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Development of a subcritical fluid extraction and GC–MS validation method for polychlorinated biphenyls (PCBs) in marine samples

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ABSTRACT

This paper describes a new procedure for extracting polychlorinated biphenyls (PCBs) from marine samples using subcritical 1,1,1,2-tetrafluoroethane (R134a). The extraction procedure was optimized at temperatures varying from 20 to 70 °C and pressures ranging from 3 to 15 MPa. The volume of the co-solvent was then optimized using 1,1,1,2-tetrafluoroethane (R134a) as the subcritical phase. PCBs were characterized by GC–MS using the optimized conditions of 3 MPa, 30 °C, and a co-solvent volume of 6 mL. The average yields of PCBs from subcritical fluid extraction of spiked oyster samples were measured and found to be greater than 90%, with relative standard deviations (RSD) of less than 10%. Detection limits of this method were in the range of 0.045–0.108 ng/g of dry mass. The method was compared to Soxhlet extraction and then applied for monitoring PCBs in oysters from Qingdao, Shandong, China.

1. Introduction

PCBs is a group of chlorinated aromatic hydrocarbons, including 209 congeners, differing in the position and number of chlorine atoms bound. In view of thermodynamic aspects and spatial configuration constraints, the number of existing congeners can range between 130 and 150 [1–4].

PCBs were first synthesized in 1881. Since then, 2 million tons of PCBs have been produced for commercial use [5]. Additionally, PCBs have been widely used as heat transfer fluids and dielectric fluids [6]. Due to their high stabilities, PCBs have been detected in air, water and organisms [7]. PCBs represent a major health problem, have shown toxic effects by interfering with hormone system in human body, and many of these are carcinogenic substance [8]. Organisms living in polluted waters that are consumed as seafood, including fish and mollusks, can store PCBs in their fatty tissues and thus pose a risk to human health when consumed [9]. Some countries have established levels (recommended maximum limits, RMLs) for PCBs in some products, such as fish (2000 ng/g), eggs (100–300 ng/g), PCBs contamination levels set by European Commission (2000 ng/g of fat, including PCB-28, PCB-52, PCB-101, PCB-118, PCB-138, PCB153, PCB-180) by a factor of 250 [10,11].

Analytical procedures for detecting PCBs from marine samples typically include three steps: extraction, purification and analysis by GC coupled with ECD (electron capture detector) or MS (mass spectrometry). Traditional methods, such as Soxhlet extraction [12] and microwave-assisted extraction [13,14], require long extraction times and tedious procedures and consume large quantities of hazardous organic solvents. Supercritical fluid extraction (SFE) technology has become an increasingly popular method because it has the advantages of a shorter extraction time and lower organic solvent consumption. The extraction of trace levels of polychlorinated contaminants using supercritical fluid technology has proven to be a promising way to recover most contaminants [15–19]. Carbon dioxide (CO_2) has been the most popular supercritical solvent and typically requires pressures of up to 500 bar for satisfactory extraction. Taking into account economic and environmental concerns, we searched for an alternative SFE solvent that would enable operation under less intense conditions.

1,1,1,2-Tetrafluoroethane (R134a) is non-toxic and non-flammable, and it has a permanent dipole moment (2.05 D) and reasonable critical properties (101 °C, 40.6 atm). These characteristics led to the evaluation of its use as an alternative to supercritical CO_2 for the extraction of polar analytes [20–22]. R134a has been used to extract β-carotenes from palm oil [23,24], PBDEs from house dust [25], and medroxyprogesterone from aquatic products [26], but there are no reports on the application of subcritical R134a for extracting PCBs from marine samples. In the experiments presented here, oysters were chosen because they are the most frequently analyzed marine matrix.

R134a has been shown to have considerable potential for the extraction of PCBs from marine samples [27]. In comparison to Soxhlet extraction, R134a extraction times were reduced from

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13 h to 1 h, and the amount of solvent used was reduced from 150 mL to less than 10 mL. The major drawback of the R134a-based method is the presence of co-extracted lipids, so the lipids must be separated from PCBs to obtain extracts that can be analyzed by GC–MS. Because subcritical R134a extraction is a new method, the traditional sulfuric acid-based purification method was used for removing the lipid impurities. A high extraction recovery was achieved, but the time involved would result in high labor costs. Consequently, silica gel column clean-up was chosen to be used to remove the impurities.

The aim of the present study was the development and validation of a new subcritical fluid extraction method for selected indicator PCBs, followed by purification using either sulfuric acid extraction or a silica gel column. Obtained extracts were quantified and validated using GC–MS. Parameters including the extraction temperature, pressure and the volume of co-solvent were optimized to achieve an efficient extraction. The newly established method was compared with Soxhlet extraction and then used to analyze PCBs in a variety of marine samples.

2. Experimental

2.1. Chemicals and reagents

PCB standards were purchased from Dr. Ehrenstorfor (Germany), a mixture containing 7 different congeners at 10 ng/μl in hexamethylene, 2,4,4′-trichlorobiphenyl (PCB-28), 2,2′,5,5′-tetrachlorobiphenyl (PCB-52), 2,2′,4,5,5′-pentachlorobiphenyl (PCB-101), 2,3′,4,4′,5-pentachlorobiphenyl (PCB-118), 2,2′,3,4,4′,5′-hexachlorobiphenyl (PCB-138), 2,2′,3,4,4′,5′-hexachlorobiphenyl (PCB-153) and 2,2′,3,4,4′,5,5′-heptachlorobiphenyl (PCB-180) (PCB numbering according to IUPAC). Working solutions of PCBs were prepared in hexane. The extraction solvent was supercritical fluid-grade R134a (INEOS England). Methanol used as the extraction solvent was purchased from Merck (Darmstadt, Germany). Silica gel and anhydrous Na_2SO_4 (supplied by Sinopharm, Shanghai, China) were activated at 150 °C for 24 h prior to use. Analytically pure grade sulfuric acid (98%) was supplied by Laiyang Economic Development Zone Fine Chemicals Factory (Yantai, China).

2.2. Sample preparation

Oysters (*Ostrea talienwhanensis Crosse*), mussels (*Mytilus edulis*), black carp (*Mylopharyngodon piceus*), and shrimp (*Trachypenaeus curvirostris*) were collected from the local market in Qingdao, China. All samples were washed with distilled water. Muscle tissue was freeze-dried for 48 h, and then it was ground. Spiked samples were made by mixing oyster muscle tissues with 200 μL of PCB standard solutions (IUPAC numbers, PCB-28, PCB-52, PCB-101, PCB-118, PCB-138, PCB-153, PCB-180). Spiked samples were used to optimize the extraction conditions. The solvent of PCB standard solutions was evaporated under a nitrogen stream.

2.3. Apparatus

PCB extraction experiments were conducted in an apparatus constructed in our laboratory. The apparatus contains a high-pressure pump (Hangzhou Zhejiang Petrochemical Equipment Co., Ltd., Hangzhou, China) with a maximum pressure of 35 MPa, which was used to regulate the R134a flow. The instrument was equipped with an extraction vessel (120 mm × 20 mm I.D.). Liquid R134a was handled with a high-pressure metering pump with a jacketed cooling head. The R134a flow rate was maintained at 1.0 mL/min for all extraction conditions.

2.4. Subcritical R134a extraction

For each extraction run, 0.5 g of a spiked oyster sample was placed into the stainless-steel extraction cell, and then co-solvent was added. Glass wool was placed into the bottom and top of the extraction cell to prevent the material from leaking, which would cause a blockage in the system. The extraction cell was placed in an oven to control the operating temperature to within ±1 °C of the set-point temperature.

Subcritical R134a fluid at ambient temperature was raised to the desired pressure using a metering pump at the beginning of the extraction. This static extraction procedure was continued for 20 min to promote static contact between the sample and subcritical R134a fluid. Next, the sample was subjected to dynamic extraction for 40 min. The extraction fluid containing the PCBs was then removed from the vessel and depressurized to ambient pressure through a restrictor. The PCBs were collected in a sample trap (20 mL amber glass vial) filled with 15 mL hexane.

2.5. Soxhlet extraction

For comparison of the developed method with an established method, Soxhlet extraction was performed on approximately 2.0 g of samples spiked with 200 μL of each PCB standard solution. The spiked sample was wrapped with filter paper and placed into an extraction thimble. Extractions were performed with 130 mL of a hexane-acetone mixture (V:V = 1:1) for 15 h [28]. Each experiment was repeated three times.

2.6. Sample clean up

2.6.1. Concentrated sulfuric acid purification

After either subcritical R134a extraction or Soxhlet extraction, the extract was purified with concentrated sulfuric acid by adding 4 mL of concentrated sulfuric acid to the extract, vortexing for 2 min, and removing the concentrated sulfuric acid layer. This purification procedure was repeated 4 times for each sample. The sulfuric acid layer was washed with hexane [29]. The remaining extract and hexane washes were combined and evaporated to 2 mL using rotary evaporation followed by evaporation to dryness under a nitrogen stream. The residue was dissolved in 1 mL hexane for GC–MS analysis.

2.6.2. Silica gel column purification

After obtaining the optimal analytical parameters for subcritical R134a extraction, the extraction samples were concentrated to 2 mL by rotatory evaporation at 40 °C and then transferred to the top of silica gel column, consisting of 3 g of 80–100 mesh silica gel column that had been pre-rinsed with hexane [30]. The silica gel column was prepared with a 1 cm layer of anhydrous sodium sulfate above and below the silica gel. Hexane was used to elute the PCBs, and the eluents were concentrated to dryness under a nitrogen stream. The residue was dissolved in 1 mL of hexane for GC–MS.

2.7. Chromatographic analysis by GC–MS

Analyses were performed on an HP6890 gas chromatograph with a 5973 mass spectrometry detector (Agilent, Germany). The gas chromatograph was equipped with an HP-5MS capillary column (30 m × 0.25 mm × 0.25 μm film thickness, Agilent). Helium was used as carrier gas at a constant flow rate of 1.2 mL/min. A sample volume of 1.0 μL was injected in splitless mode. The sample injection port was kept at 250 °C. The oven temperature program was as follows: 70 °C for 2 min, increased to 150 °C at a rate of 25 °C/min, then increased to 200 °C at a rate of 3 °C/min

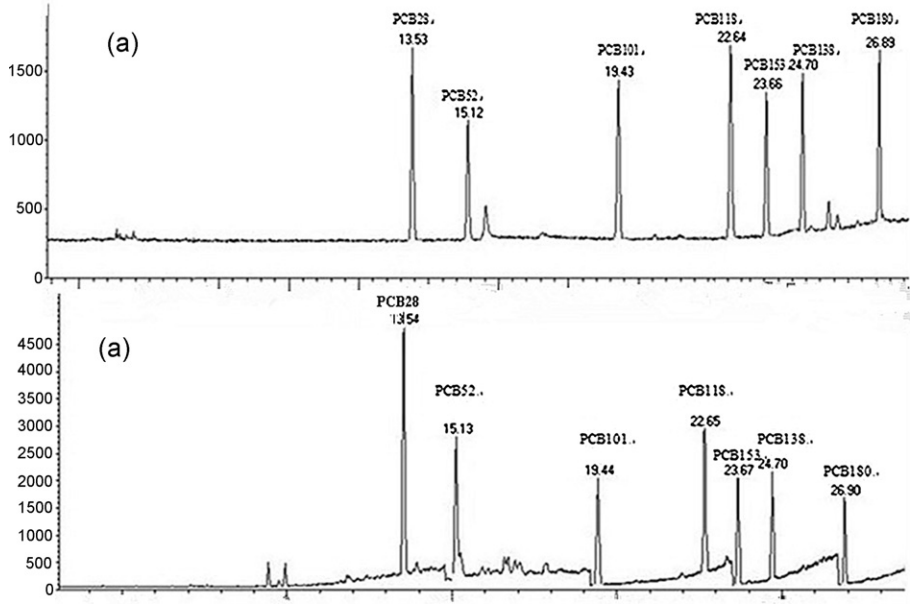

Fig. 1. Gas chromatograms of PCBs: (a) gas chromatograms of the standard PCBs; (b) gas chromatograms of PCBs standard added in sample.

and finally ramped to 280 °C at a rate of 80 °C/min and held at this temperature for 10 min. Ions were obtained by electron impact ionization at 70 eV. The transfer line, source and quadrupole temperature were set at 280, 230 and 150 °C, respectively. Extracts were analyzed under the SIM mode. Individual PCB compounds were identified based on their retention times. Quantitation was achieved using external calibration curves obtained by regression analysis of peak areas versus injected standard solutions. Standard solutions were in the range of 4–200 μg/L. The standard working curves were established at each run before sample injection

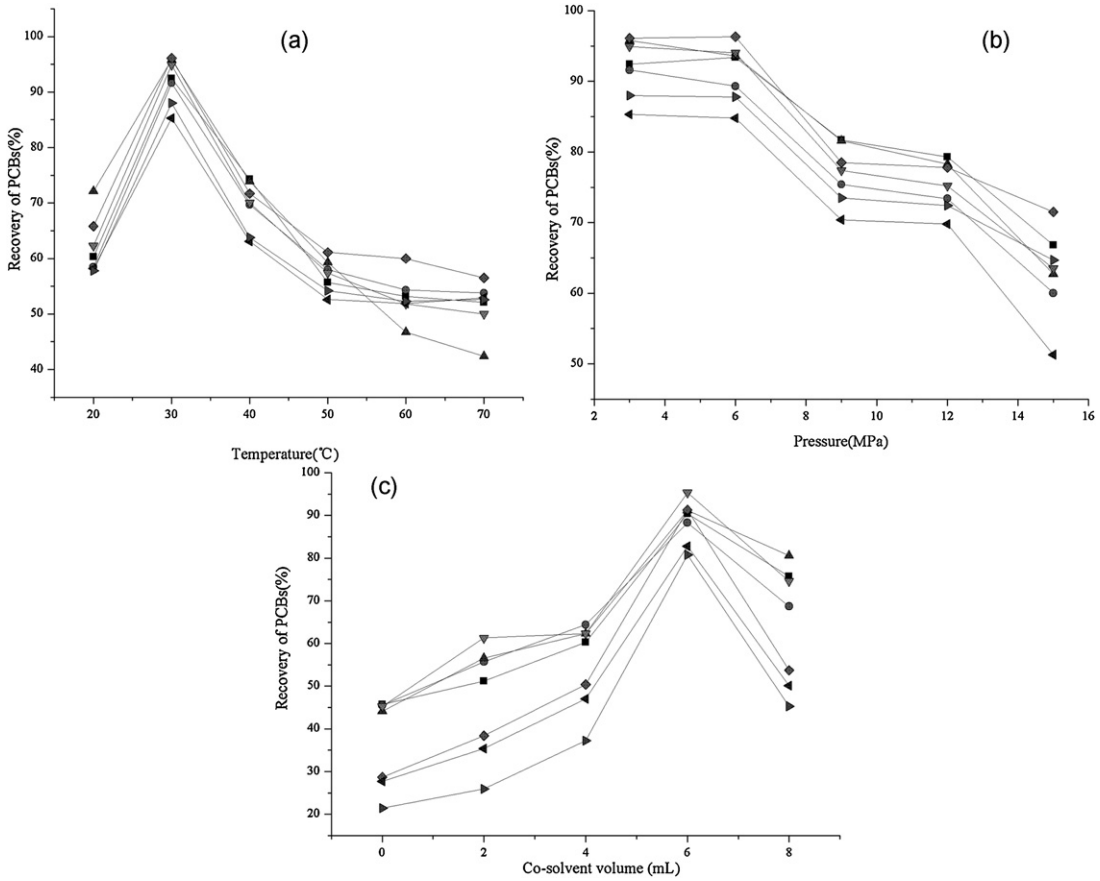

Fig. 2. Subcritical R134a extraction optimization plot of recovery vs extraction conditions: (a) extraction temperature, (b) extraction pressure, and (c) volume of co-solvent (RSD ≤ 5%). (—■—) PCB28, (—●—) PCB52, (—▲—) PCB101, (—▼—) PCB118, (—◆—) PCB138, (—◀—) PCB153, and (—▶—) PCB180.

Table 1
Comparison of purification methods and extraction methods.

PCBs	Concentrated sulfuric acid				Silica-gel column	
	Soxhlet extraction		Subcritical R134a extraction		RSD (%)	Mean recoveries (%)
	RSD (%)	Mean recoveries (%)	RSD (%)	Mean recoveries (%)		
28	4.5	88.1	4.5	93.4	3.8	87.7
52	3.8	86.3	4.3	92.5	7.1	92.3
101	4.9	88.8	6.7	102.3	7.3	93.4
118	4.1	93.1	5.3	97.2	6.6	95.4
153	5.3	90.9	4.1	96	5.3	98.1
138	5.4	92.3	3.2	91.9	4.4	97.1
180	3.1	91.5	4.4	93.1	3.7	94.6

to eliminate the instability of the instrument as a variable in the analysis.

2.8. Method validation

The method detection limits (MDLs) of the subcritical R134a extraction were determined using the EPA standard procedure. Samples from the spiked oyster muscle tissue were injected seven times to evaluate the standard deviation (SD).

Oyster tissue samples spiked with PCB standard solutions at levels of 1.0 μg/g, 1.5 μg/g, and 2.0 μg/g were extracted with subcritical R134a, and analyzed by GC–MS. Each level was repeated three times. At the same time, different concentrations of 200, 100, 20, 10, 4 μg/L PCBs were injected into GC/MS to make a calibration curve.

To show the applicability of the process to other marine samples, a study was also completed for other marine samples. mussels (*M. edulis*), black carp (*M. piceus*), and shrimp (*T. curvirostris*) from local market were analyzed.

To test the recovery of PCBs in the presence of the spiked samples, samples didn't contain any PCB congeners that can be determined by GC/MS.

2.9. Determination of lipid content

To determine the amount of lipids in the samples, 2.0 g of each sample was Soxhlet-extracted with petroleum ether for 12 h. The solvent was evaporated, and the amount of lipids was gravimetrically determined for each sample. The results were expressed as dry masses.

Table 2
Extraction recoveries of PCBs from samples.

PCBs	Fortification level (μg/g)	Recovery (%)	RSD (%)
PCB28	1	90.5	9.9
	1.5	91.4	7.7
	2	93.4	4.5
PCB52	1	91.2	4.5
	1.5	90.8	2.9
	2	92.5	4.3
PCB101	1	91.3	9.6
	1.5	92.7	3.1
	2	102.3	6.7
PCB118	1	90.4	7.9
	1.5	91.5	8.5
	2	97.2	5.3
PCB153	1	93.1	5.5
	1.5	95.5	2.1
	2	96.0	4.1
PCB138	1	90.8	6.2
	1.5	92.6	2.6
	2	91.9	3.2
PCB180	1	90.6	5.7
	1.5	90.2	4.9
	2	93.1	4.4

3. Results and discussion

3.1. Optimization of subcritical R134a extraction

The goals of this study were to optimize the subcritical R134a extraction conditions to obtain a maximum recovery of PCBs, achieve superior extraction efficiency compared to Soxhlet extraction, and investigate the recovery of the PCBs after silica gel column purification compared with the sulfuric acid extraction method.

The temperature, pressure and co-solvent volume are the significant variable parameters in subcritical R134a extraction. To optimize the extraction temperature, 0.5 g of oyster samples, spiked with 200 μL of the PCB solutions, were extracted at 3 MPa with 6 mL of co-solvent while the extraction vessel temperature was progressively increased (20, 30, 40, 50, 60 and 70 °C) in separate experiments. Fig. 2(a) demonstrates the changes in recovery with respect to temperature for each of the PCBs extracted from samples. It was found that the recovery of PCBs increased when the temperature was increased from 20 °C to 30 °C. This phenomenon is due to increased diffusion and penetration power with increased temperature, which increases the chance of the contact between the R134a fluid and the solute. However, the recovery of PCBs decreased as the temperature increased past 30 °C. With higher temperature, the density of the R134a solvent decreases and, consequently, the solvent power decreases. Therefore the highest recovery was obtained at a relatively low temperature of 30 °C.

PCBs were extracted while the subcritical R134a pressure was raised incrementally (3, 6, 9, 12 and 15 MPa) in separate experiments, keeping other parameters constant. Fig. 2(b) shows changes in the recovery with respect to pressure for each extracted PCB. We observed that the recoveries of all PCBs decreased with increasing subcritical R134a pressure. The viscosity of R134a increases with increasing pressure, which prevents the solute from diffusing into the fluid. The critical pressure of R134a is 4 MPa, and higher pressure were far from the subcritical state. This change could have affected the recovery of PCBs. It is interesting to note the highest recovery was achieved at 3 and 6 MPa. Considering the power consumption and safety factors 3 MPa was chosen as the optimal pressure for further experiments.

The effect of co-solvent volume was determined using different volumes (0, 2, 4, 6 and 8 mL) at 30 °C and 3 MPa. As shown in Fig. 2(c), the recovery of the 7 extracted PCBs increased markedly between 0 and 6 mL. This was most likely due to the increased polarity of the solvent mixture when compared to R134a alone. Methanol can form H-bonds with the matrix, so the dissolution of PCBs in fluid was enhanced [31]. However, volumes over 6 mL have the opposite effect on recovery. During experiments, it was observed that the color of the extract using volumes of methanol greater than 6 mL was yellow in color and contained large quantities of impurities, whereas extracts using smaller volumes of methanol were clear. Thus, a higher fluid polarity results in the extraction of more impurities, which may compete with

Table 3
The concentration of PCBs in real samples.

Samples	The concentration of PCBs (ng/g)							Total amount of PCBs
	PCB28	PCB52	PCB101	PCB118	PCB153	PCB138	PCB180	
Oyster	0.046	0.075	0.029	0.135	ND	0.032	ND	0.320
Mussel	0.049	0.037	0.081	0.064	ND	0.025	ND	0.257
Black carp	ND	ND	0.033	ND	ND	0.022	ND	0.055
Shrimp	0.035	ND	0.057	ND	0.126	ND	ND	0.218

PCBs for extraction and result in decreased recovery. Additionally, the large amounts of co-solvent may change the critical fluid parameters, thus decreasing the dissolving capacity of subcritical R134a fluid.

In summary, we found that the highest recovery of PCBs is obtained at 30 °C, 3 MPa, and with 6 mL methanol.

3.2. Comparison of recovery with both subcritical and Soxhlet extraction

The traditional approach to PCB extraction utilizes Soxhlet extraction, which is time-consuming and uses large volumes of toxic solvents. We compared the recovery of PCBs with both subcritical R134a extraction and Soxhlet extraction. Subcritical R134a extraction was performed using the previously mentioned optimized conditions. The recovery data showed in Table 1 indicate that subcritical R134a extraction has the same excellent extraction efficiency as Soxhlet extraction (Table 1). The Soxhlet extraction method requires use of large solvent volumes (about 150 mL) and long extraction times (up to 13 h), Subcritical R134a extraction used much less organic solvent and requires 2 h. It is a labor saving and environmental method for extracting PCBs from oyster samples.

3.3. Comparison of purification methods

Another purification method was performed using a silica gel column. An oyster sample (0.5 g) was spiked with 200 μL of PCB standard solutions. Triplicate samples were extracted using subcritical R134a extraction and purified with a silica gel column or sulfuric acid. A comparison of the recoveries and RSD is presented in Table 1.

As subcritical R134a extraction is a new method, we decided to compare it to a traditional and reliable purification method to evaluate the appropriateness of this new method for the extraction of PCBs from marine samples.

Table 1 showed that each of these methods can achieve a high recovery and show a good reliability. The consumption of organic solvents is equal in the two purification methods. However, the hexane used in the silica gel column method is safer when compared to sulfuric acid. Furthermore, the hexane is expected to be reusable.

3.4. Results of method validation

The method was validated under the optimized conditions (30 °C, 3 MPa, 6 mL of co-solvent). The accuracy and precision of the method were determined using oyster samples spiked with PCB standard solutions at levels of 1, 1.5 and 2 μg/g. The MDLs evaluated for the subcritical R134a extraction method ranged from 0.045 to 0.108 ng/g of dry mass for GC–MS. In the range of 4–200 μg/L, the concentration of PCB congeners had a good linearity ($r^2 > 0.996$). The mean recovery of PCBs was above 90%, with RSD values below 10% (Table 2). Fig. 1 shows gas chromatograms of the standard PCBs and gas chromatograms of PCBs standard added in sample.

3.5. Application of the new method to several marine samples

The validity of this method was studied using real marine samples. The samples were analyzed using the optimized conditions and had lipid contents of 4.2, 5.8, 7.6 and 3.7% by weight for oysters (*O. talienwhanensis Crosse*), mussel (*M. edulis*), black carp (*M. piceus*), and shrimp (*T. curvirostris*), respectively. The quantitative values of PCBs in different real samples are shown in Table 3. In the oyster and mussel samples, 5 groups of PCBs (28, 52,101, 110, 138) were detected. In the shrimp samples, only 3 groups of PCBs (28, 101, 153) were detected, while only 2 group of PCBs (101, 138) were detected in black carp. With the exception of the PCB 118 in oyster and mussel and the PCB 153 in shrimp, all PCBs were below the MDLs. Therefore, these samples represented matrices with low PCB concentrations. The total content of the 7 PCBs in the samples were 0.32, 0.25, 0.21, and 0.05 ng/g for oyster, mussel, shrimp, and black carp, respectively. The PCBs levels in these marine samples was far below the established safety level listed in part one.

4. Conclusions

A rapid and simple method for the determination of PCBs in marine samples has been established using subcritical R134a extraction and GC–MS. Under the optimized conditions, the recovery was excellent compared to the traditional methods and requires less time and less toxic solvents. This method is feasible for the analysis of PCBs.

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