system in the PBD.

Table 1
Plackett-Burman factorial design for the significant factors.

Run order	Factor						Recovery (%)		
	V	m	t	T	E	D	4-*n*-OP	4-*n*-NP	BPA
1	−	−	−	+	+	+	84	94	60
2	−	−	+	+	+	−	90	97	77
3	+	+	−	+	+	−	71	79	39
4	−	+	−	−	−	+	86	92	54
5	−	+	+	−	+	+	81	91	53
6	+	+	+	+	−	+	63	71	34
7	+	+	−	−	+	−	74	67	41
8	+	−	+	−	−	−	57	68	31
9	−	−	−	−	−	−	105	116	76
10	+	−	−	+	−	+	76	85	44
11	+	−	+	−	+	+	62	69	36
12	−	+	+	+	−	−	64	77	46

As for the determination in real samples, the role of ultrasonication was significant to the thorough extraction of target analytes from fish tissues, which could achieve better extraction efficiency and was used in this study.

3.2. Optimization of the significant variable by single factor experiment

The single factor experiment was done to find the optimal extraction volume. As shown in the PBD experiment, extraction volume has a negative effect on the extraction efficiency, so it was superior to choose low level values, considering to the volume loss of acetonitrile during the extraction and cleaning up steps, at least 5 mL of acetonitrile was needed to make sure that the extract left is enough for the following derivatization and subsequently GC-MS analysis. So 5 mL of acetonitrile was set as the lowest extraction volume in this study. Values of 5, 5.5, 6, 6.5 and 7 mL were chosen to get the best recovery rate.

From the single factor experiment (Fig. 3), it can be seen that the best recovery rate was obtained when 5 mL of acetonitrile was used and the corresponding recovery rate ranged from 55% to 90%. So the extraction volume was set as 5 mL at the next experiment.

3.3. Optimization of the cleaning up steps

As far as we can see, lipid components are the most common coexist substances in fishes, considering the little fat partitions, acetonitrile was used as the extraction solvent and salt NaCl was used to get phase separation. Fatty acid is one of the most common components in lipid and they usually get co-extracted in the pretreatment, so complete removal of them is crucial for the accurate analysis of trace pollutants contained in fish species. In this study, optimization of the clean-up step was performed using different sorbents and different amount of the chosen sorbents. Different sorbents (Cleanert-NH_2 silica gel powder + $MgSO_4$, neutral Al_2O_3 + $MgSO_4$, PSA + C18 + $MgSO_4$) were evaluated and different amounts of sorbents (50 mg PSA, 150 mg C18), (100 mg PSA, 100 mg C18), (50 mg PSA, 100 mg C18) and (100 mg PSA, 120 mg C18) were also tested through spiking the blank fish tissues (5 g) in two replicates at 4 ng/g level of the target mixture. Recoveries of the tested analytes from different treatments were

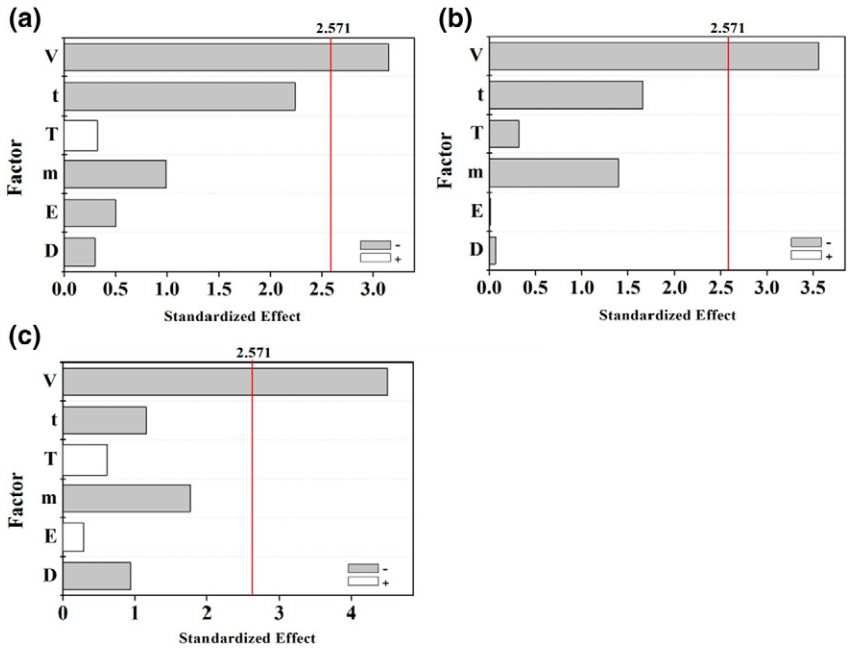

Fig. 2. Standardized ($P < 0.05$) significant effects Pareto charts of the Plackett-Burman design ($n = 2$) for 4-*n*-octylphenol (a), 4-*n*-nonylphenol (b) and bisphenol A (c).

compared to finalize the optimum d-SPE sorbents, procedural blank and solvent blank were also done each time.

As it can be seen in Fig. 4a, the combination of PSA and C18 displayed better cleaning up efficiency with the recoveries of target analytes ranging from 52% to 95%, which were higher than that of the rest. Compared with the NH_2-silica-gel powder and Al_2O_3 cleaning up, the matrix interferences was significantly minimized through cleaning up by PSA + C18, as shown in Supplementary material Fig. S1. It was because that the combination of PSA and C18 could effectively remove fatty acid and nonpolar lipid co-extractives [27], and the PSA removes more matrix co-extractives than NH_2 silica gel powder because PSA has both a primary and secondary amine [20].

To achieve the best cleanup efficiency and relative good recovery, different amount of PSA and C18 were compared via the recovery experiment. The spiking level is the same as aforementioned, results were shown in Fig. 4b, the satisfactory cleanup efficiency was achieved when the sorbent composed with 100 mg PSA, 100 mg C18 and 200 mg $MgSO_4$, which may owe the limited fatty acid and large amount of nonpolar organic co-extractives.

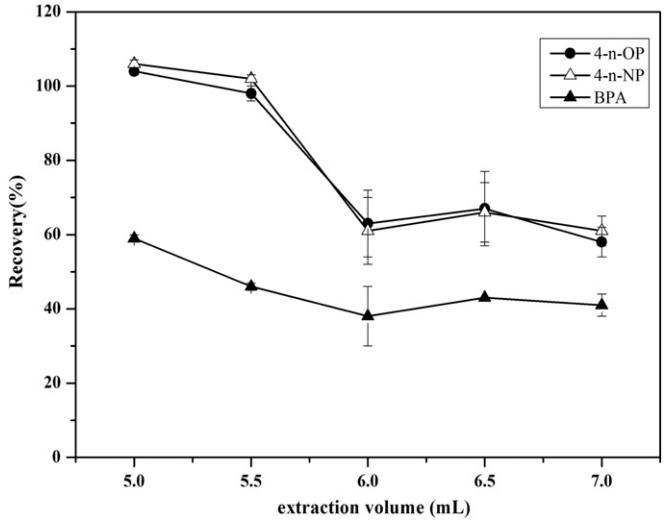

Fig. 3. Influence of extraction volume on extraction efficiency of target analytes from fish samples.

3.4. Calibration and method validation

Good linearity ($r^2 > 0.99$) was obtained for all three tested analytes and the MDL and MQL of the three analytes ranged from 0.18– 0.54 ng/g ww and 0.60– 1.80 ng/g ww respectively, of which MDL was defined as three times signal to noise while MQL was ten times of it, both parameters were determined in blank fish samples ($n = 10$).

The recovery experiment was carried out by spiking the fish tissues in five replicates with mixture standard at three concentration level, that is, 4, 10, 15 ng/g and the internal standard (BPA-D16) was added after extraction, the calculations of the recovery were performed through internal standard matrix-matched calibration curve, it takes concentration of target analytes as x-axis and peak area ratio as y-axis, internal standard (added after extraction) used in our study was mostly used to minimize the matrix effect and monitor the possible error caused by derivatization, injection or instability of the instrument during analysis, with recovery ranged from 50% to 113% and RSD ranged from 2% to 11%, as shown in Table 2. Good precision (from 3% to 5% and from 2% to 6%) were also obtained in both the intra-day and inter-day experiment.

It can be concluded from the recovery experiment that the developed method is fit for the measurement of alkylphenols in real samples like fish and other complex matrices. The MDL and MQL of each analyte agree with that obtained by Gu et al. [28], the MDL of 4-*n*-OP, 4-*n*-NP and BPA was 0.2, 0.3, 0.5 ng/g and MQL was 0.5, 0.8, 2.0 ng/g respectively, which were achieved through LC-MS/MS for fresh biota samples (fish, prawn and Mollusc) from coastal cities of China. MQL of 4-*n*-OP, 4-*n*-NP and BPA was 1.7, 2.3 and 3.3 ng/g (dry weight) for biota samples from a European river basin which were also determined through LC-MS/MS.

3.5. Matrix effect

The presence of matrix interference, especially when analyzing complicated matrices such as fish, can lead to false quantitative results. In

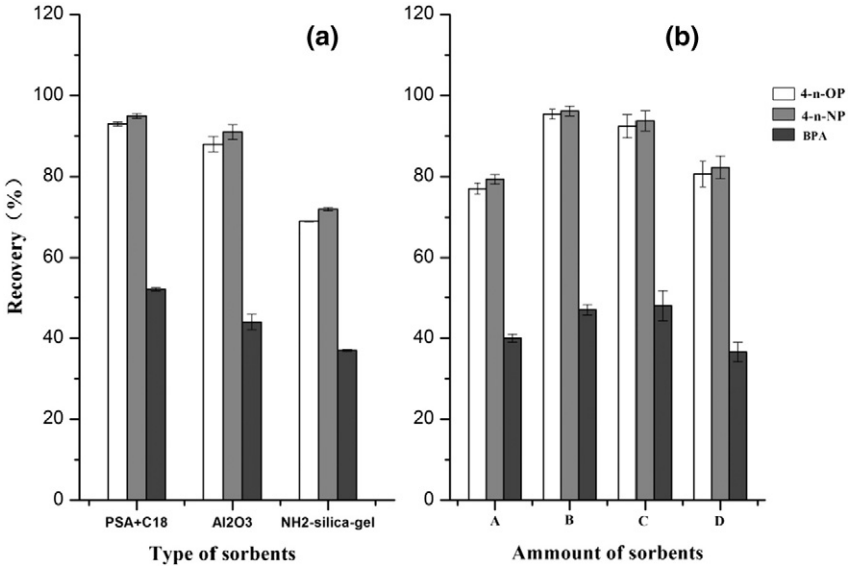

Fig. 4. Recoveries of three different sorbents (a) and different amount of the chosen sorbents (A-50 mg PSA + 150 mg C18, B-100 mg PSA + 100 mg C18, C-50 mg PSA + 100 mg C18and D-100 mg PSA + 120 mg C18) (b) for the cleanup of fish samples (n = 3).

order to reduce this effect, matrix matched analytical solutions were used to obtain the analytical curves to avoid quantitation problems.

In this study, we used an internal standard matrix-matched calibration, which combines the advantage of internal standard calibration and matrix-matched, minimizing the matrix effects for all the target analytes. As shown in Supplementary material Fig. S2, the chromatogram of the matrix-matched standard almost agreed with that of the standard analytes in solvent and the Spiked. Four types of common fish were used to study the effect of matrix, that is, Carassius, Cyprinoid, black carp, Milter. The preparation steps were the same as discussed in Section 2.3. Aliquots of extract (1 mL) after cleaning up were spiked with analytes of known amount respectively. Concentration of analytes was derived from an internal standard calibration curve which was prepared using standards constituted by *n*-hexane.

Compared to standard analytes in solvent, signal enhancements were observed in all three analytes (except for 4-*n*-nonylphenol of black carp), especially for bisphenol A. Co-extracted matrix components were found to have minimal effect on the analytical response of early-eluting analytes as described in other study [29]. The percentage of matrix effect was then calculated according to the equation: $ME\% = (A_{matrix} / A_{solvent} - 1) \times 100$ (where A stand for slope of calibration curve), as can be seen in Table 3. The matrix-matched calibration curves obtained show good coefficient of $r^2 \geq 0.99$.

The data showed that matrix effect was insignificant for 4-*n*-octylphenol and 4-*n*-nonylphenol (ME > −3 to <10%) but significant for bisphenol A (ME > 60%) according to the five categories of matrix effect result, that is, high signal suppression (ME < −50%), moderate suppression (ME < −10 to −50%), no matrix effect (ME > −10 to ME < 10%), moderate signal enhancement (ME > 10 to ME < 50%) and high signal enhancement (ME > 50%) [12]. To minimize the mistakes caused by matrix, the quantitation of analytes was performed using the matrix-matched internal calibration curve. Compared with work done by Munaretto et al. [30], minimal matrix effect was obtained after cleaning up with less sorbent (combination of PSA and C18).

3.6. Concentration of target analytes in real samples

To evaluate the analytical precision, three replicates analysis of fish samples spiked at 4 ng/g level (sample number 3 and 11) were conducted every fourteen samples, with recovery ranged from 51% to 96% and RSD from 1% to 5% as shown in Table 4.

Detection frequencies of 4-*n*-OP, 4-*n*-NP, BPA in these 28 fish samples were 75%, 86%, 75%, with concentration ranges of not detected (n.d.) to 1.78 ng/g, not detected to 3.27 ng/g and not detected to 4.95 ng/g ww respectively. Fig. 5 were the distribution of the concentration level of target analytes and mean concentration level of APs (total of 4-*n*-OP, 4-*n*-NP, BPA) from S1 to S28, compared to 4-*n*-OP, higher concentration level of 4-*n*-NP was detected which may owe its high usage when producing alkylphenol ethoxylates, usually 80% of NP and 20% of OP. Moreover, the higher levels of NP were also related to the widespread application of NPEOs and the persistence of NP in the environment [6]. Improper usage or abuse of fish bait for enclosure culture may be an important source for EDC contamination [31] in water environment, which might lead to contamination of fish indirectly, accordingly, higher 4-*n*-NP and BPA were detected in fishes raised in aquafarm (S1, S12, S21, S25) than those species raised in nature lake or rivers. This phenomenon agrees with that in river and fish bile [7] in Pearl River Delta, South China. The concentrations of each analyte in biota samples were higher than that in river or sediment, indicating the accumulation of alkylphenols in high trophic level organisms like human, animal and marine life. As we all know, BPA was used to make polycarbonate and epoxy resins most of the time. Many consumer products like reusable water bottles, protective linings inside metal-based food and beverage cans are all related with BPA [32]. Exposure of BPA into river, soil and foodstuff [33] threatened human and animal's health potentially.

Table 2
Validation parameters of the optimized method.

Analytes	Recovery (%) (%RSD)			MDL (ng/g)	MQL (ng/g)
	4 ng/g	10 ng/g	15 ng/g		
4-*n*-octylphenol	110 (3)	82 (5)	80 (11)	0.18	0.60
4-*n*-nonylphenol	113 (2)	77 (5)	74 (10)	0.25	0.82
Bisphenol A	50 (2)	53 (5)	58 (9)	0.54	1.80

Table 3
Matrix effect (%) of different fish species.

Analytes	Carassius	Cyprinoid	Black carp	Milter
4-*n*-octylphenol	6.6	10.4	4.4	9.6
4-*n*-nonylphenol	7.0	0.6	−3.4	1.3
Bisphenol A	60.1	59.8	103.7	67.0

Table 4
Concentrations (ng/g, ww) of 4-*n*-OP, 4-*n*-NP and BPA found in different fish samples.

Sampling site	Sample name	Living pattern	4-*n*-OP	4-*n*-NP	BPA
S1	*Tilapia*	Bottom	nd[a]	2.19	2.15
S2	Grass carp	Middle, bottom	1.78	<MQL[b]	2.14
S3 (n = 3)	Grass carp	Middle, bottom	0.84 (65)	1.89 (51)	2.13 (56)
S4	Black carp	Middle, bottom	nd	<MQL	4.95
S5	*Carassius auratus*	Demersal	0.83	1.58	3.86
S6	*Coreius heterodon*	Bottom	0.87	0.96	1.69
S7	Cyprinoid	Demersal	<MQL	0.82	<MQL
S8	Grass carp	Middle, bottom	nd	<MQL	2.37
S9	Cyprinoid	Demersal	nd	<MQL	3.60
S10	Milter	Middle, up	0.94	2.25	nd
S12	Grass carp	Middle, bottom	0.82	2.11	<MQL
S13	Snakehead	Middle, up	0.79	2.57	<MQL
S14	*Carassius auratus*	Demersal	0.84	nd	<MQL
S11 (n = 3)	Grass carp	Middle, bottom	nd (96)	<MQL (63)	nd (66)
S15	Cyprinoid	Demersal	nd	nd	3.05
S16	*Carassius auratus*	Demersal	0.87	<MQL	<MQL
S17	Catfish	Demersal	0.77	<MQL	<MQL
S18	Cyprinoid	Demersal	0.87	<MQL	nd
S19	*Amblycephala*	Middle, bottom	0.89	nd	<MQL
S20	*Carassius auratus*	Demersal	0.76	<MQL	nd
S21	Black carp	Middle, bottom	0.76	2.54	1.93
S22	Cyprinoid	Demersal	0.85	2.78	<MQL
S23	Grass carp	Middle, bottom	nd	nd	3.72
S24	Cyprinoid	Demersal	1.17	2.11	<MQL
S25	Milter	Middle, up	0.88	3.27	<MQL
S26	Cyprinoid	Demersal	1.01	<MQL	nd
S27	*Carassius auratus*	Demersal	0.72	<MQL	<MQL
S28	*Carassius auratus*	Demersal	0.88	2.47	nd

[a] nd - not detected.
[b] MQL-limit of method quantitation.

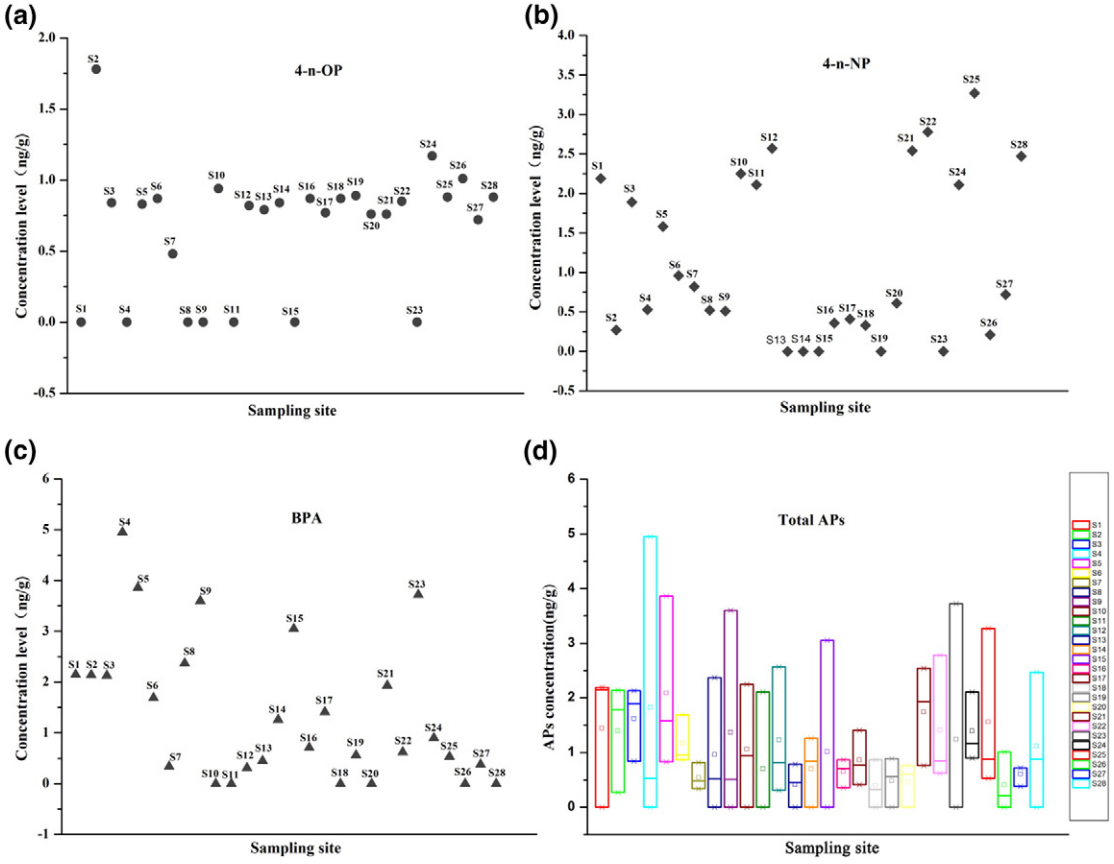

Fig. 5. Distribution of the concentration level of 4-*n*-octylphenol (a), 4-*n*-nonylphenol (b), bisphenol A (c) and mean concentration level of the total APs (d) in sampling sites from S1 to S28.

From the APs figure (Fig. 5d) it was observed that mean concentration level of target analytes in fish samples were mostly ranged from 0.5 ng/g to 1.5 ng/g and may not cause health risk for grown people according to human health risk assessment [9] but except for children aged around seven, indicating high intake risk for children. Moreover, the estimated NP and BPA intake were 520 ng/kg bw/day and 43 ng/kg bw/day for an average (63 kg) Chinese adult [33], which was well below the tolerable daily intake (TDI) value of 5 μg/kg bw/day recommended by EFSA [34].

4. Conclusion

This ultrasonic-assisted QuEChERS method was found to be efficient in analysis of complicated matrices like fish, using less sorbents for cleaning up and more importantly, less time to operate, offering a reliable, convenient and sensitive method coupled with GC/MS. Low MDL and MQL were also achieved through derivatization. The developed QuEChERS-based cleaning up procedures only use a small amount of sorbents (combination of PSA + C18 + $MgSO_4$) and take less time to process samples, but it still need to be optimized to minimize matrix effect. Extractive solvent acetonitrile (5 mL) reduced the cost compared with that using acetonitrile containing 1% glacial acetic acid [21] or some amount of water to some extent. Generally speaking, this developed method is effectual in quantitation and monitoring of trace EDCs in complex matrices like fish and other aquatic ecosystem.

Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of, the manuscript entitled.

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