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Liquid Chromatography

DETERMINATION OF THE MIGRATION OF BISPHENOL A FROM POLYCARBONATE BY DISPERSIVE LIQUID-LIQUID MICROEXTRACTION COMBINED WITH HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

Jiangping Cao,[1] Shuhui Liu,[1,2] Weiwei Bai,[1] Jie Chen,[1] and Qilong Xie[1]
[1]*College of Science, Northwest A&F University, Yangling, Shanxi, China*
[2]*State Key Laboratory of Crop Stress Biology in Arid Areas, Yangling, China*

A novel method for the determination of the migration of bisphenol A (BPA) from polycarbonate water bottles has been developed by using dispersive liquid–liquid microextraction (DLLME) followed by high performance liquid chromatography for compliance with the regulatory specific migration limit. Experimental parameters, including the type and volume of extractants and dispersers, the sample solution pH, addition of salt, extraction time, and temperature, were examined and optimized. Under the optimum conditions, average recovery rates for the real samples varied from 82% to 98%, with relative standard deviation values less than 3.6%. The method offered excellent linearity over a range of 0.8–600 µg L^{-1} with a correlation coefficient of $r = 0.9999$. Intra-day and inter-day repeatability values expressed as relative standard deviation were 3.9% and 6.9%, respectively. The method quantitation limit and detection limit were 0.7 and 0.2 µg L^{-1}, respectively. The developed method was successfully applied to the determination of the migration of bisphenol A from 26 polycarbonate water bottles collected locally. The findings indicated the migration from the used bottles was significantly higher than the new ones, and the migration amounts from one sample was very close to the regulatory specific migration limit, and the amounts from seven samples exceeded the daily intake limit for infants.

Keywords: Bisphenol A; Dispersive liquid–liquid microextraction; Polycarbonate water bottles

INTRODUCTION

Bisphenol-A (BPA) is an industrial intermediate principally used for the manufacture of polycarbonate and epoxy resins, which are utilized to produce common plastic products, such as baby bottles, water bottles, and the linings of most food and beverage cans. Because of the incomplete reactions, plastic products contain

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Address correspondence to Shuhui Liu, College of Science, Northwest A&F University, No. 3 Taicheng Road, Yangling, Shanxi 712100, China. E-mail: shliu815@126.com

the unreacted BPA, which can migrate into food and drink from the plastic containers during the use process.

BPA has well-characterized estrogenic and endocrine disrupting activities, resulting in a serious threat to human health (Le et al. 2008; Tominaga et al. 2006; Wetherill et al. 2007). To reduce the risk of possible health problems associated with BPA, the tolerable daily intake for BPA was established at $50\,\mu g\,kg^{-1}$ body weight day^{-1} by the United States (U.S. Environmental Protection Agency 1993), and at $10\,\mu g\,kg^{-1}$ body weight day^{-1} by the European Commission's Scientific Committee on Food (SCF) (2002). The European Commission (EC) established a specific migration limit (SML) for BPA of 0.6 mg/kg of food or food simulant in Commission Directive 2004/19/EC (2004). In addition, Canada, the European Union, the U.S. and some other countries banned the use of polycarbonate baby bottles successively from 2008 onward.

However, the high amounts of production and the wide application of polycarbonate water bottles still exist, which may be the main route of BPA exposure to people in addition to canned food. In addition, the BPA contamination from polycarbonate water bottles is augmented severely because BPA can also be released by polycarbonate hydrolysis. The related studies showed the migration of BPA from polycarbonate bottles ranged from <1 ppm to as high as 599 ppm (Biles et al. 1997; Kawamura et al. 1998; Howe et al. 1998). The wide range of migration levels from article to article may be due in part to processing conditions as well as raw material sources. Therefore, developing a reliable, fast, and green analytical method to support the implementation of the aforementioned internationally accepted directives are of particular importance for ensuring that the migration amounts of BPA are lower than SML and the tolerable daily intake.

The quantitative determination methods of BPA are mainly HPLC-UV (Rezaee et al. 2009), HPLC-MS/MS and GC/MS (Shao et al. 2007; Xiao et al. 2011; Cunha and Fernandes 2010). The crucial step for measuring the migration of BPA from plastic cups and food packaging is the preconcentration of the target analyte from sample matrix, for which liquid–liquid extraction (LLE) (Zafra et al. 2003), solid-phase extraction (SPE) (Lin et al. 2012), solid-phase microextraction (SPME) (Tan et al. 2009), stir bar sorptive extraction (SBSE), and hollow fiber liquid-phase microextraction (HF-LPME) were often utilized (Coensel, David, and Sandra 2009; Kawaguchi et al. 2008). Because of the disadvantages involved in these sample preparation techniques including time- or solvent-consumption as well as tedious operation processes, pursuing a new approach to extract and concentrate BPA from sample matrix is necessary.

As dispersive liquid-liquid micro-extraction (DLLME) was proposed by Assadi and coworkers in 2006 (Rezaee et al. 2006), this method has quickly gained popularity, as evidenced by its appearance in a continually growing numbers of original papers and reviews (Viñas et al. 2012; Herrera-Herrera et al. 2010; Rezaee, Yamini, and Faraji 2010). Owing to its significant advantages including less solvent usage, shorter extraction times and a higher enrichment ratio, DLLME has been widely used for the extraction of organic pollutants in environmental samples (Zhou, Pang, and Xiao 2009; Najafia et al. 2012).

A few research papers dealing with the extraction BPA by using DLLME have been reported by Zhou, Gao, and Xie (2011) who used temperature-controlled ionic liquid as an extractant to determine BPA in environmental water samples; by Rezaee et al. (2009), who used chloroform as an extractant to determine BPA in domestic water; and by López-Darias et al. (2010), who contrasted the extraction efficiency of BPA between DLLME and single-drop microextraction using 1-decanol as an extraction solvent to determine BPA from seawaters. Owing to the tedious DLLME experimental procedure, or carcinogenic extractant used, or unconvenience of DLLME performance caused by low melting point of the extractant, the DLLME methods developed previously are not suitable as a routine technique for the determination of the migration of BPA from plastic water bottles.

Therefore, the objective of this study was to develop a reliable, fast, low-cost, and green analytical method for the determination of the migration of BPA from polycarbonate bottles by using DLLME followed by HPLC-UV, and to evaluate the quality of 26 polycarbonate water bottles collected locally based on SML and the tolerable daily intake for BPA.

MATERIALS AND METHODS

Reagents and Chemicals

Bisphenol A was purchased from the Aladdin Reagent Company (Shanghai, China). Cetyltrimethylammonium bromide (CTAB), sodium dodecyl sulfate (SDS), triton X-114, 1-dodecanol, and 1-octanol were supplied from Sigma-Aldrich (St. Louis, MO, USA). Analytical-grade sodium chloride was obtained from Tianjin BODI Chemical Reagent Co. Ltd. (Tianjin, China). HPLC grade methanol was provided by Kemiou Chemical Reagent Co. Ltd. (Tianjin, China). Disposable polyethylene pipets were purchased from Xinjian Medical Plastic Factory (Jiangsu, China).

To prepare a stock standard solution (1 mg mL^{-1}), 10 mg of bisphenol A was accurately weighed and dissolved in 10 mL of methanol and then stored at 4°C. Further dilutions were later made using water.

The 10 new polycarbonate water bottles from different brands were purchased from local supermarkets. The 16 used polycarbonate water bottles from different brands were collected on the campus of Northwest A&F University.

Instrumentation

The quantitative analysis was performed on a Shimadzu Prominence LC-20A (Kyoto, Japan) equipped with two LC-20AT pumps, a 7725i manual sample injector, and a SPD-M20A photodiode array detector. Separations were carried out on an Inertsil C_{18} column (150 mm × 4.6 mm, with 5 µm particle size). A mixture of water and methanol (35:65) at a flow rate of 1.0 mL/min was used as a mobile phase in isocratic elution mode. The injection volume was 20 µL for all the solutions and the detection was performed at the wavelength of 214 nm. An ultrasonic cleaning machine (Model KQ-5200E, Kunshan City Ultrasonic Instruments Inc., China) was used. Centrifugation was done with a Beckman Allegra X-12 system (Beckman Coulter Inc., Fullerton, USA). A 100 µL and 500 µL Hamilton microsyringe (Reno, USA) was used to collect and measure the volume of extraction solvent. The pH meter of Model 213 (Hanna, Italy) was used. Water was purified using a Millipore Direct-Q 3 system (Millipore Corporation, Bedford, MA, USA).

Migration from Polycarbonate Water Bottles

Water was used as the food simulant for the migration testing according to Directive 82/711/EEC and 85/572/EEC (1982; 1985). Two migration experiments were performed with the polycarbonate water bottles.

Migration based on directive 82/711/EEC. All of the 26 polycarbonate water bottles were used to examine the migration based on Directive 82/711/EEC. After cleaning with ultrapure water, each of the bottles was filled fully with boiling ultrapure water and the cap was screwed tightly. The bottles were then placed into an oven at 100°C for 4 hours. After that the water was cooled to room temperature, and the extraction and concentration procedure of BPA were followed.

Migration based on directive 82/711/EEC after use under severe condition. After cleaning with ultrapure water, the 10 new polycarbonate bottles fully filled with ultrapure water were continuously heated in an oven at 100°C for 7.5 days (180 hours). Then the water bottles were emptied. The following step was the same as previously described.

A blank sample was also prepared with ultrapure water placed in a glass bottle at the same temperature and conditions previously described.

Extraction Procedures

The 3.5 mL of water sample was added into a disposable polyethylene pipette with a long and narrow neck, to which 120 μL of 1-octanol (as an extraction solvent) was injected rapidly with a 500-μL syringe. Then, the pipette was put in a centrifugal tube to seal its tip mouth using the tube lid. After shaking for 5 min, the resultant cloudy solution was centrifuged for 3 min at 5000 rpm. After centrifuging, the extraction phase (112 μL ± 2 μL) floated on the top of the narrow neck of the pipette was withdrawn and completely transferred into a sample vial using a microsyringe, during which the extraction phase volume was precisely measured. Then, the sample extracted was diluted with the same volume of methanol and injected into the HPLC system. All experiments were performed in triplicate.

Enrichment Factor and Recovery and Migration Amount

In this study, the enrichment factor (EF) of the DLLME was calculated by the ratio of the analyte concentration in the organic phase (C_O) and the initial concentration of analyte (C_W) in the water samples:

$$\mathrm{EF} = \frac{C_O}{C_W}$$

The results of the migration tests were calculated by applying the following formula:

$$M = \frac{m \times a_2}{a_1 \times q} \times 1000$$

where M is the migration in mg/kg; m is the mass in mg of substance released by the sample as determined by the migration test; a_1 is the surface area in dm^2 of the sample in contact with the foodstuff or simulant during the migration test; a_2 is the surface area in dm^2 of the material or article in real conditions of use; and q is the quantity in grams of foodstuff in contact with the material or article in real conditions of use. In this study, because the water bottles tested were filled fully with water, a_1 was equal to a_2.

Validation Assay

A calibration curve and linear regression equation were determined for external BPA standards using five dilutions ranging from 0.2–20 µg/L. The injection volume was 20 µL.

Instrumental precision was determined by injecting a standard solution at a concentration of $500\,\mu g\,L^{-1}$ for six replicate experiments. The repeatability of the extraction procedure was evaluated by using the relatively standard deviation (RSD) of six measurements of a real sample taken on one day or six continuous days.

Three real samples were chosen to determine the recovery capacity of the method. Water containing 20, $100\,\mu g\,L^{-1}$ BPA were filled fully into the selected polycarbonate bottles and performed as described in section of migration based on Directive 82/711/EEC. Every concentration level was carried out in triplicate, and the corresponding DLLME was performed three times. Recovery rates were calculated by the equation $R\% = [(C_s - C_p)/C_a] \times 100$, where R (%) is the percent recovery of added BPA, C_s is total BPA content in the spiked simulant sample (water), C_p is endogenous BPA content in simulant water without BPA spiked, and C_a is the amount of BPA standard added to water.

RESULTS AND DISCUSSION

Optimization of Extracting Conditions of DLLME

Selection of extracting solvent. The characteristics of an extractant are one of the most important factors influencing the extraction efficiency of DLLME. Extractant solvents used mainly are halogenated hydrocarbons such as chlorobenzene, chloroform, carbon tetrachloride, tetrachloroethylene, and chlorobenzene, which can be easily sedimented at the bottom of the test tube by centrifugation after extraction because of their heavier density than water. However, these extractant solvents are highly toxic, and some are even possible human carcinogens. To overcome this drawback, researchers have recently attempted to use solvents with the density lower than that of water (octanol, decanol, etc.) (Yiantzi et al. 2010; López-Darias et al. 2010) and ionic liquid as extractants (Zhou et al. 2009).

In this experiment, 70 µL of 1-octanol or 1-dodecanol was added into water sample solution containing 15% NaCl (*w*/*v*) and 0.1 mM CTAB (pH 6.5, without adjusting) to examine the extraction efficiency of BPA. The results show that the extraction efficiency achieved by using 1-octanol and 1-dodecanol were 97% and 92%, respectively. The polarity of 1-octanol is slightly stronger than that of 1-dodecanol, which results in the higher solubility of BPA in 1-octanol in comparison with 1-dodecanol. In addition, it was more convenient to perform the DLLME with the former at room temperature because of the latter solidification caused by its lower melting point. Therefore, 1-octanol was selected.

Selection of disperser solvent. In DLLME, the application of the disperser solvent can reduce the surface tension between two phase interfaces, increase contact area, and improve extraction efficiency. In this study, 70 μL of 1-octanol were added into 3.5 mL of water sample solution (15% NaCl, pH 6.5) containing three types of surfactants (0.1 mM SDS, CTAB and Triton X-114) to investigate the extraction efficiency. Meanwhile no disperser solvent and 200 μL of methanol as a disperser solvent were also tested. As can be seen in Fig. 1, the addition of a disperser did not improve the extraction efficiency, showing a disperser was not required in this DLLME procedure.

Influence of water sample pH. BPA has a pK_a from 9.59 to 11.30 (Staples et al. 1998). To extract BPA from aqueous solution to organic solvent effectively, it is reasonable to keep environmental pH levels at acidic and neutral condition. The 70 μL of 1-octanol were added into 3.5 mL of sample solution containing 15% NaCl (*w*/*v*) with three different pH (1.0, 3.0, 6.5) to examine the influence of pH on the extraction efficiency. The results showed the pH did not obviously influence the extraction efficiency of BPA. Therefore, pH 6.5 was chosen for the following experiment because of the easy performance.

Influence of ionic strength. Changing the ionic strength of an aqueous solution can cause variance of the solubility of the analyte and affect the extraction efficiency. Under the aforementioned optimized extraction conditions obtained, the extraction efficiency of BPA was investigated when the concentration of sodium chloride varied from 0 to 30% (*w*/*v*). According to the results illustrated in Fig. 2, the extraction efficiency of BPA decreased while the concentration of sodium chloride was increased. Therefore, salt was not added in the subsequent experiments.

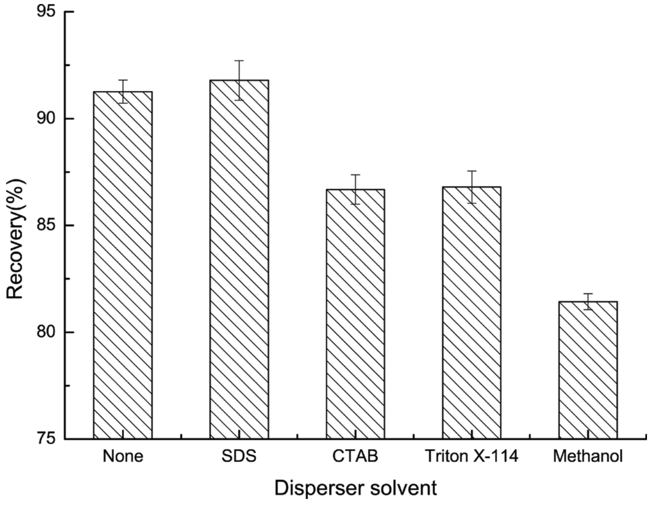

Figure 1. Effect of disperser solvent on the extraction efficiency.

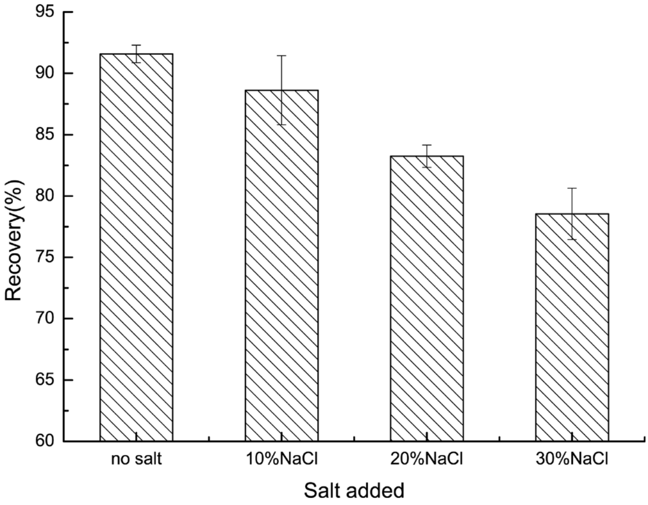

Figure 2. Effect of ionic strength on the extraction efficiency.

Selection of the extractant volume. The influence of the extractant volumes on the extraction efficiency was investigated in the range of 30–150 μL. As can be seen in Fig. 3, the extraction efficiency increased when 1-octanol volume was increased from 30 μL to 120 μL. With the volume over 120 μL, the extraction efficiency leveled off. Therefore, 120 μL of 1-octanol were appropriate.

Selection of shaking time and extraction temperature. During the extraction procedure, mass transfer between the two phases is a key step for the efficient extraction, which makes both shaking time and extraction temperature important DLLME parameters. In this study, shaking times in the range of 3–10 min were examined. When shaking time increased from 3 to 5 min, the extraction

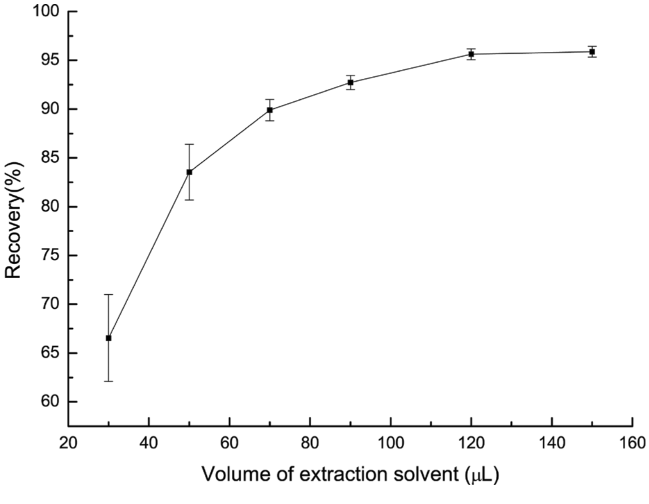

Figure 3. Effect of the extractant volume on the extraction efficiency.

efficiency increased; with the period being longer, the extraction efficiency did not increase accordingly. Therefore, a shaking time of 5 min was optimal.

With regard to the extraction temperature, generally the extraction efficiency is improved with increasing temperature because of the reduced surface tension and enlarged contact area. The comparison of the extraction efficiency resulted from different extraction temperature (20, 60°C) indicated that extraction temperature did not obviously affect the result. Therefore, the extraction procedure was performed at room temperature.

Performance Characteristics for the Validated Method

Linearity, precision and repeatability. Analytical characteristics were obtained under optimum conditions. The calibration curve in the concentration range of 0.8–600 $\mu g\,L^{-1}$ of BPA was established with a regression equation of $Y = 73467X + 14643$ ($r = 0.9999$). The RSD values for the instrumental precision were 3.2%.

The BPA content in the water sample was determined using six replicates during one day to obtain the intra-day precisions with a RSD value of 3.9%. The inter-day precisions were carried out during a week and the RSD value obtained was 6.9%. All of the RSDs were adequate for the levels of analytes in samples.

Recovery rate. Three samples were chosen to determine the recoveries of this method (see Table 1). The average recovery rates were 82%–98% and 83%–88% when the spiked contents were 20 and 100 $\mu g\,L^{-1}$, respectively, and the RSD value was in the range of 1.1–3.6%.

Enrichment factor and method quantitation limit. Under the optimal experiment conditions, an EF of 31 was achieved.

The method quantitation limit (MQL), estimated as $X_b + 10SD_b$ (X_b average and SD_b standard deviation of the eight blank samples), was determined to be 0.7 $\mu g\,L^{-1}$. The method detection limit (MDL), estimated as $X_b + 3SD_b$, and was found to be 0.2 $\mu g\,L^{-1}$. Table 2 compares LODs, LRs, sample volumes, extractant solvent, and enrichment factor for previously reported DLLME methods dealing with the extraction and determination of BPA in water samples. In Rezaee's report,

Table 1. Recovery rates of bisphenol A in real water samples

Samples	Spiked ($\mu g\,L^{-1}$)	Total found ($\mu g\,L^{-1}$)	Average recovery (%)	% RSD ($n = 3$)
S12	0	2.81	—	3.3
	20	19.30	82	1.7
	100	86.34	83	1.1
S22	0	23.19	—	4.8
	20	40.38	86	2.5
	100	111.41	88	2.1
S17	0	71.27	—	3.8
	20	90.98	98	3.6
	100	156.33	85	1.1

Table 2. Comparison of this DLLME procedure with previously reported DLLME methods

Methods	LOD ($\mu g\,L^{-1}$)	LR ($\mu g\,L^{-1}$)	Sample volume (mL)	Extractant solvent (μL)	EF	Reference
DLLME-HPLC-FLD	0.23	1.0–100	10.0	$[C_8MIM][PF_6]$	–	Zhou et al. 2011
DLLME-HPLC-UV	0.07	0.5–100	10.0	Chloroform	150	Rezaee et al. 2009
DLLME-HPLC-UV	0.70	2.0–20	5.0	1-Decanol	89	López-Darias et al. 2010
DLLME-HPLC-UV	0.20	0.2–20	3.5	1-Octanol	31	This study

(–) Was not shown.

although a lower LOD had been achieved, the extractant solvent was chloroform. In Zhou's (2011) work, ionic liquid was utilized with temperature-controlling mode, which was not as simple as the method we developed. In López-Darias's (2010) work, extractant solvent employed was 1-decanol, which was easily solidified at room temperature caused by its lower melting point. Therefore, the method we developed is reliable, simple, environmentally benign, low cost, and highly sensitive.

Application of DLLME-HPLC in Real Samples

Migration based on the directive of EC. In this study, the proposed DLLME-HPLC method was applied for determining the migration of BPA for 10 brand new polycarbonate bottles and 16 used ones according to the basic rules set by council directive 82/711/EEC; the results are shown in Table 3 and Fig. 4. The

Table 3. BPA migration amounts from real samples

New samples	BPA migration amount[a] ($\mu g\,kg^{-1}$) Brand new[b]	After using[c]	Used samples	BPA migration amount[a] ($\mu g\,kg^{-1}$)
S1	1.98 ± 0.07	6.84 ± 0.05	S11	5.78 ± 0.17
S2	0.97 ± 0.05	4.13 ± 0.08	S12	2.57 ± 0.04
S3	1.04 ± 0.04	3.52 ± 0.03	S13	9.55 ± 0.47
S4	3.64 ± 0.12	4.04 ± 0.02	S14	68.89 ± 1.26
S5	0.98 ± 0.02	2.34 ± 0.11	S15	45.37 ± 2.38
S6	1.16 ± 0.02	2.30 ± 0.07	S16	509.22 ± 11.72
S7	0.95 ± 0.01	9.86 ± 0.50	S17	71.57 ± 3.2
S8	1.30 ± 0.15	3.18 ± 0.10	S18	13.22 ± 0.35
S9	0.87 ± 0.08	4.78 ± 0.19	S19	7.39 ± 0.30
S10	1.77 ± 0.04	10.77 ± 0.15	S20	22.31 ± 0.91
			S21	6.00 ± 0.06
			S22	12.70 ± 0.33
			S23	85.64 ± 2.96
			S24	53.85 ± 1.02
			S25	8.60 ± 0.43
			S26	2.57 ± 0.05

[a] Mean ± %RSD ($n = 3$).

[b] Migration obtained when first using.

[c] Migration obtained after severe treatment.

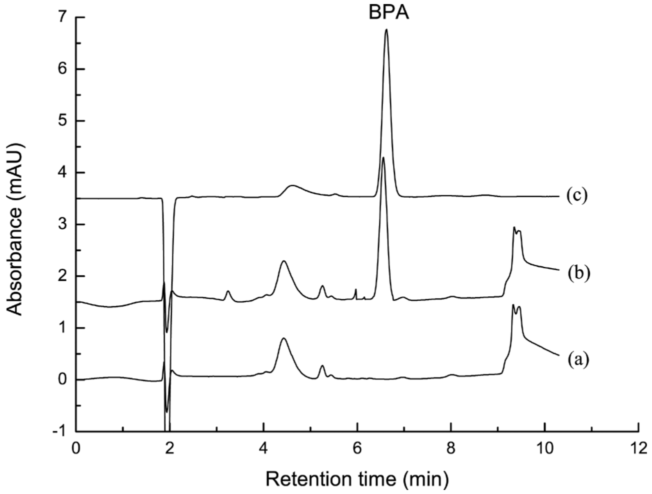

Figure 4. Chromatogram of a blank water sample extracted by DLLME (a), a real sample extracted by DLLME (b) and direct analysis of the standard solution without DLLME (c).

migration in the new polycarbonate bottles ranged from 0.87 to 3.64 µg/kg, and the migration in the used ones ranged from 2.57 to 509.22 µg/kg, which showed the migration level of the latter sample set increased significantly in comparison with the former set. The wide range of migration levels may be due in part to processing and/or usage conditions as well as raw material sources.

EC established a specific migration limit (SML) for BPA of 0.6 mg/kg of food or food simulant in Commission Directive 2004/19/EC (2004). The BPA migration level of sample 16 was close to SML (0.6 mg/kg), while the migration levels from other samples were much lower than the limit. The daily intake of BPA was estimated by using the quantity of consumed drinking water that are 2 kg per day for adults (60 kg body weight) and 1 kg per day for infants (10 kg body weight) according to the U.S. EPA (U.S. EPA, 2006). The Tolerable Daily Intake set by the European Commission's Scientific Committee on Food (SCF) is 0.01 mg/kg. According to this level, the tolerable BPA concentration in drinking water should be lower than 300 µg/kg for adults and 50 µg/kg for infants, respectively. Sample 16 exceeded this limit for adults and samples 14, 16, 17, 23, and 24 exceeded the limit for infants. These findings indicated health issues resulted from exposure to BPA by drinking with polycarbonate bottles should not be ignored.

Brede et al. (2003) compared the migration levels of BPA from new polycarbonate bottles and ones that were dishwashed and brushed and boiled, showing that the migration level of BPA varied from 0.23 to 8.4 $\mu g\,L^{-1}$ (dishwashed 51 times) and 6.7 $\mu g\,L^{-1}$ (dishwashed 169 times), respectively. It was considered that the nomadic BPA remained on the surface of bottle during the manufacturing process. The surfaces of the bottles eroded during the usage for the scouring of the drinking water, which accelerated the migration of BPA because of the expansion of the contact area with water. This might be the reason why the migration from the used polycarbonate bottles was much higher than that from the new samples.

Migration based on the directive of EC after use under severe conditions. The migration level of BPA was also examined after 10 new polycarbonate bottles were treated at 100°C for 7.5 days; the results are shown in Table 3. After the severe treatment, the migration level varied from the original range of 0.87–3.64 µg/kg to the range of 2.30–10.77 µg/kg, increasing by one to ten-fold Nam, Seo, and Kim (2010) determined the migration of BPA from polycarbonate baby bottle with repeated use at 95°C for 30 min, indicating that the migration curve included lag effect region (0.13–1.11 ppb, <10 times), steady region (1.11 ppb, 10–60 times), and aging region (1.11–3.08 ppb, >60 times). In this experiment, the migration of the brand new polycarbonate bottles belonged to the lag effect region; after being treated under the exaggerated condition, the migration level was within aging region, which could explain the reason why the migration was increased tremendously after the treatment.

CONCLUSIONS

In this present study, a DLLME-HPLC-UV method for the determination of the migration of BPA in polycarbonate water bottles was developed. The 1-octanol, which is relatively less toxic in comparison with the widely used chlorinated solvents in DLLME, was utilized as the extractant; additionally, the addition of a disperser and salt and the adjustment of the sample solution pH were not required. Moreover, a commercial available pipette was used to perform DLLME without needing a special device. All these features made this DLLME procedure environmentally benign, simple, rapid, and inexpensive. It is also a reliable and sensitive method because of the excellent precision and low LOD achieved. This DLLME-HPLC-UV method can be applied as a routine technique for evaluating the quality of polycarbonate water bottles based on SML and the tolerable daily intake for BPA.

Findings from the real samples assay indicated the migration amounts from the used bottles was significantly higher than the new ones, and some were close to the regulatory specific migration limit or exceeded the daily intake limit. More attention should be paid to BPA exposure to people who use polycarbonate water bottles.

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