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Short communication

Dispersive liquid–liquid microextraction combined with high-performance liquid chromatography-UV detection as a very simple, rapid and sensitive method for the determination of bisphenol A in water samples

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ABSTRACT

Dispersive liquid–liquid microextraction (DLLME) coupled with high-performance liquid chromatography (HPLC)-UV detection was applied for the extraction and determination of bisphenol A (BPA) in water samples. An appropriate mixture of acetone (disperser solvent) and chloroform (extraction solvent) was injected rapidly into a water sample containing BPA. After extraction, sedimented phase was analyzed by HPLC-UV. Under the optimum conditions (extractant solvent: 142 μL of chloroform, disperser solvent: 2.0 mL of acetone, and without salt addition), the calibration graph was linear in the range of 0.5–100 μg L^{-1} with the detection limit of 0.07 μg L^{-1} for BPA. The relative standard deviation (RSD, $n = 5$) for the extraction and determination of 100 μg L^{-1} of BPA in the aqueous samples was 6.0%. The results showed that DLLME is a very simple, rapid, sensitive and efficient analytical method for the determination of trace amount of BPA in water samples and suitable results were obtained.

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1. Introduction

Bisphenol A (BPA) is a chemical used in polycarbonate plastics, epoxy resins and also in various industrial products. In 1993, Krishnan et al. reported that BPA exhibited estrogenic activity and is released from polycarbonate flasks during autoclaving [1]. In addition, the estrogenic activity of BPA has been extensively evaluated by a variety of assays [2–3].

Although many studies have been done for detection of BPA in environmental samples [4–7], the effect of BPA on environmental samples still remains a controversial issue. Highly reliable methods are required for the detection of trace compounds with estrogenic activity. Some sample preparation techniques as liquid–liquid extraction (LLE) [8], solid-phase extraction (SPE) [9–11] and molecularly imprinted solid-phase extraction (MISPE) [12,13] have been developed for the extraction of BPA from various matrices. Recently, solventless and solvent minimized polymer sorption techniques such as solid-phase microextraction (SPME) [14] and stir bar sorptive extraction (SBSE) [15] have been successfully applied for the extraction of BPA from water samples. Liquid-phase microextraction (LPME), that use only a single droplet of a solvent has been developed for the extraction of different analytes from water samples [16]. Also, LPME has been applied successfully to the extraction of BPA in water samples [17].

A novel microextraction technique, termed dispersive liquid–liquid microextraction (DLLME) as a powerful preconcentration technique was demonstrated by Rezaee et al. [18]. The performance of DLLME was illustrated by extraction of different organic and inorganic compounds [18–25] from water samples.

In the present study, the applicability of the DLLME combined with HPLC-UV for the extraction and determination of BPA in water samples was investigated.

2. Experimental

2.1. Chemicals and reagents

Bisphenol A was purchased from Merck (Darmstadt, Germany). HPLC-grade solvents were used throughout of the experiments and were obtained from Merck. The ultra-pure water used was purified on a model Aqua Max-Ultra Youngling Ultra-Pure water purification system (Dongan-gu, South Korea). Proper amounts of BPA were dissolved in methanol to obtain stock solution of the analyte with a concentration of 250 mg L^{-1}. Working standard solutions were freshly prepared by diluting the standard solution of the analyte with ultra-pure water to the required concentration.

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2.2. HPLC system

Chromatographic separations were carried out on a Varian HPLC equipped with a 9012 HPLC pump (Mulgrave, Australia), a 9010 autosampler (having a 20 μL sample loop) and a Varian 9050 UV–vis detector. Separations were carried out on a Zorbax Extend C_{18} column (15 cm × 4.6 mm, with 3 μL particle size) from Agilent (Wilmington, DE, USA). A mixture of water and acetonitrile (55:44) at a flow rate of 1 mL min^{-1} 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 224 nm.

2.3. Dispersive liquid–liquid microextraction procedure

A 10.0 mL of ultra-pure water was placed in a 40 mL glass tube with conical bottom and spiked at the level of 100 μg L^{-1} of BPA. Acetone (2.0 mL), as disperser solvent, (containing 142 μL chloroform) was injected rapidly into the sample solution using a 5.0 mL gastight Hamilton syringe (Bonaduz, Switzerland). The produced cloudy solution was centrifuged for 5 min at 6000 rpm by applying the model 2010 D Centurion Scientific Centrifuge (West Sussex, UK). After centrifuging, the sedimented phase (about 30 ± 2 μL) was completely transferred into another test tube and after evaporation of the solvent in a water bath; the residue was dissolved in 30 μL of HPLC-grade methanol and injected into the HPLC. All the experiments were performed in triplicates and means of the results were calculated and reported.

3. Results and discussion

Preconcentration factor (PF) and percent extraction recovery (ER%) as analytical responses were calculated based on the following equations:

$$PF = \frac{C_{sed}}{C_0} \quad (1)$$

$$ER\% = C_{sed} \times V_{sed}/C_0 \times V_{aq} \times 100 \quad (2)$$

where C_{sed} and C_0 are concentration of the analyte in the sedimented phase and initial concentration of the analyte in the aqueous sample, respectively. V_{sed} and V_{aq} are the volume of the sedimented phase and volume of the aqueous sample, respectively. C_{sed} is calculated from a calibration curve which was obtained by direct injection of BPA with the concentrations in the range of 5–25 mg L^{-1}.

On the other hand the relative recovery (RR) was obtained from the following equation:

$$RR\% = \frac{C_{found} - C_{real}}{C_{added}} \times 100 \quad (3)$$

where C_{found}, C_{real} and C_{added} are the concentrations of analyte after addition of known amount of standard in the real sample, the concentration of analyte in real sample and the concentration of known amount of standard which was spiked to the real sample, respectively.

3.1. Selection of extractant solvent

In the present study, chlorobenzene (density, 1.11 g mL^{-1}), carbon tetrachloride (density, 1.59 g mL^{-1}) and chloroform (density, 1.48 g mL^{-1}) were selected as extractant solvents. The study was performed by using 2.0 mL of acetone containing different volumes of the extractant solvent to produce about 30 μL of the sedimented phase. Thereby, 71, 76 and 142 μL of chlorobenzene, carbon tetrachloride and chloroform were used, respectively. ER% using chlorobenzene, carbon tetrachloride and chloroform were 33.9%, 28.3% and 45.2%, respectively. The results revealed that chloroform has the highest extraction recovery in comparison with the other tested solvents. It is probably because of higher solubility of BPA in chloroform in comparison with chlorobenzene and carbon tetrachloride. Also, evaporation of chloroform is easier than the other tested solvents. Therefore, chloroform was selected as the extraction solvent.

3.2. Selection of disperser solvent

Acetone, acetonitrile and methanol, which are miscible with water and the extractant solvents, were selected as disperser solvents. A series of sample solutions were prepared by the injection of 2.0 mL of each disperser solvent containing 142 μL chloroform (as extractant solvent) into the sample solution. Considering the sedimented phase volume, it was found that with combination of chloroform–acetonitrile, the sedimented phase volume was very higher than 30 μL and the cloudy state was not formed well, whereas in the case of chloroform–methanol, and chloroform–acetone, the sedimented volume was about 30 μL. Therefore, acetone and methanol could be selected as disperser solvents for further studies. Further experiments revealed that the ER% in the presence of acetone and methanol were 46.5% and 36.7%, respectively. According to the results, acetone has the higher percent recovery, lower toxicity and lower cost in comparison with methanol. Therefore, acetone was selected for further studies.

3.3. Effect of extractant solvent volume

To examine the effect of extractant solvent volume on the ER%, solutions containing different volumes of chloroform (122, 132, 142, 152 and 162 μL) and fixed volume of acetone (2.0 mL) were used for DLLME procedures. By increasing the volume of chloroform from 122 to 162 μL, the volume of the sedimented phase increases from 10 to 56 μL. Also, according to Fig. 1, by increasing the volume of chloroform, the ER% of analyte increases. On the other hand, preconcentration factor decreases by increasing the volume of chloroform (Fig. 1) due to increase in the sedimented phase volume. The volume of extractant solvent has to be selected to obtain high PF and ER%. In the following studies, 142 μL of chloroform was selected as an optimal volume of the extractant solvent.

3.4. Effect of disperser solvent volume and extraction time

Variation of the volume of acetone causes changes in the volume of sedimented phase. To obtain a constant volume of sedimented phase, the volumes of acetone and chloroform were changed, simultaneously. The experimental conditions were fixed and included the use of different volumes of acetone (0.50, 1.0, 2.0 and 4.0 mL) containing 100, 120, 142 and 158 μL of chloroform, respectively. The obtained results showed that ER% of BPA increases by increasing of the volume of acetone and then decreases by further increasing of

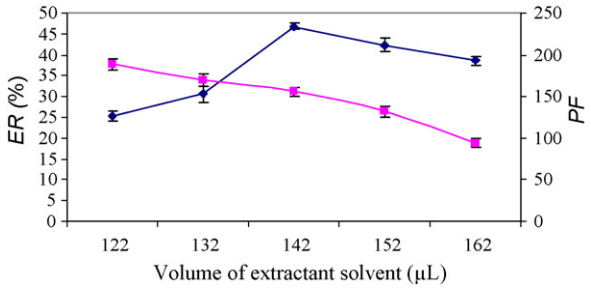

Fig. 1. Effect of the extractant solvent ($CHCl_3$) volume on the PF (■) and ER% (♦) of BPA. Extraction conditions: water sample volume, 10.0 mL; disperser solvent (acetone) volume, 2.0 mL; concentration of BPA, 100 μg L^{-1}.

Table 1
Determination of BPA in river and tap water samples.

Sample	Concentration of BPA ($\mu g\,L^{-1}$)	Added BPA ($\mu g\,L^{-1}$)	Found BPA ($\mu g\,L^{-1}$) (± RSD%[d]) ($n=3$)	Relative recovery (%)
River water[a]	n.d.[c]	1.00	0.95 (±8.40)	95.3
		5.00	4.83 (±7.60)	96.6
Tap water[b]	n.d.[c]	1.00	0.98 (±9.10)	98.2
		5.00	4.67 (±6.80)	93.4

[a] Kolakchal river water (Tehran, Iran).
[b] The water was taken from Tarbiat Modares University (Tehran, Iran).
[c] Not detected.
[d] Standard deviation.

the volume of acetone. It seems that, in the lower volumes of acetone, a cloudy state is not formed well, thereby, the recovery is low. In higher volumes of acetone, solubility of BPA in aqueous solutions increases. Therefore, the extraction efficiency decreases due to the decrease of distribution coefficient. A 2.0 mL of acetone was chosen as optimum volume.

According to the other reports [18–25], time has no influence on the extraction efficiency, because in DLLME, the surface area between the extractant solvent and the aqueous phase is infinitely large. In the present method, centrifuging of the sample solution is time determining step, which is about 3 min.

3.5. Salt addition

The effect of salt addition on the extraction recovery of BPA was evaluated by adding NaCl (0–8%, w/v) into the aqueous solution containing 100 $\mu g\,L^{-1}$ of BPA and applying the DLLME procedure. By increasing of NaCl%, the volume of sedimented phase increases (from 30 to 50 μL), because of the decrease in solubility of the extractant solvent in the presence of salt. Fig. 2 shows that PF decreases in the presence of salt; because of increasing in the volume of the sedimented phase. No significant effect on ER% was observed when different amounts of sodium chloride were added into the sample solution (Fig. 2).

3.6. Quantitative analysis

Calibration curves were obtained under the optimized conditions with linear dynamic range of 0.5–100 $\mu g\,L^{-1}$ and correlation of determination (r^2) of 0.997. The PF and ER% of the method were 150 and 45.2%, respectively, at the concentration level of 100 $\mu g\,L^{-1}$ of BPA and the sample volume of 10.0 mL. The relative standard deviation (RSD, $n=5$) at the concentration level of 100 $\mu g\,L^{-1}$ was 6.0%. The limit of detection (LOD) based on signal-to-noise ratio (S/N) of 3 was 0.07 $\mu g\,L^{-1}$.

3.7. Real water analysis

River and tap water samples were collected from Kolakchal River and Tarbiat Modares University (Tehran, Iran), respectively, and analyzed by the DLLME combined with HPLC-UV. The results showed that both samples were free from BPA contamination. Thus, they were spiked with BPA standards to assess matrix effects. Fig. 3 shows the chromatograms obtained for the river water samples before and after spiking with two different concentrations of BPA (1 and 5 $\mu g\,L^{-1}$). Also, the results of relative recoveries of the river and tap water samples are tabulated in Table 1. The data in Table 1 show that the relative recoveries of BPA were in the ranges of 93.4%–98.2%, demonstrating that the river and tap waters matrices had little effect on the DLLME.

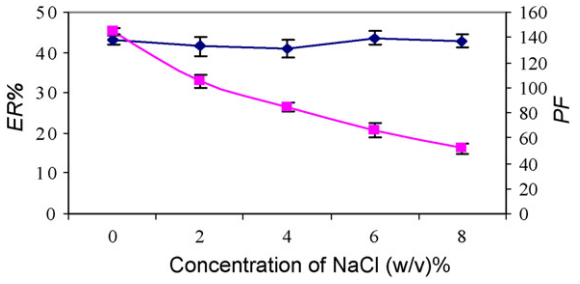

Fig. 2. Effect of salt addition on the PF (■) and ER% (♦) of BPA. Extraction conditions: water sample volume, 10.0 mL; disperser solvent (acetone) volume, 2.0 mL; extractant solvent ($CHCl_3$) volume, 142 μL; concentration of BPA, 100 $\mu g\,L^{-1}$.

3.8. Comparison of DLLME with LPME, SPME and SBSE

Table 2 compares LODs, LRs, RSDs%, extraction times, and sample volumes for LPME [26], SBSE [25], SPME [27,14] and DLLME method for the extraction and determination of BPA in water samples. The

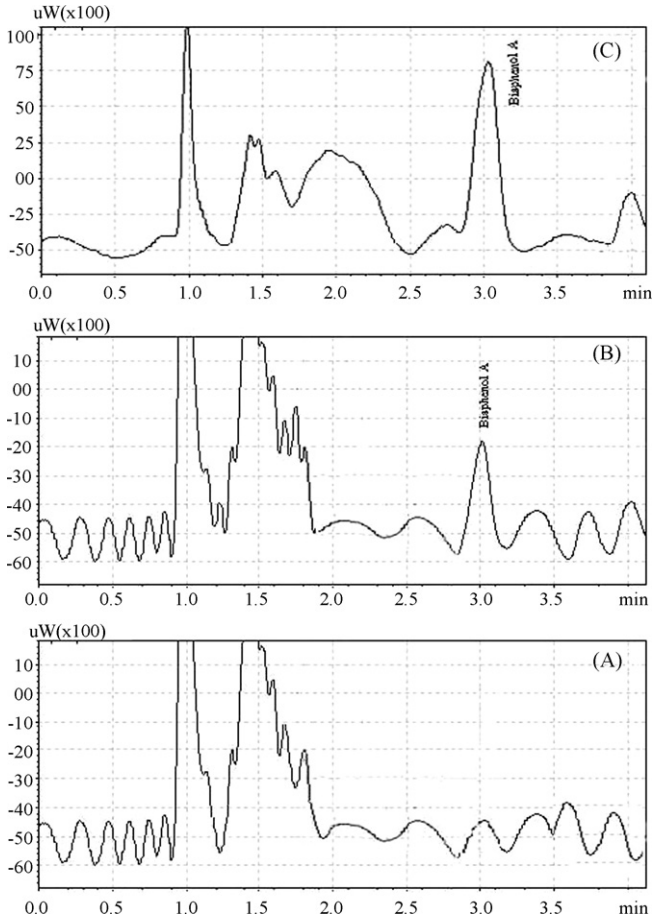

Fig. 3. HPLC chromatograms of BPA in river water before spiking (A) and after spiking with 1 $\mu g\,L^{-1}$ (B) and 5 $\mu g\,L^{-1}$ (C) using DLLME method combined with HPLC-UV under optimum conditions.

Table 2
Comparison of DLLME–HPLC-UV with other similar methods.

Methods	LOD ($\mu g\,L^{-1}$)	LR ($\mu g\,L^{-1}$)	RSD (%)	Extraction time (min)	Sample volume (mL)	Reference
LPME without derivatization-GC–MS	0.2	1–1000	3.2–8.9	90	10	
LPME with in situ derivatization-GC–MS	0.002	0.01–10	3.2–8.9	90	10	[26]
SBSE without derivatization -GC–MS	0.5	2–100	<10	45	2	[15]
SBSE with in situ derivatization -GC–MS	0.005	0.02–10	<10	45	2	[15]
SPME-GC–MS	0.04–1	0.027–195	10	60	10	[14]
SPME-HPLC	0.9	10–500	22	20	10	
DLLME-HPLC	0.07	0.5–100	6.0	<3 (equilibrium)	10	10 the present method

results show that the extraction time in DLLME is very short and less than 3 min. While, extraction time for SPME, LPME and SBSE ranged from 20 to 90 min, without equilibrium in most cases. The RSDs for the DLLME is low and approximately the same as SPME, LPME and SBSE. DLLME has acceptable LOD ($0.07\,\mu g\,L^{-1}$) and good liner range ($0.5–100\,\mu g\,L^{-1}$) without using derivatization reagents and applying very sensitive determination methods like GC–MS and HPLC-MS. It is worthy to note that the derivatization process needs to spend more time and consume chemical reagent that complicated the extraction process. The volume of sample solution required for DLLME is about 10 mL, which is similar to that of SPME, LPME and SBSE method. In contrast to SPME, LPME and SBSE, the stirring speed has no influence in DLLME efficiency. In addition to other advantages of DLLME, it is very simple, rapid, inexpensive and easy to use.

4. Conclusion

This paper describes the application of the DLLME method combined with HPLC-UV, for determination of trace amounts of BPA in water samples. The relative recoveries for BPA in the ranges of 93.4%–98.2% and demonstrated that the river and tap waters matrices had little effect on the DLLME.

Comparing to the other methods, in DLLME, consumption of toxic organic solvents is minimum. Also the proposed method has lowered LOD and much shorter extraction time.

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References

[1] A.V. Krishnan, P. Stathis, S.F. Petmath, L. Tokes, D. Feldman, Endocrinology 132 (1993) 2279.
[2] C. Sonnenschein, A.M. Soto, J. Steroid Biochem. Mol. Biol. 65 (1998)143.
[3] S.C. Nagel, F.S. Vomsaal, K.A. Thayer, M.G. Dhar, M. Boechler, W.V. Welshons, Environ. Health Perspect. 105 (1997) 70.
[4] O.P. Heemken, H. Reincke, B. Stachel, N. Theobald, Chemosphere 45 (2001)245.
[5] R.A. Rudel, S.J. Melly, P.W. Geno, G. Sun, J.G. Brody, Environ. Sci. Technol. 32 (1998) 861.
[6] H.M. Kuch, K. Ballschmiter, Environ. Sci. Technol. 35 (2001) 3201.
[7] P. Paseiro Losada, P. Lopez Mahia, L. Vazquez Oderiz, J. Simal Lozano, J. Simal Gandara, J. AOAC Int. 74 (1991) 925.
[8] J.L. Vilchez, A. Zafra, A. Gonzalez-Casado, E. Hontoria, M. del Olmo, Anal. Chim. Acta 431 (2001) 31.
[9] U. Bolz, W. Korner, H. Hagenmaier, Chemosphere 40 (2000) 929.
[10] S.N. Pedersen, C. Lindholst, J. Chromatogr. A 864 (1999) 17.
[11] H.G.J. Mol, S. Sunarto, O.M. Steijger, J. Chromatogr. A 879 (2000) 97.
[12] M. Kawaguchi, Y. Hayatsu, H. Nakata, Y. Ishii, R. Ito, K. Saito, H. Nakazawa, Anal. Chim. Acta 539 (2005) 83.
[13] B. San Vicente, F. Navarro Villoslada, M.C. Moreno-Bondi, Anal. Bioanal. Chem. 380 (2004) 115.
[14] P. Braun, M. Moeder, S. Schrader, P. Popp, P. Kuschk, W. Engewald, J. Chromatogr. A 988 (2003) 41.
[15] M. Kawaguchi, K. Inoue, M. Yoshimura, R. Ito, N. Sakui, N. Okanouchi, H. Nakazawa, J. Chromatogr. B 805 (2004) 41.
[16] M.A. Jeannot, F.F. Cantwell, Anal. Chem. 68 (1996) 2236.
[17] C. Basheer, H.K. Lee, J. Chromatogr. A 1057 (2004) 163.
[18] M. Rezaee, Y. Assadi, M.R. Milani Hosseini, E. Aghaee, F. Ahmadi, S. Berijani, J. Chromatogr. A 1116 (2006) 1.
[19] H. Jiang, Y. Qin, B. Hu, Talanta 74 (2008) 1160.
[20] P. Liang, J. Xu, Q. Li, Anal. Chim. Acta 609 (2008) 53.
[21] M. Shamsipur, M. Ramezani, Talanta 75 (2008) 294.
[22] M.G. Lopez, I. Rodriguez, R. Cela, J. Chromatogr. A 1166 (2007) 9.
[23] D. Nagaraju, S.D. Huang, J. Chromatogr. A 1161 (2007) 89.
[24] L. Farina, E. Boido, F. Carrau, E. Dellacassa, J. Chromatogr. A 1157 (2007) 46.
[25] M.A. Farajzadeh, M. Bahram, J.A. Jonsson, Anal. Chim. Acta 591 (2007)69.
[26] M. Kawaguchi, R. Ito, N. Endo, N. Okanouchi, N. Sakui, K. Saito, H. Nakazawa, J. Chromatogr. A 1110 (2006) 1.
[27] C. Nerin, M.R. Philo, J. Salafranca, L. Castle, J. Chromatogr. A 963 (2002) 375.