Solid phase extraction with pyrenebutyric acid-bonded silica for analysis of polychlorinated biphenyls in sewage water by gas chromatography–mass spectrometry

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1. Introduction

Polychlorinated biphenyls (PCBs) are ubiquitous pollutants in the environment due to the persistence although their production was almost banned worldwide in the mid 1970s. Investigations in many parts of the world have revealed widespread diffusion of PCBs in the environment (WHO, 1993). A variety of adverse biological effects have also been described on wildlife and human for these contaminants including cytochrome P450 enzyme induction (Hallgren and Darnerud, 1998), endocrine effects on thyroid and steroid hormone (Darnerud et al., 2001; Zhou et al., 2001), immunotoxicity (Fowles et al., 1994) and neurotoxicity (Tilson and Kodavanti, 1997). PCBs have been classified by the International Agency for Research on Cancer as probably carcinogenic to humans and may have also non-carcinogenic health effects, particularly as endocrine disruptors (IARC, 1998).

In generally, enrichment and clean-up procedure are necessary for the analysis of PCBs by gas chromatography–mass spectrometry (GC–MS). Solid phase extraction (SPE) is a simple, cost effective and versatile method for enrichment and clean-up of chemical analysis (Liška, 2000). Some stationary phases of SPE have been developed to analyze PCBs including C18 and/or NH2 (Dmitrovic et al., 2002; Čonka et al., 2005).

Pyrenebutyric acid-bonded silica (PYB) is one stationary phase with π-acidic character (Yu et al., 2005). Pyrenebutyric acid-bonded silica (PYB) as sorbent was developed to determine 23 polychlorinated biphenyls (PCBs) in sewage water by gas chromatography–mass spectrometry (GC–MS). Factors were optimized in SPE procedures including elution solvent, pH, and cartridge burden. The recoveries of 23 PCB congeners were 69.44–111.91% under optimized conditions. Comparisons were also conducted among PYB-SPE, C18-SPE and United States Environmental Protection Agency 608 (USEPA608) methods in the analysis of PCBs in sewage water samples. The results showed that the performance of PYB-SPE method was similar with USEPA608 method and better than C18-SPE method. Both PYB-SPE and USEPA608 methods were then employed to analyze PCBs in real spiked sewage water samples. The recoveries of PCB congeners determined by PYB-SPE method ranged from 70.6% to 92.4% in real spiked sewage water samples which were identified to be in accordance with USEPA608 method. Limits of detection (LOD) were in the range of 0.06–0.22 ng L−1 for PCB congeners. The optimized PYB-SPE method was successfully applied to the determination of PCBs in sewage water samples.

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A B S T R A C T

A solid phase extraction (SPE) method using pyrenebutyric acid-bonded silica (PYB) as sorbent was developed to determine 23 polychlorinated biphenyls (PCBs) in sewage water by gas chromatography–mass spectrometry (GC–MS). Factors were optimized in SPE procedures including elution solvent, pH, and cartridge burden. The recoveries of 23 PCB congeners were 69.44–111.91% under optimized conditions. Comparisons were also conducted among PYB-SPE, C18-SPE and United States Environmental Protection Agency 608 (USEPA608) methods in the analysis of PCBs in sewage water samples. The results showed that the performance of PYB-SPE method was similar with USEPA608 method and better than C18-SPE method. Both PYB-SPE and USEPA608 methods were then employed to analyze PCBs in real spiked sewage water samples. The recoveries of PCB congeners determined by PYB-SPE method ranged from 70.6% to 92.4% in real spiked sewage water samples which were identified to be in accordance with USEPA608 method. Limits of detection (LOD) were in the range of 0.06–0.22 ng L−1 for PCB congeners. The optimized PYB-SPE method was successfully applied to the determination of PCBs in sewage water samples.

2. Material and methods

2.1. Chemical reagents and materials

PYB was prepared according the method described before (Yu et al., 2005). PYB-SPE cartridges were then packed at 300 mg/
were activated with 5 mL isopropanol and H2O. C18 Sep-Pak cartridges were obtained from Waters (Millford, MA, USA). PCB standards including 23 congeners were purchased from AccuStandard (New Haven, CT, USA). The concentration series from CS1 to CS5 were 1, 5, 25, 100, 500 ng mL−1 for PCB99, 138, 180, 183, 187, were 2, 10, 50, 200, 1000 ng mL−1 for PCB28, 52, 77, 81, 101, 105, 114, 118, 123, 126, 153, 156, 157, 167, 169, 170, 189, and were 4, 20, 100, 400, 2000 ng mL−1 for PCB128, respectively. The solvents used in the study were pesticide grade from Tedia (Fairfield, OH, USA). The water used in the study was Milli-Q water.

PYB-SPE cartridges were in turn conditioned by 15 mL toluene, n-hexane, dichloromethane and n-hexane:dichloromethane (85:15, v/v) after packed, respectively. Before use, the cartridges were activated with 5 mL isopropanol and H2O. C18 Sep-Pak cartridges (5 cc) were obtained from Waters (Millford, MA, USA). Before use, the cartridges were pretreated following the manual.

2.2. Sample collection and pretreatment

Sewage water was collected from a municipal wastewater plant in Wuhan, China. After transferred into lab, the sewage water was filtrated through glass microfiber filter with core size of 0.7 μm (Whatman, Maidstone, UK) to remove particles. The samples were then adjusted to pH 2 with 1 M HCl to avoid biological degradation and stored at 4 °C in darkness. The samples were analyzed in 48 h. An aliquot of 200 mL sewage water was adjusted to pH 8 with 1 M NaOH. The sample was then loaded onto PYB-SPE cartridge at a flow rate of 10 mL min−1. After loading sample, the cartridge was washed by 2 × 5 mL of H2O and methanol: H2O (3:7, v/v) twice and dried by vacuum for 30 min. The analytes were eluted by 2 × 5 mL of n-hexane:dichloromethane (85:15, v/v). The elution was blew to nearly dryness under a gentle nitrogen stream and reconstituted to 30 μL in nonane for GC–MS analysis. Another aliquot of 200 mL sewage water was loaded onto C18-Sep-Pak cartridge at a flow rate of 10 mL min−1. After loading sample, the cartridge was also washed by 2 × 5 mL of H2O and methanol: H2O (3:7, v/v) twice and dried by vacuum for 30 min. The analytes were eluted by 5 mL of n-hexane, 3 mL of n-hexane:dichloromethane (1:1, v/v) and 5 mL of dichloromethane in sequence. The elution was concentrated to 30 μL in nonane under a gentle nitrogen stream.

A more aliquot of 200 mL sewage water was pretreated according to USEPA608 method (USEPA, 1999a). The final volume of analytes was also adjusted to 30 μL in nonane under a gentle nitrogen stream.

2.3. GC–MS analysis

The GC–MS analysis was performed on Agilent 6890-5973N GC–MS system equipped with a DB-5 ms column (30 m × 0.2 mm I.D. × 0.25 μm film thickness). The temperatures of injector and ion source were 250 °C and 230 °C, respectively. One microliter sample was injected by an autosampler in splitless mode. The carrier gas was helium at 1 mL min−1. The oven temperature was programmed as follows: initial temperature 120 °C for 1 min, heated to 220 °C at 3 °C min−1, then ramped at 6 °C min−1 to 280 °C and held for 5 min. Selected ion monitoring (SIM) was used to quantify the analytes.

A series of standard solutions was prepared to obtain the linear range of each PCB congener. Linear correlation coefficients (r2) of these congeners were varied between 0.9926 and 0.9979. Limits of detection (LODs) were also defined as signal to noise ratio of 3:1 and ranged from 0.05 to 0.22 ng L−1 for the PCB congeners. Limit of quantification (LOQ) was calculated as three times of LOD for each PCB congener.

3. Results

3.1. Optimization of PYB-SPE procedure

To obtain the best performance of PCBs analysis, PYB-SPE procedure was optimized including elution solvent, pH, and cartridge burden before the application of PYB-SPE cartridge in sewage water sample analysis.

3.2. Elution solvent

Dichloromethane and n-hexane are the most commonly used solvents in PCBs analysis. In the study, these solvents were also employed to elute PCBs from PYB-SPE cartridge. The recoveries of PCB congeners were compared among dichloromethane, n-hexane,
n-hexane:dichloromethane (85:15, v/v) and n-hexane:dichloromethane (50:50, v/v) which were loaded at 3 × 5 mL on the cartridge.

As the results shown in Fig. 1, different solvents showed different elution efficiency. The recoveries of most PCB congeners eluted by n-hexane were less than 50%. Performance of dichloromethane were better than n-hexane. The average recovery exceeded 60% for dichloromethane. n-Hexane is known as an non-polar solvent and dichloromethane is an polar solvent with a polarity index of 3.1 (http://www.chemical-ecology.net/java/solvents.htm). Therefore, n-hexane was employed to pre-elute silica gel column in some analysis of PCBs which are polar chemicals (USEPA, 1999b). Meanwhile, dichloromethane was not chosen to elute PCBs alone. In fact, the mixture of n-hexane and dichloromethane was often used to elute PCBs from silica gel column due to its proper polarity index. Both mixtures of n-hexane and dichloromethane also resulted in good recoveries of PCB congeners. The average recoveries were near 80% for both mixtures of n-hexane and dichloromethane. The performance of n-hexane:dichloromethane (85:15) was a little better than that of n-hexane:dichloromethane (50:50). Therefore, the mixture of n-hexane:dichloromethane (85:15) was used to elute PYB-SPE cartridge in the following tests.

Fig. 2. Elution curve of five PCB congeners indicating five PCB homologues. Condition: 200 mL water spiked with 50 μL of CS3 was loaded onto the cartridge before elution.

Fig. 3. Effects of pH values on the recoveries of 23 PCBs. Condition: 200 mL water spiked with 50 μL of CS3 was loaded onto the cartridge before elution.
3.3. Volume of elution solvent

The volume of elution solvent should vary among PCB congeners due to their different characters originated from the chemical configuration. To identify the proper elution volume of n-hexane:dichloromethane (85:15), eight aliquots of 2 mL n-hexane:dichloromethane (85:15) were used to in turn elute PCBs from PYB-SPE cartridge after loaded 200 mL water spiked with 50 μL of PCBs standard solution CS3. The concentrations of PCB congeners were then determined in each aliquot. The recoveries of PCB28, 81, 99, 156 and 189 were chosen to indicate the results of tri, tetra, penta, hexa and hepta chlorinated biphenyls in Fig. 2, respectively.

From the results, most proportion of each congener was eluted by 8 mL of n-hexane:dichloromethane (85:15). The recoveries of PCB congeners all exceeded 65% in 8 mL of n-hexane:dichloromethane (85:15). The rest of each PCB congener was less than 3% in last three aliquots. Therefore, 10 mL of n-hexane:dichloromethane (85:15) was used to elute PCBs from PYB-SPE cartridge in the following tests.

3.4. pH

Fifty microliters of PCBs standard solution CS3 was spiked into 200 mL of phosphate buffers with pH 2.0, 3.5, 5.0, 7.0 and 8.0, respectively. These buffers were then loaded onto PYB-SPE cartridges and eluted by 10 mL of n-hexane:dichloromethane (85:15). The results of PCBs recoveries in elution were shown in Fig. 3. The recoveries increased with the elevation of pH value. The phosphate buffer with pH 8.0 resulted in the best performance among all of the tests. The recoveries for PCB congeners all exceeded 73% at the pH value.

In PYB molecules, there are amido groups and amino groups. These groups bind with protons under acid conditions (Yu et al., 2006). Protonation of these groups would prevent PCBs from binding with PYB molecules and reduce the retention of PCBs in PYB stationary phase. On the contrary, PYB is transformed to neutralized molecules to validate the binding with PCBs in weak alkaline solution. Meanwhile, the binding between PCBs and PYB would be strengthened by hydrophobicity and charge-transfer ability of PYB molecule in weak alkaline solution. Therefore, the best recovery for each PCB congener was obtained at pH 8.0.

3.5. Cartridge burden

In the present study, PYB-SPE cartridges were packed with stationary phase at 300 mg/5 mL. To determine the cartridge burden, 200 mL water spiked with 10, 20, 50, 70, and 100 μL of PCBs standard solution CS3 was loaded onto PYB-SPE cartridges, respectively. The concentrations of PCB congeners were then detected in the elution solution.

From the results shown in Fig. 4, the average recoveries of PCB congeners were near 80% for the PYB-SPE cartridges loaded on 10, 20, and 50 μL of CS3. However, the average recoveries of PCB congeners sharply decreased for the PYB-SPE cartridges loaded on 70 μL of CS3 and above. If the amount of PCB congeners exceeds the cartridge burden, the rest of PCB congeners could not bind with the stationary phase and result in the loss of PCB congeners in the elution solution. Therefore, the cartridge burden of PYB-SPE cartridge

![Table 1](image)

The congeners not listed in the table were not detected by the three methods.
at 300 mg/5 mL was determined as 50 μL of PCBs standard solution CS3. Under the condition of 50 μL of PCBs standard solution CS3, the recoveries of PCBs ranged from 69.44% to 111.91%.

### 3.6. Determination of PCBs in sewage water samples

After optimized, PYB-SPE cartridge was employed to analyze PCBs in sewage water. Meanwhile, the concentrations of PCBs were also determined in the samples by C18-SPE and USEPA608 methods to compare the performances of these different methods.

The concentrations of PCB congeners in sewage water samples were shown in Table 1. The recoveries of PCB congeners ranged from 69.44% to 111.91%. The performance of PYB-SPE method was similar with USEPA608 method and better than C18-SPE method in the analysis of real sewage water. PYB-SPE method was only employed to analyze PCBs in sewage water in the study, but it is expected to be applied to other matrices with proper modification.

### References


