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Determination of Bisphenol A in Food and Environmental Samples Using Combined Solid-Phase Extraction–Dispersive Liquid–Liquid Microextraction with Solidification of Floating Organic Drop Followed by HPLC

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Abstract A solid-phase extraction (SPE) coupled with dispersive liquid–liquid microextraction based on the solidification of floating organic drop (DLLME-SFO) method has been developed for extraction and determination of organic bisphenol A (BPA) in different real samples. Bisphenol A was determined by high-performance liquid chromatography-fluorescence detector (HPLC-FL). Variables affecting the performance of both steps were thoroughly investigated. The analytical characteristics of the method were determined. The calibration graph is linear in the range of 0.005–10 ng g^{-1} with detection limit of 0.002 ng g^{-1}. The relative standard deviation (RSD) for of BPA in different samples are in the range of 6.8–9.6 % (n=5). The obtained results show that SPE-DLLME-SFO combined with HPLC-FL is a fast and simple method for the determination of BPA in food and environmental samples.

Keywords Dispersive liquid–liquid microextraction · Solid-phase extraction · Solidification of floating organic drop · Bisphenol A (BPA)

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Introduction

During the past years, endocrine disruptors (EDs) have been attracting much more attention because of their possible negative effects on human health (Grumetto et al. 2008). Endocrine disruption is one of the highest priority research topics for the United States Environmental Protection Agency (US EPA), and a detailed research strategy has been developed to guide the placement of resources over the next several years (Kavlock 1999). The effects of bisphenol A (BPA) as a endocrine disruptor are mediated by both genomic and non-genomic estrogen-response mechanisms with the disruption of the cell function occurring at doses as low as 1 pM (0.23 ng L^{-1}) (Vom Saal and Hughes 2005). BPA is nowadays deeply disseminated in the products of modern consumer society, in epoxy resins, polycarbonate plastic, and other plastics (polysulfone, alkylphenolic, polyarylate, polyester-styrene, and certain polyester resins), used in food and beverage containers, among other products (EFSA 2013). Unfortunately, heat and contact with either acid or basic compounds speed up the hydrolysis of the ester bond linking BPA molecules in polycarbonate and resins, leading that small amounts of BPA can leach out from food containers into the foods and beverages (Biedermann-Brem et al. 2008). Endocrine disruption is one of the highest priority research topics for the US EPA, and a detailed research strategy has been developed to guide the placement of resources over the next several years (Kavlock 1999). The European Union and the US EPA have ordered further evaluation for their endocrine disruption role of a “priority” list (Fiamegos and Stalikas 2007). Due to their endocrine disruptor potential, it is necessary to develop rapid, simple, and efficient methods for determination of BPA in foods and environmental matrices.

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Occurrence of BPA in different foods such as canned vegetables and fruits (Cunha and Fernandes 2013; Yoshida et al. 2001), honey (Inouea et al. 2003), fish (Chen et al. 2013), soft drinks (Cunha et al. 2011; Raouf Yazdinezhad et al. 2013), and water samples (Li and Song 2013; Rezaee et al. 2009; Zhou et al. 2011) has been widely reported. Some sample preparation techniques as liquid–liquid extraction (LLE) (Vilchez et al. 2001), solid-phase extraction (SPE) (Lázaro Martínez et al. 2009; Wen et al. 2012), molecularly imprinted solid-phase extraction (MISPE) (Kawaguchi et al. 2005; San Vicente et al. 2004) have been developed for the extraction of BPA from various matrices. Recently, solvent less and solvent-minimized polymer sorption techniques such as solid-phase microextraction (SPME) (Liu et al. 2008a, b; Mudiam et al. 2011; Viñas et al. 2010) and stir bar sportive extraction (SBSE) (Kawaguchi et al. 2004) have been successfully applied for the extraction of BPA from water samples. But, some need lots of time, some are expensive, and some require large amounts of organic solvents. Consequently, most of the analysis time is spent on the sample preparation steps alone. Fortunately, liquid-phase microextraction (LPME) has become an ideal alternative for BPA analysis (Basheer and Lee 2004; Kawaguchi et al. 2008). Among LPME, dispersive liquid–liquid microextraction (DLLME) (Daneshfar et al. 2011; Li et al. 2011; Song et al. 2011), as a relatively new sample preparation technique, showing remarkable advantages in small amounts of organic solvent (μL level), short extraction time, low cost, simplicity of operation, high enrichment factor, and environmental benignity, has been rapidly developed and applied for trace analysis of BPA (Cunha et al. 2012; Cunha and Fernandes 2010; Ma et al. 2012; Wang et al. 2009). Moreover, not only is DLLME a suitable sample preparation technique for a wide range of analytical instruments but also it can be easily combine with most other sample preparation methods.

In conventional DLLME, the density of extraction solvent should be higher than water; the applications of DLLME in most cases were limited to water samples, and the volume of the sedimented phase in some cases was dependent on the surrounding temperature. In addition, the high-density extraction solvents, being mostly halogenated, are generally hazardous to laboratory personnel and the environment (Pirsaheb et al. 2013). Recently, less toxic solvents, such as alcohols, alkanes, etc., have a density less than those of aqueous solutions and are collected on the upper surface of the sample solution as a microdrop or a thin film after centrifuging. Removal and determination of the collected phase volume are not as simple as for high-density extraction solvents. Different techniques such as using a capillary tube (Farajzadeh et al. 2010) or specialized extraction vessel (Farajzadeh et al. 2009) were reported for removal of lighter extraction solvents. However, complete removal of the collected phase is difficult or impossible in most cases. One of these methods that is called solidified floating organic drop (SFO) (Leong and Huang 2008), a small volume of an organic solvent with a melting point near room temperature (10–30 °C), is floated on the surface of an aqueous solution (Pirsaheb et al. 2013). However, for complex matrices such as highly saline solution, savage, and biological samples, DLLME cannot be used successfully. Therefore, in the analysis of these samples, a cleanup step is necessary before DLLME. Combination of SPE and DLLME as a novel sample pretreatment method leads to high enrichment factor (>1500) and can be used successfully in complex matrices.

In this work, combination of SPE and DLLME based on solidification of floating organic drop (SPE-DLLME-SFO) was employed as a sample preparation method for high-performance liquid chromatography (HPLC). The applicability of the method was demonstrated for the rapid determination of ultra trace amounts of BPA in different matrices.

Experimental

Reagents and Materials

Bisphenol A was purchased from Sigma-Aldrich (Milwaukee, WI, USA), methanol (for spectroscopy), acetone (suprasolv for gas chromatography), acetonitrile (hypergrade for liquid chromatography), 1-undecanol, 1-dodecanol, 1-decanol; hydrochloric acid and sodium chloride were obtained from Merck (Darmstadt, Germany). The ultra-pure water (six times distillated) used was purchased from Shahid Ghazi Pharmaceutical Co. (Tabriz, Iran). Stock standard solution of bisphenol A was prepared in methanol (5.0 mL), with concentration of 1000 ppm. The working solutions were prepared daily by appropriate dilution of the stock standard solution.

Instrumentation

The analysis of bisphenol A was performed by an HPLC Knauer with Chromgate software version 3.1 having binary pumps Smartline-1000-1 and Smartline-1000-2 and RF-10 AXL multiwavelength fluorescence detector (Berlin, Germany), an online solvent vacuum degasser and manual sample injection with a 20 μL injection loop (model 7725i, Rheodyne, Cotati, CA, USA). Separations were carried out on an H5-ODS C_{18} column (15 cm×4.6 mm, with 5 μm particle size) from Anachem (Luton, UK). A mixture of water and acetonitrile (55:45) at a flow rate of 1 mL min^{-1} was used as a mobile phase in isocratic elution mode. The detection wavelength was performed at λ_{ex}=224 nm and λ_{em}=308 nm. The pH values were measured with a Metrohm pH meter (model 692, Herisau, Switzerland) supplied with a glass-combined electrode. A Hettich Zentrifugen (EBA20, Tuttlingen, Germany) was used for centrifugations.

Manual Sample Injection with a 20 mL Injection Loop

Extraction Procedure Solid-phase extraction cartridge used for the extraction of BPA molecules from the water samples was 500 mg C_{18} sorbent (6 mL syringe barrel, Finisterre, Spain). The SPE sorbent was conditioned with 4.0 mL of acetone and water respectively. The water samples (100 mL), containing 1 ng g^{-1} of bisphenol A, prepared by diluting the stock solution was loaded at the flow rate of about 15 mL min^{-1} with the aid of vacuum pump (Vaccubrand, Germany). In the case of food and environmental sample, the final volume reached to 100 mL in a volumetric flask. Final solution was subjected to SPE-DLLME-SFO procedure. After drying the solid phase by passing air through it for several minutes, the target analytes were subsequently eluted with 1.0 mL acetone and were collected into 10 mL screw cap glass test tubes with conical bottom. Then, 30 μL of 1-undecanol (extraction solvent) was added to the acetone, which was used as disperser solvent in the test tube. Then, 5.0 mL pure water was injected rapidly into the test tube with a 5.0 mL syringe (gastight, Hamilton, Bonaduz, Switzerland). A cloudy solution, resulting from the dispersion of the fine 1-undecanol droplets in the aqueous solution, was formed in the test tube. After extracting for a few seconds, the phase separation was performed by centrifugation at 5500 rpm for 10 min. Accordingly, the dispersed fine droplets of the extraction phase were collected on the top of the conical test tube (27± 2 μL). The test tube was transferred into a beaker containing ice pieces, and the organic solvent was solidified after 5 min. Then, the solidified solvent was transferred into a vial by laboratory spatula, where it melted immediately. Finally, the extraction solvent was injected into an HPLC for analysis. The extraction setup for SPE-DLLME-SFO is illustrated in Fig. 1.

Sampling

Canned foodstuffs belonging to different categories were analyzed, namely fruits (pineapple, peach), tomato, powdered milk, soft drinks, honey, and fish, and samples from different manufactures were randomly purchased in different local markets. Sealed samples were stored at room temperature (±20 °C) protected from light and opened only on the moment of analysis. Once opened, the whole content of canned fruits and only the solid portions of canned vegetables were triturated with an electric chopper and an aliquot was taken for analysis.

Preparation of Real Samples

Tomato and Fruit Cans

The sample preparation procedure employed entails the following steps: (i) weight 10 g of thoroughly homogenized sample into a 40 mL screw-capped glass vial; (ii) add 5 mL of deionized water; (iii) seal the vial, vortex it for 5 min, and centrifuge at 3500× *g* for 2 min; (iv) add 10 mL MeCN, 4 g of anhydrous $MgSO_4$, and 1 g of NaCl; (v) seal the vial and shake vigorously by hand for 15 min; and (vi) centrifuge the vial at 3500 rpm for 2 min. The final volume reached to 100 mL in a volumetric flask. Final solution was subjected to SPE-DLLME-SFO procedure.

Fish Samples

According to previously reported work, an aliquot of 0.2 g dry weight (accurate to three significant figures) was extracted (Wei et al. 2011). Each sample was extracted with 10 mL acetonitrile in a pre-washed 50 mL PP centrifuge tube. The sample was extracted in an ultrasonic bath for 30 min and was then mixed in a digital reciprocating shaker for 30 min at 300 mot min^{-1} at room temperature. The solution was centrifuged at 1000 rpm for 15 min. The supernatant (the acetonitrile phase) was saved. The extraction was repeated twice, and all the extracts pooled. Then, extracted phase was mixed with 15 mL n-hexane and was shaken vigorously for 30 min to remove lipids. The acetonitrile layer was then reached to 100 mL in a volumetric flask with ultra-pure water, and the pH of samples was adjusted in the interval 6–7 with some drops of sodium hydroxide (50 %, *w*/*v*). Final solution was subjected to SPE-DLLME-SFO procedure.

Honey

Each honey sample (10 g) was mixed with 100 mL deionized water, stirred in a homogeneous solution, and filtered through a 0.22-μm membrane. All the samples were stored in darkness at 4 °C. The pH of samples was adjusted in the interval 6–7 with some drops of sodium hydroxide (50 %, *w*/*v*). Final solution was subjected to SPE-DLLME-SFO procedure.

Powdered Milk

Powdered milk was prepared as reported (Szymański et al. 2006). For analysis of powdered milk, an accurately weighed sample (1.0 g) was dissolved in 5 mL 50:50 % (*v*/*v*) ethanol–water. The sample solution was mixed for 2 min in an ultrasonic chamber, centrifuged for 40 min at 5000 rpm, and finally filtered through a 3-W membrane filter. Then, the final volume reached to 100 mL in a volumetric flask. The pH of samples was adjusted in the interval 6–7 with some drops of sodium hydroxide (50 %, *w*/*v*). Final solution was subjected to SPE-DLLME-SFO procedure.

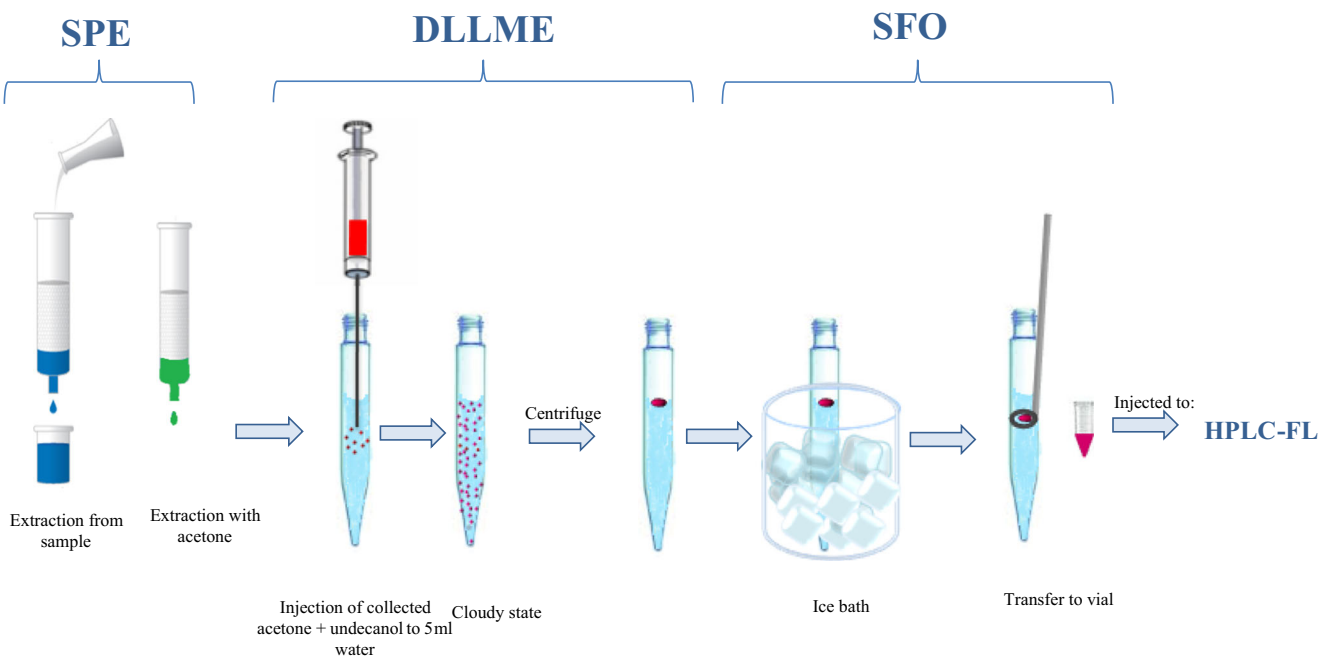

Fig. 1 Schematic diagram of SPE-DLLME-SFO extraction method

Soft Drinks

Soft drinks samples were stored unopened at room temperature until analysis. One hundred milliliters of carbonated samples were degassed by sonication for 30 min. Samples containing orange or lemon were centrifuged for 15 min at 5000 rpm to separate the fruit pulps. The pH of samples ranged in the interval 2.5–3.5, and it was adjusted in the interval 6–7 with some drops of sodium hydroxide (50 %, *w*/*v*). Final solution was subjected to SPE-DLLME-SFO procedure.

Water Samples

The bottled purified water samples were purchased from local supermarket, including three different brands. Tap water samples were collected from our laboratory after flowing for about 5 min. River water samples were collected into glass bottle from Kani Rizeh River (Javanrud, Iran); then, they were all filtrated through a 0.45-μm PTFE syringe filter. The samples were directly analyzed or stored at 4 °C for use. The pH of samples was adjusted in the interval 6–7 with some drops of sodium hydroxide (50 %, *w*/*v*). Final solution was subjected to SPE-DLLME-SFO procedure.

Results and Discussion

In this research, SPE-DLLME-SFO method combined with HPLC-FL was developed for determination of bisphenol A in different matrices. To attain a high extraction recovery and enrichment factor with the employment of SPE-DLLME-SFO, the influence of different parameters affecting the extraction conditions for SPE and DLLME including flow rates, breakthrough volume, type and volume of elution solvent, type of extraction and disperser solvents and their volume, pH, extraction time, and salt addition were optimized.

The extraction recovery (ER) was defined as the ratio between the amount of the analyte in the floating phase (n_{floated}) and the initial amount of the analyte (n_0) within the sample.

$$ER\% = \frac{n_{floated}}{n_0} \times 100 = \frac{C_{floated} \cdot V_{floated}}{C_0 \cdot V_{sample}} \times 100$$

where C_{floated} and C_0 are the concentration of analyte in the floating phase and initial concentration of the analyte in the sample; V_{floated} and V_{sample} are the volumes of the floating phase and sample, respectively.

The relative recovery (RR) was obtained from the following equation:

$$RR\% = \frac{C_{found} - C_{real}}{C_{added}} \times 100$$

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

Optimization of SPE Parameters

Effect of the Flow Rates

The flow rate of sample solution must be low enough to perform an effective retention of the BPA molecules. On the other hand must be high enough not to waste time. The effect of flow rate on recoveries of BPA was investigated in the flow rate range of 5–30 mL min^{-1}. It was found that in the range of 5–15 mL min^{-1}, bisphenol A recovery by the cartridge was not affected considerably by the sample solution flow rate. As a result (results not shown), 15 mL min^{-1} was used as the optimized sample flow rate.

The flow rate of elution solvent was investigated, and quantitative desorption of analytes from the cartridge was achieved in a flow rate of 1 mL min^{-1}, using 1.0 mL of acetone. At higher flow rates, quantitative desorption of analytes needed larger volumes of acetone. Therefore, a flow rate of 1 mL min^{-1} was chosen for further studies.

Effect of Breakthrough Volume

Breakthrough volume depends on the nature of the sorbent material and the type and concentration of sample constituents. The effect of breakthrough volume (from 50 to 500 mL, containing a constant amount of BPA molecules on extraction recovery was investigated. The results showed that the acceptable extraction recovery was observed when sample volumes were increased to 350 mL. It seemed to be the tolerated volume for breakthrough. Considering the analytical time and trace level of BPA in water samples, 100 mL was used as the optimized breakthrough volume.

Effect of Sample Solution pH

Sample pH is another important parameter that might affect the extraction efficiency, because the analytes will be present at different forms at different pH environments. A series of experiments were performed to investigate the effect of pH. The sample pH was designed in the range of pH 3–9. The data were shown in Fig. 2. It was found that the peak areas of BPA had no significant effect in the range of pH 3–6 but slightly deceased in the range of pH 6–9. The pKa value of BPA was 9.5. Usually, the solution pH is three units lower than the pKa value which could make the analyte existing as molecular form. According to the results, the pH 6 was chosen as the optimum pH value for further studies.

Effect of Elution Solvent Type and Volume

Since the eluent of SPE should be used as the dispersant in the following DLLME-SFO procedure, in SPE combined with DLLME-SFO, acetone, acetonitrile, and methanol were investigated as elution solvents. The SPE cartridge was eluted using 1.0 mL of each elution solvent. The results of this study are shown in Fig. 3, which clearly shows that the recovery by using acetone is higher than that for acetonitrile and methanol. Consequently, acetone was selected as the optimum elution (disperser) solvent.

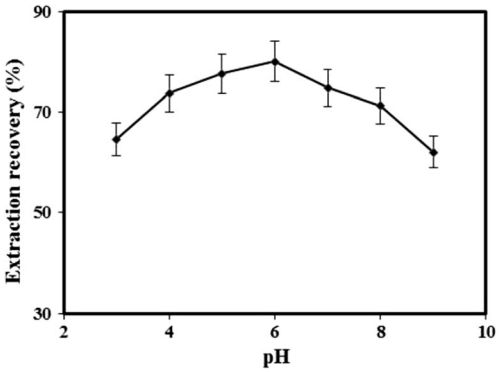

Fig. 2 Effect of pH on the extraction recovery of BPA obtained from SPE-DLLME-SFO. Extraction conditions: water sample volume, 100 mL; elution or disperser solvent (acetone) volume, 1.0 mL; extraction solvent (1-undecanol) volume, 30.0 μL; sedimented phase volume, 27±2 μL; room temperature; concentration of BPA, 1 ng g^{-1}

The volume of elution (disperser) solvent must be high enough to perform an effective elution of BPA molecule. On the other hand, it should be low enough so that it maintains the enrichment factor as high as possible. For this purpose, various experiments were performed by using different volumes of acetone (0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mL). The obtained results are illustrated in Fig. 4. As seen, the acetone volumes of lower than 1.0 mL cannot elute cartridge completely and the extraction recovery decreases. Also, by using more than 1.0 mL acetone, in DLLME stage, the solubility of analyte in water samples increases and it causes and hence the extraction efficiency decreases. Thus, 1.0 ml of acetone was selected as the volume of elution (disperser) solvent in subsequent experiments.

Effect of Salt Addition

Salt addition is frequently used to adjust the ionic strength, improve the extraction efficiency, and reduce the detection limit. Depending on the nature of the target analytes, addition of salt to the sample solution can decrease the solubility of the analytes and therefore enhance extraction because of the salting-out effect. To study the effect of ionic strength, the experiments were conducted at different sodium chloride concentrations in the sample solution, ranging from 0 to 6 % (*w*/*v*). The extraction recovery for BPA obtained highest values when 3 % of sodium chloride was added to the samples. Further addition of sodium chloride did not result in an increase in extract efficiency. Therefore, subsequent experiments were carried out with adding 3 % (*w*/*v*) salt.

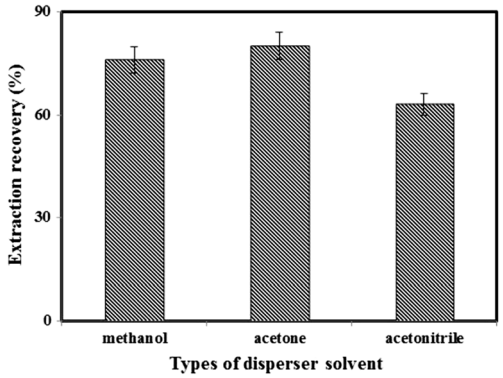

Fig. 3 Effect of type of elution or disperser solvent on the extraction recovery of BPA obtained from SPE-DLLME-SFO. Extraction conditions: similar to those in Fig. 1, except for a sample solution pH of 6

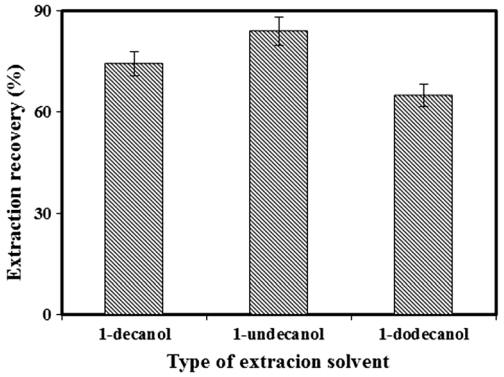

Fig. 5 Effect of the type of extraction solvent on the extraction recovery of BPA obtained from SPE-DLLME-SFO. Extraction conditions are similar to those of Fig. 2

Optimization of DLLME Parameters

Effect of the Type and Volume of Extraction Solvent

Selecting a suitable extraction solvent is crucial in this method. The extracting solvent must have low volatility, low water solubility, high solubility in dispersive solvent, capable of formation of cloudy solution in water in the presence of dispersive solvent, a melting point near to room temperature (in the range of 10–30 °C), no interference with the analytical techniques used for the determination of analyte, and a density lower than water. Accordingly, several extracting solvents, including 1-decanol, 1-dodecanol, and 1-undecanol, were investigated. Average extraction recovery (n=5) and standard deviation (SD) for different extraction solvents are shown in Fig. 5. The results revealed that 1-undecanol has the highest extraction recovery in comparison with the other tested solvents. Therefore, 1-undecanol was chosen for further experiments.

To examine the effect of extraction solvent volume on the extraction recovery, solutions containing different volumes of 1-undecanol (20, 30, 40, 50, 60, and 70 μL) with a fixed volume of disperser solvent were used with the same SPE-DLLME-SFO procedure. As shown in Fig. 6, by increasing the volume of 1-undecanol from 20 to 70 μL, the volume of the floated phase increased approximately from 14 to 61 μL. The result shows that extraction recoveries decrease with increasing the volume of 1-undecanol; it is clear that by increasing the volume of 1-undecanol, the volume of the floated phase increases. However, because of dilution effect, an increase in the extraction solvent volume resulted in a slight decrease of the extraction recovery. Subsequently, at low volume of the extraction solvent (30 μL), high enrichment factors and recoveries were obtained. A volume of less than 30 μL of 1-undecanol resulted in a floated volume less 20 μL which was insufficient for determination by the HPLC system with a 20 μL injection loop. Thus, in order to have a high enrichment factor and good repeatability, 30 μL of 1-undecanol was selected as the optimum volume of the extracting solvent.

Effect of Type and Volume of Disperser Solvent

In SPE-DLLME, disperser solvent was the solvent used in SPE stage as elution solvent. For this purpose, acetone, acetonitrile, and methanol were used as disperser (elution)

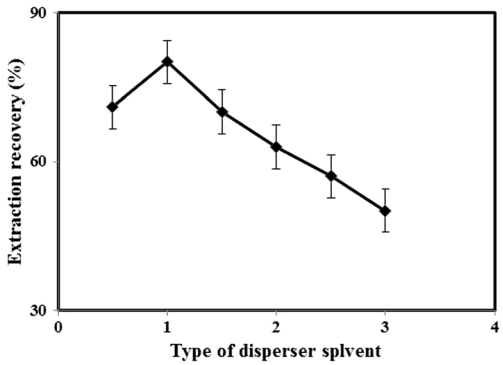

Fig. 4 Effect of the volume of elution or disperser solvent (acetone) on the extraction recovery of BPA obtained from SPE-DLLME-SFO. Extraction conditions are similar to those of Fig. 2

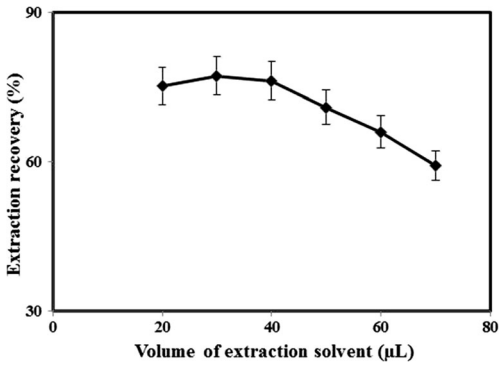

Fig. 6 Effect of the volume of extraction solvent (1-undecanol) on the extraction recovery of BPA obtained from SPE-DLLME-SFO. Extraction conditions are similar to those of Fig. 2.

solvent. Acetone as an optimum elution for SPE, acetone was selected as disperser solvent. Since disperser solvent in SPE stage is used as elution solid phase, its volume should be enough to elute the solid phase completely. Meanwhile, the volume of disperser solvent should be low enough not to increase floated phase volume. According to the results in "Effect of Elution Solvent Type and Volume" section, a volume 1.0 mL of acetone was selected as an optimum volume of disperser solvent.

Effect of Extraction Time

Extraction time is one of the most important factors in most extraction procedures. In DLLME, extraction time is defined as the time between injection mixture of disperser and extraction solvent and starting to centrifuge. The influence of the extraction time was examined in the range of 0–20 min with the experimental conditions remaining constant. The results showed that the extraction time has no significant effect on the extraction efficiency. It was revealed that after the formation of the cloudy solution, the surface area among the extraction solvent and the aqueous phase was essentially large. Thereby, transfer of BPA molecules from aqueous phase to extraction solvent is fast. This fact was one of the advantages of the DLLME technique.

Analytical Performance

The analytical performance was investigated in terms of repeatability, linearity, correlation coefficient, limit of detection (LOD), and enrichment factor (EF) under optimized experimental conditions shown in Fig. 7 and Table 1. An excellent linear relationship was obtained between peak areas and the corresponding concentrations of BPA in the wide range 0.005–10 ng g^{-1} (R^2=0.996). A high EF, defined as the ratio between the BPA concentration in the final organic phase and the initial concentration of the sample of BPA and formed a well-stable cloudy solution, could be obtained of 1940 at the concentration level of 1 ng g^{-1} of BPA. And at the same concentration, the repeatability was investigated on five replicate experiments with the relative standard deviations (RSDs) of 4.7 %. The LOD, calculated as the analyte concentration for which the peak area was three times the background noise, was 0.002 ng g^{-1}, which is much lower than the maximum admissible dose for BPA in diet-related makings, namely 0.05 mg kg^{-1} or L^{-1} formulated by the US Food and Drug Administration (FDA) and 0.01 mg L^{-1} by the Health Department of the Chinese Government (Li and Song 2013).

Determination of BPA in Food and Environmental Samples

The proposed SPE-DLLME-SFO combined with HPLC-FL methodology was applied for determination of BPA in different samples. The concentrations of BPA in different real samples were determined (Table 2 and Table 3). The analyte recovery of this procedure was evaluated by spiking different levels of standard analyte to samples in replicates of five. The corresponding relative recoveries are summarized in Table 2 and Table 3. As seen, the relative recoveries for BPA in spiked real samples are between 95.4 and 102.1 %. These results demonstrate that matrix, in our present context, has no significant effect on SPE-DLLME-SFO followed by HPLC-FL for determination of the BPA. Figure 8 shows the obtained chromatograms of powdered milk solution and spiked powdered milk solution at the concentration level of 2 ng g^{-1} for BPA.

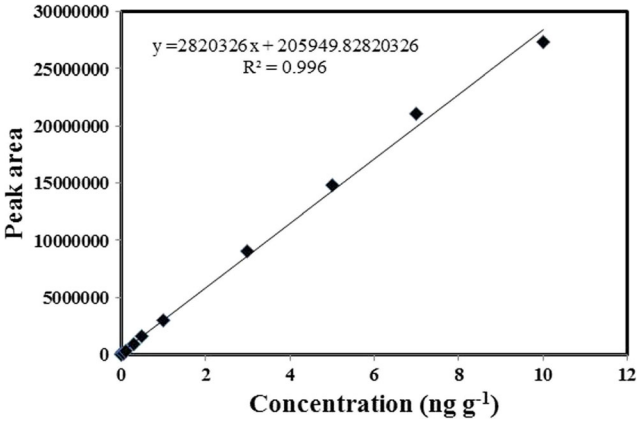

Fig. 7 Calibration curve of BPA obtained under optimized conditions

All canned food samples tested showed detectable levels of BPA, in a broad range of concentrations from 3.7 to 8.3 ng g^{-1}. The highest levels of BPA were also found in canned vegetables (tomato can) with an average of 7.6 ng g^{-1} compared to 5.7 ng g^{-1} in canned fruits (peach and pineapple cans). The dependence of BPA on food types are consistent with previously reported data (Cunha and Fernandes 2013). The reported low BPA levels found in canned fruits could be explained by the fact that usually food industry uses electrolytic tinplate instead of epoxy phenolic films in canned fruit containers (Oldring and Nehring 2007). In general, variability was observed between the two food

Table 1 Analytical characteristics of SPE-DLLME-SFO-HPLC for determination of BPA

Parameter	Analytical feature
Linear range (ng g^{-1})	0.005–10
R^2	0.996
Limit of detection (ng g^{-1}) (3σ, n=5)	0.002
Repeatability (RSD[a], %) (n=5)	4.7
Enrichment factor	1940
Sample volume (mL)	100

[a] BPA concentration was 1 ng g^{-1} for which RSD was obtained

Table 2 Concentration of BPA in different real samples with and without spiking of target analysis

Sample	Brand	Concentration of BPA mean±SD (ng g^{-1})	Added BPA (ng g^{-1})	Found BPA mean±SD (ng g^{-1})	Relative recovery (%)
Honey	A1	0.9±0.06	2	2.8±0.3	96.5
	A2	1.5±0.1	2	2.4±0.2	96.0
	A3	n.d.	2	2.0±0.2	100
Fish	B1	1.9±0.2	1	2.8±0.2	96.5
	B2	1.5±0.1	1	2.5±0.2	100
	B3	2.2±0.2	1	3.2±0.3	100
Tomato cans	C1	8.3±0.8	0.5	8.6±0.8	97.7
	C2	6.6±0.6	0.5	7.0±0.7	98.6
	C3	7.8±0.8	0.5	8.2±0.8	98.8
Pineapple in syrup	D1	6.5±0.6	0.5	6.7±0.6	95.7
	D2	4.1±0.4	0.5	4.7±0.5	102.1
	D3	7.7±0.8	0.5	7.9±0.8	96.3
Peach in syrup	E1	4.9±0.5	0.5	5.5±0.6	101.8
	E2	3.7±0.3	0.5	4.2±0.5	100
	E3	7.2±0.7	0.5	7.6±0.7	98.7
Powdered milk	F1	1.3±0.1	2	3.1±0.3	93.9
	F2	2.4±0.2	2	4.2±0.4	95.4
	F3	n.d.	2	1.9±0.2	96

SD standard deviation (n=5), *n.d.* not detected

types and also within the food types. Pineapple and peach had 2-fold difference between brands, while tomato had only 1.2-fold different between brands. These differences could be related with can variables, e.g., coating type, amount of coating, manufacturing, and processing of the can, and also with sterilization conditions (temperature and time) (Geens et al. 2010; Cunha and Fernandes 2013). In fact, the lowest level of BPA (3.7 ng g^{-1}) was observed in peach can. In contrast, the highest level of BPA in canned fruits was observed in pineapple in syrup (7.7 ng g^{-1}). In general, the range of BPA levels reported for canned fruit samples were similar to those reported in literature: <6.8 μg kg^{-1} in four samples from Austria (Braunrath et al. 2005).

Regarding the canned vegetable (tomato) samples studied, BPA occurred at levels ranging from 6.6 to 8.3 ng g^{-1}. As previously reported, the presence of bisphenols in canned tomatoes is due to a release from packaging (Braunrath et al. 2005; Thomson and Grounds 2005). The passage of bisphenols from cans to food may be related to can material quality, and/or to the temperature of heat preserving process, and/or to the pH of the content. The BPA levels in canned tomato were lower than those reported by Cunha and

Table 3 Concentration of BPA in aqueous samples with and without spiking of target analysis

Sample	Brand	Concentration of BPA mean±SD (ng mL^{-1})	Added BPA (ng mL^{-1})	Found BPA mean±SD (ng mL^{-1})	Relative recovery (%)
Bottled water	G1	n.d.	2	1.9±0.2	95
	G2	n.d.	2	2.0±0.2	100
	G3	n.d.	2	1.9±0.2	95
Tap water		n.d.	2	2.0±0.2	100
Spring water (KaniRizeh)		n.d.	2	1.9±0.2	95
Lemon carbonated drink		1.4±0.1	1	2.3±0.2	95.8
Orange carbonated drink		0.5±0.05	1	1.5±0.1	100
Energy drink		0.5±0.04	1	1.4±0.1	93.3

SD standard deviation (n=5), *n.d.* not detected

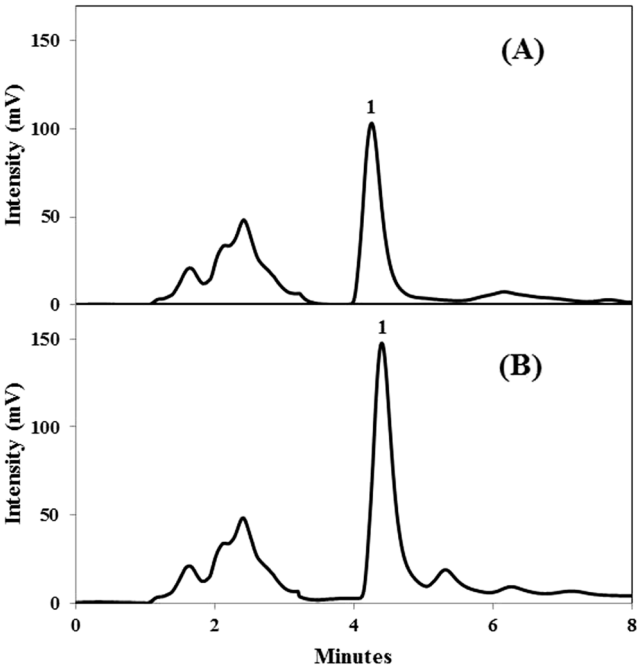

Fig. 8 The chromatograms of the powdered milk solution (**a**) and the spiked powdered milk solution at the concentration level of 2 ng g^{-1} for BPA (**b**), obtained using SPE-DLLME combined with HPLC-FL. Extraction conditions: water sample volume, 100 mL; sample solution pH, 6; eluent or disperser solvent (acetone) volume, 1.0 mL; extraction solvent (1-undecanol) volume, 30 μL; volume of the aqueous solution for DLLME, 5.0 mL; sedimented phase volume, 27 μL; room temperature. Peak identification: (1) BPA

Fernandes (2013). It is possible that the differences in BPA concentrations with previously reported works simply represent differences in can size, can lot, or manufacturer. However, they may also represent differences in sample processing or analytical methodology.

The results obtained for powdered milk showed that the BPA were present in two samples in the range of 1.3–2.4 ng g^{-1}, but levels were in all cases below concern. The concentrations here detected are similar to those reported by Liu et al. (2008a, b)) and Maragou et al. (2006).

Comparison of SPE-DLLME-SFO with Other Extraction Methods

Characteristics of the proposed method have also been compared with other methods reported for the preconcentration and determination of BPA in different samples. Table 4 compares the limit of detection, linear range, repeatability, and enrichment factor. As is obvious, the limit of detection of the proposed SPE-DLLME-SFO method with a sample volume of 100 mL is much better than the detection limits of the other methods. The enrichment factor in SPE-DLLME-SFO is very high and the extraction time is relatively short, compared to the literature reports. Hence, the proposed method would be a good alternative for the determination of ultra traces of BPA in different matrices.

Conclusions

In the present study, SPE method was combined with DLLME-SFO technique. This combination was successfully applied to extraction and preconcentration of bisphenol A from different samples prior to analysis by

Table 4 Comparison of SPE-DLLME-SFO with other extraction methods for determination of BPA in different matrices

Methods	LOD (ng g^{-1})	LR (ng g^{-1})	RSD %	Enrichment factor	Samples	Reference
DLLME-GC-MS	0.004	0.01–10	3.2–7.6	1720	Milli-Q water, tap water, bottled water, river water	Li and Song 2013
HF-LLLME-HPLC-UV	0.055	0.2–500	6.9	376	Sand lake sediment, sand lake fish, commercial fish	Chen et al. 2013
SPE-LC-GC-MS	0.37	0.5–400	0.6–12		Fish, mollusk, prawn	Gu et al. 2014
RP-DLLME-HPLC-DAD	2.5	10–500	1.9–5.9	–	Sunflower oil, peanut oil, rape seed oil, olive oil, *Sesamum indicum*, blend oil	Liu et al. 2013
QuEChERS DLLME-GC-MS	0.3	1–300	>20	–	Canned vegetables and fruits	Cunha and Fernandes 2013
SPE-DLLME-SFO-HPLC	0.002	0.005–10	4.7	1940	Honey, powdered milk, canned fruit, fish, soft drink, tap water, bottled water, river water	This work

LOD limit of detection, *LR* linear range, *RSD* relative standard deviation, *DLLME-GC-MS* dispersive liquid–liquid microextraction-gas chromatography-mass spectroscopy, *HF-LLLME-HPLC-UV* hollow fiber liquid-liquid-liquid microextraction-high-performance liquid chromatography-ultraviolet detector, *SPE-LC-GC-MS* solid phase extraction-liquid chromatography-gas chromatography-mass spectroscopy, *RP-DLLME-HPLC-DAD* reversed phase-dispersive liquid–liquid microextraction-diode array detector, *QuEChERS DLLME-GC-MS* the "Quick Easy Cheap Effective Rugged and Safe"-dispersive liquid–liquid microextraction-gas chromatography-mass spectrometry, *SPE-DLLME-SFO-HPLC* solid-phase extraction- dispersive liquid–liquid microextraction-solidification of floating organic drop-high performance liquid chromatography-fluorescence detector

HPLC. High preconcentration factor was obtained easily through this method, and a detection limit at ng g^{-1} level was achieved with 100 mL of sample. The high-preconcentration factor and the low detection limit were the major advantages of the technique. In this method, the sample preparation time (less than 15 min) as well as the consumption of the toxic organic solvents (at microliter level) was minimized without affecting the method sensitivity. As a conclusion, the proposed method possesses great potential in analysis of trace organic compounds in real samples.

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Compliance with Ethics Requirements

Conflict of Interest Marzieh Sadeghi has received research grants from the Department of Chemistry, Razi University, Kermanshah, Iran. Ziba Nematifar declares that he has no conflict of interest. Nazir Fattahi declares that he has no conflict of interest. Meghdad Pirsaheb declares that he has no conflict of interest. Mojtaba Shamsipur declares that he has no conflict of interest.

Ethical Approval This article does not contain any studies with human or animal subjects.

Informed Consent Not applicable.

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