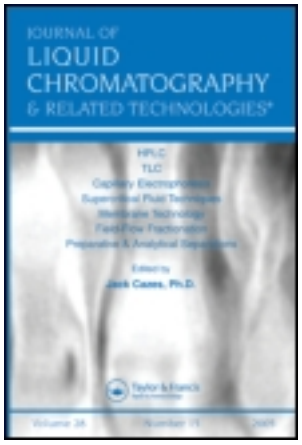

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DISPERSIVE LIQUID-LIQUID MICROEXTRACTION BASED ON SOLIDIFICATION OF FLOATING ORGANIC DROP COMBINED WITH COUNTER-ELECTROOSMOTIC FLOW NORMAL STACKING MODE IN CAPILLARY ELECTROPHORESIS FOR THE DETERMINATION OF BISPHENOL A IN WATER AND URINE SAMPLES

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DISPERSIVE LIQUID-LIQUID MICROEXTRACTION BASED ON SOLIDIFICATION OF FLOATING ORGANIC DROP COMBINED WITH COUNTER-ELECTROOSMOTIC FLOW NORMAL STACKING MODE IN CAPILLARY ELECTROPHORESIS FOR THE DETERMINATION OF BISPHENOL A IN WATER AND URINE SAMPLES

Usama Alshana,[1] Ibrahim Lubbad,[1] Nilgün G. Göğer,[1] İsmet Çok,[2] Uğur Tamer,[1] and Nusret Ertaş[1]

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□ *Dispersive liquid–liquid microextraction method based on solidification of floating organic drop (DLLME-SFO) was combined for the first time with counter-electroosmotic flow normal stacking mode (counter-EOF NSM) in capillary electrophoresis (CE) for preconcentration and determination of bisphenol A (BPA) in water and urine samples. Several parameters affecting extraction efficiency, including type and volume of the extraction and disperser solvents, pH, volume of sample and back-extraction solutions, and ionic strength, were systematically studied. In-vial back-extraction of the target analyte from the resulting organic drop into an aqueous phase facilitated the direct application of DLLME-SFO with CE. Under optimum conditions, improvement factors of 1250 (water) and 430 (urine) as compared to conventional capillary zone electrophoresis (CZE) were obtained. Calibration graphs were linear up to 100 $\mu g L^{-1}$ with coefficients of determination (R^2) $\geq$ 0.9989 and relative standard deviation (RSD %) $\leq$ 1.9. Limits of detection (LOD) of 0.8 $\mu g L^{-1}$ (water) and 2.5 $\mu g L^{-1}$ (urine) were achieved. Because this method required simple and inexpensive devices and very small volumes of nontoxic organic solvents, it is an affordable, efficient, and convenient method for extraction and determination of trace amounts of BPA in water and human urine samples.*

Keywords bisphenol A, capillary electrophoresis, counter-electroosmotic flow normal stacking mode, dispersive liquid-liquid microextraction, solidification of floating organic drop, urine

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INTRODUCTION

Over the past few decades, there has been increasing interest in the determination of endocrine disrupting chemicals (EDCs) in different matrices because of their potential adverse effects on the endocrine systems of humans and wildlife.[1,2] Among phenolic EDCs, bisphenol A [BPA, (4,4′-(propane-2,2-diyl) diphenol)] has generated the most concern from regulatory agencies and scientists due to its high production, widespread use, and ubiquitous occurrence in the environment.[3] BPA is a principal component of both polycarbonate and epoxy resins and is widely used for plastic products such as water bottles, baby bottles, food containers, and dental sealants.[4] It can easily migrate into the human body and produce adverse health effects including increased risks of diabetes mellitus, cardiovascular diseases, and liver-enzyme abnormalities.[5] The increased global concern about BPA highlights the importance of developing sensitive analytical methods to detect trace amounts of this compound in environmental and biological samples.

To date, different analytical methods have been developed for the determination of BPA in various matrices, most of which were based on high performance liquid chromatography (HPLC)[6,7] and gas chromatography (GC).[8,9] Recently, there has been increasing interest in the application of capillary electrophoresis (CE) for the determination of EDCs including BPA due to its extremely high separation efficiency, short analysis time, low operating costs, wide application range and minimal requirement of sample volume (in the nanoliter range).[10–12] Nevertheless, one of the drawbacks of CE with direct UV detection is the poor concentration sensitivity resulting from minute injection volumes needed to maintain high separation efficiency and a short optical pathlength equal to the capillary diameter. In order to overcome this sensitivity problem, several on-line preconcentration strategies, such as stacking[13] and sweeping[14] have been developed. The simplest and most commonly used sample stacking technique is normal stacking mode (NSM), also referred to as field-amplified sample stacking (FASS).[15] It is based on the concept that ions electrophoretically migrating through a low-conductivity solution (sample plug) into a high-conductivity background electrolyte (BGE) slow down dramatically at the boundary of the two solutions. This technique has been successfully applied for the on-line preconcentration of tetracyclines,[16] fluoroquinolone antibiotics,[17] sulfonamides,[18] biogenic amines,[19] and so forth. Although sample stacking and sweeping[20] have enjoyed some degree of success in CE as efficient online sample preconcentration techniques, there is still a major problem when directly applied to complex sample matrices without a sample pretreatment step as they suffer tremendously from matrix effects.[21]

Liquid–liquid extraction (LLE)[22] and solid-phase extraction (SPE)[23,24] have been the main extraction techniques used to extract and/or preconcentrate BPA prior to its determination. Shortcomings associated with LLE such as emulsion formation, use of large sample volumes and toxic organic solvents make it labor-intensive, expensive, time-consuming, and environmentally-unfriendly. Although SPE uses much less solvent than LLE, it can still be considered significant, and normally an extra step is needed to preconcentrate the analytes further into smaller volumes. SPE is also time-consuming and relatively expensive.[25]

Recently, much research has been directed toward efficient, economic and "green" miniaturized extraction techniques. Liquid–liquid microextraction (LLME) with its different operating modes, such as single drop microextraction (SDME),[26] hollow fiber-based liquid-phase microextraction (HF-LPME),[27] solvent-bar microextraction (SBME),[28] and dispersive liquid–liquid microextraction (DLLME),[29] among others, has attracted increasing attention as a novel sample preparation technique. SDME is inexpensive and has minimal exposure to organic solvents. However, the major disadvantage of this method is that a small organic drop held at the tip of a needle is unstable and may be dislodged during extraction.[30] This drawback has been partially overcome using hollow fiber-based methods. Nevertheless, HF-LPME and SBME are also limited by the small contact surface of the fiber, which necessitates long extraction times. Furthermore, the formation of air bubbles on the surface of the hollow fiber can reduce the transport rate and influence the reproducibility of the extraction. For real samples, such as urine, adsorption of hydrophobic substances on the fiber surface may block the pores of the fiber.[31]

In DLLME the surface area between the extraction solvent and sample solution are infinitely large initially because a cloudy solution is formed. Therefore, extraction equilibrium can be reached quickly. This method has attracted much attention due to its advantages including fast extraction, low consumption of organic solvent, and simplicity.[32] Yet, the extraction solvent is in most cases limited to solvents with higher density than water such as chlorobenzene, chloroform, tetrachloromethane, and carbon disulfide, all of which are highly toxic and environmentally unfriendly.[33]

Lately, a simple, quick, and inexpensive dispersive liquid–liquid microextraction method based on solidification of floating organic drop (DLLME-SFO) has been developed by Leong and Huang,[34] in which a mixture of an organic extraction solvent with lower density than water, low toxicity, and proper melting point near room temperature (in the range of 10–30°C) and a disperser solvent was used. In this method, a small volume (10–100 μL) of the extraction solvent was floated on the surface of an aqueous solution containing the analytes. The aqueous solution was

stirred for a selected time. After extraction, the floated extraction solvent drop could easily be collected by solidifying it at low temperature. The solidified organic solvent melted immediately at room temperature and was then analyzed.

This work presents the first attempt to combine DLLME-SFO with the online preconcentration technique of counter-electroosmotic flow normal-stacking mode (counter-EOF NSM) in CE for preconcentration and determination of bisphenol A in different water samples and human urine. Several parameters affecting extraction efficiency, including type and volume of the extraction and disperser solvents, pH, volume of sample and back-extraction solutions, and ionic strength, were systematically studied and optimized. In-vial back-extraction of the target analyte from the resulting organic drop into an aqueous phase facilitated the direct application of DLLME-SFO with CE.

EXPERIMENTAL

Reagents and Materials

Bisphenol A (solubility in water at $25^{\circ}C < 0.1\,g/100\,g$; $logP = 4.0$; $pK_a = 9.7$) was purchased from Sigma-Aldrich (99.9%, Munich, Germany). HPLC-grade methanol (Lab-Scan, Gliwice, Poland), acetonitrile (Sigma-Aldrich, St. Louis, MO, USA) and acetone (Merck, Darmstadt, Germany) were used. Sodium chloride was purchased from Merck (Darmstadt, Germany). 1-undecanol (1-UN) (99.0%), 1-dodecanol (1-DO) (98.0%), and diphenyl ether (DPE) (99.0%) were obtained from Sigma-Aldrich (Steinheim, Germany). A stock solution of the BPA was prepared by dissolving an appropriate amount in methanol to obtain a 1000 mg L^{-1} solution that was stored in the dark at $-20^{\circ}C$. Aliquots of this stock solution were daily diluted with deionized water to prepare standard solutions. All other reagents and solvents used were at least of analytical reagent grade unless otherwise specified. The sample solution for the DLLME-SFO extraction experiments was prepared by spiking the analyte in deionized water. Samples of tap water were taken from Gazi University (Ankara, Turkey); spring and bottled water were purchased from a local market. Borate buffer was prepared from $Na_2B_4O_7 \cdot 10H_2O$ obtained from Sigma-Aldrich (Steinheim, Germany). All background electrolytes (BGE) and solutions were prepared in deionized water and were stored in the dark at $4^{\circ}C$. When necessary, pH of the solutions was adjusted with 0.1 M NaOH (Merck, Darmstadt, Germany) and 0.1 M HCl (Sigma-Aldrich, Steinheim, Germany). All solutions and samples were degassed using a sonicator (Sonorex Bandelin Electronic, Walldorf, Germany) and filtered through 0.20-μm filters (Econofilters, Agilent Technologies, Waldronn, Germany) before use.

Instrumental

The experiments were carried out on an HP^{3D} CE (Agilent Technologies, Waldbronn, Germany). Conventional capillary zone electrophoresis (CZE) and counter-EOF NSM were performed using uncoated fused-silica capillaries (Postnova Analytics, Landsberg, Germany) of 75 μm i.d. and 64.5 cm length with effective length to the detector of 56 cm. Online UV diode-array detector (DAD) operated at a wavelength of 194 nm was used. Optimum wavelength for the target analyte was determined using "Isoabsorbance" and "3D" plots in the instrument's "Data Analysis" software (Agilent Technologies, Waldbronn, Germany). Pressure injection was employed throughout the experiments. A Thermo Orion, 720A pH meter (Beverly, MA, USA) equipped with a glass electrode was used for measuring the adjusted pH of all aqueous and buffer solutions used throughout the experiments. Deionized water (18.2 MΩ · cm) treated with Millipore (Simplicity, 185) Milli-Q water purification system (Milford, MA, USA) was used for all aqueous solutions.

New capillaries were successively flushed with deionized water (10 min), 1.0 M NaOH (20 min), deionized water (20 min), and finally with the BGE (20 min). To assure good reproducibility, the capillary was successively flushed, at the end of each run, with deionized water (1 min), 1.0 M NaOH (1 min), deionized water (2 min), and the BGE (2 min).

In conventional CZE, the capillary was conditioned with a BGE (25 mM borate buffer containing 5.0% methanol, pH* 9.3); the sample, prepared in this BGE, was injected for 5 s at 50 mbar and a positive voltage of 20 kV was applied for separation. The analyte migrated in a homogeneous conductivity medium and detected at the outlet end.

In counter-EOF NSM, the capillary was conditioned with 25 mM borate buffer containing 5.0% methanol; the sample present in a low-conductivity medium was injected for 50 s at 50 mbar; BPA stacked at the boundary between the low-conductivity sample plug and the high-conductivity BGE. The following separation occurred at 20 kV by the CZE mode.

DLLME-SFO Procedure

The experimental procedure for DLLME-SFO was as follows: A sample (10 mL) of BPA-free deionized water was placed in a glass test tube and spiked with BPA at a concentration of 20 μg L^{-1}. Next, pH of this solution was adjusted to 4.0 using 0.1 mol L^{-1} HCl solution; a mixture containing 90 μL 1-UN (as the organic extraction solvent) and 1.5 mL acetone (as the disperser solvent) was rapidly pipetted into the sample solution using a micropipette; the tube was sealed and vortex mixed for 1 min. A cloudy suspension (consisting of water, acetone and 1-UN) that resulted from

the dispersion of fine 1-UN droplets in the aqueous solution formed in the test tube. After centrifugation for 5 min at 5000 rpm, the test tube placed in the freezer at −20°C and the floating organic drop was solidified after 5 min; the drop was separated using a small medicine.

Back-Extraction

The solidified organic drop melted rapidly at room temperature and was transferred into a glass insert inside a CE vial (Agilent Technologies, Waldbronn, Germany). BPA was back-extracted into 20 μL of 0.10 mol L^{-1} NaOH solution (hereafter referred to as back-extraction solution: BES) after vortex mixing for 1 min and centrifugation at 4000 rpm for 1 min. Finally, the aqueous phase containing the analyte was directly injected into CE without the need to separate the organic phase.

Urine Sample Preparation

Urine samples were collected from a healthy male volunteer (37 years old) and were frozen at −20°C. Samples were allowed to thaw at room temperature prior to analysis. 4.0 mL of the supernatant transparent solution were transferred into a test tube and were spiked with prescribed concentrations of BPA. pH of this solution was adjusted to 4.0 using 0.1 mol L^{-1} HCl solution. Next, the solution was mixed with acetonitrile at 2:1 (*v:v*) ratio and the ionic strength was increased by adding 1.0 g of NaCl in order to promote a salt-induced phase separation between acetonitrile and the aqueous phase after the solution was vortex mixed for 1 min and centrifuged for 1 min at 4000 rpm. The resultant 1.0 mL of acetonitrile was transferred into a glass test tube and the DLLME-SFO procedure was applied. It is noteworthy that acetonitrile here served as the disperser solvent in the subsequent DLLME-SFO procedure.

RESULTS AND DISCUSSION

Optimization of DLLME-SFO Conditions

In order to obtain the most effective extraction, it is important to determine the optimum DLLME-SFO conditions for the analysis of BPA including type and volume of the extraction and disperser solvents, pH and volume of sample and back-extraction solutions, and ionic strength. Peak area was used to evaluate the influence of those variables on the extraction efficiency of the DLLME-SFO technique.

Type and Volume of the Extraction Solvent

Organic solvents that are appropriate for microextractions based on solidification of the floating organic drop are selected according to the following characteristics: to have low volatility and low solubility in water for them to be stable during the extraction process; to have a high extraction efficiency for the analytes; to be separated from the analyte peaks in chromatographic applications; to have melting points (m.p.) near room temperature (preferably in the range 10–30°C). Accordingly, 1-UN (mp: 13–15°C; density: 0.830 g mL^{-1}) and 1-DO (mp: 24–27°C; density: 0.833 g mL^{-1}) were investigated. In addition, DPE (m.p.: 25–27°C; density: 1.060 g mL^{-1}, solubility in water: 0.002 g in 100 mL of water at 25°C) seemed to be a promising extraction solvent for DLLME-SFO applications. It is worthy to note, however, that DPE is denser than water and sediments at the bottom of the extraction tube or floats at the surface depending on salt content in the sample solution due to proximity of its density to that of water. 1-UN gave the highest extraction efficiency (Figure 1). Moreover, because of its stability, low vapor pressure and low water solubility at the extraction conditions, 1-UN was selected as the extraction solvent in the present study.

In DLLME-SFO, volume of the extraction solvent is a key parameter that affects extraction kinetics and therefore enrichment factors. Its effect on the analytical signal of BPA was studied in the range of 10–120 µL. As can be seen in Figure 2, the analytical signal of the target analyte increased by increasing volume of the solvent in the range of 10–90 µL before it decreased afterward. Based on these observations, a volume of 90 µL was set optimum for further experiments.

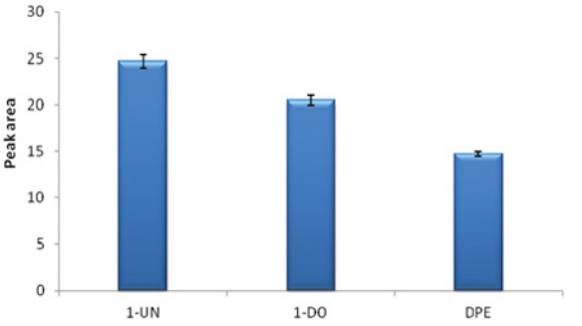

FIGURE 1 Effect of extraction solvent type on extraction efficiency. Samples spiked to 10 µg L^{-1} of BPA. Extraction conditions: aqueous sample volume 10 mL; extracted with each extraction solvent in 1.5 mL acetone; extraction time: 1 min; no salt addition; BES: 20 µL of 0.10 mol L^{-1} NaOH. (Color figure available online.)

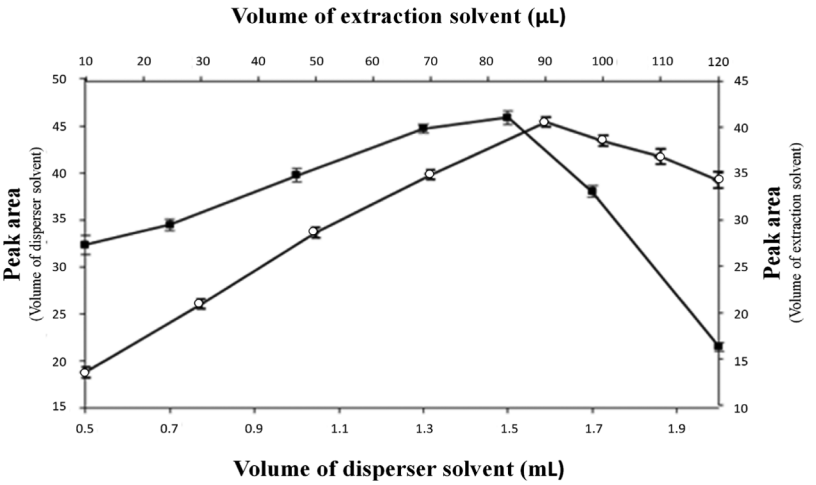

FIGURE 2 Effect of the volume of extraction (○) and disperser (■) solvents on extraction efficiency. Samples spiked to 10 µg L^{-1} of BPA. Extraction conditions: aqueous sample volume 10 mL; extracted with different volumes of 1-UN in different volumes of acetone; extraction time: 1 min; no salt addition; BES: 20 µL of 0.10 mol L^{-1} NaOH.

Type and Volume of Disperser Solvent

Miscibility of disperser solvent with extraction solvent and sample solution was the most important criteria when selecting the disperser solvent in DLLME-SFO. Thereby, acetone, acetonitrile, and methanol, which have this property, were suitable as disperser solvents. A series of sample solutions was extracted using 1.5 mL of each disperser solvent containing 90 µL 1-UN. Acetone was found to give the best extraction efficiency (Figure 3); it also has lower toxicity and is cheaper than methanol and

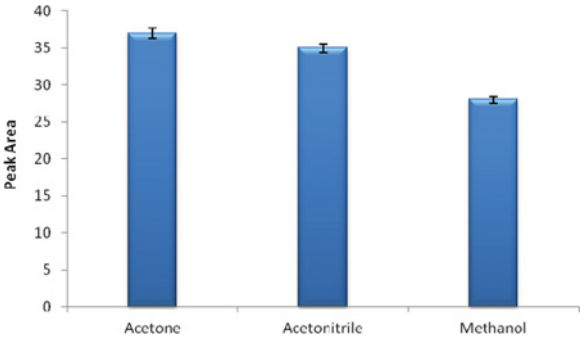

FIGURE 3 Effect of disperser solvent type on extraction efficiency. Samples spiked to 10 µg L^{-1} of BPA. Extraction conditions: aqueous sample volume 10 mL; extracted with 90 µL 1-UN, in 1.5 mL of each disperser solvent; extraction time: 1 min; no salt addition; BES: 20 µL of 0.10 mol L^{-1} NaOH. (Color figure available online.)

acetonitrile. Through investigations of the effect of disperser solvent volume on extraction efficiency, various volumes of acetone (0.5–2.0 mL) were used as shown in Figure 2. Increasing the volume from 0.5 to 1.5 mL resulted in a gradual increase in extraction efficiency, but increasing the volume beyond this point decreased the extraction efficiency steadily. This was thought to be due to the increase of the solubility of extraction solvent in water with the increase of the volume of acetone. The optimized sensitivity was achieved when 1.5 mL acetone was used.

pH of Sample and Back-Extraction Solutions

pH of sample solution played an important role since extraction efficiency was greatly affected by the charge on the studied analyte. Based on its pK_a value of 9.7, BPA is completely present in its neutral form in acidic media (pH $\leq$ 5.4) and more than 97.6% of it in its negatively charged form in highly alkaline media (pH $\geq$ 12.0). pH of sample solution was studied over the range 3.0–9.0. The highest extraction efficiency was obtained at pH 4.0. Afterward, the analyte was back-extracted into an aqueous solution (BES) containing varying concentrations of NaOH in the range of 0.01–0.20 mol L^{-1}; maximum extraction efficiency was obtained at a concentration of 0.10 mol L^{-1} as such these values were set optimum for subsequent experiments.

Volume of Sample and Back-Extraction Solutions

In three-phase LPME, higher enrichment factors can be achieved by increasing the volume ratio of the aqueous sample to the back-extraction solution. However, in many cases at equilibrium the maximum recovery can be limited by the distribution coefficient of the analyte between the donor and acceptor phases.[35] Volume of sample solutions was increased from 5 to 15 mL at a constant volume of 20 μL for BES. The results showed that the largest analytical response was obtained at a sample volume of 10 mL (Figure 4). The effect of volume of back-extraction solution was studied over the range 20 to 60 μL. It can be seen from Figure 4 that, extraction efficiency gradually decreased with increasing the volume which was due to dilution. Lower volumes than 20 μL could have resulted in higher extraction efficiency but when lower volumes were used, a microdrop of the aqueous phase was surrounded by the organic phase which resulted in a current drop when separation voltage was applied. Therefore, a volume of 20 μL was set optimal for further experiments.

Salt Addition

The addition of salt into the sample solution has been widely applied in LLE in order to improve the extraction efficiency of the analytes due to the salting-out effect.[36] However, it has shown no effect or even controversy

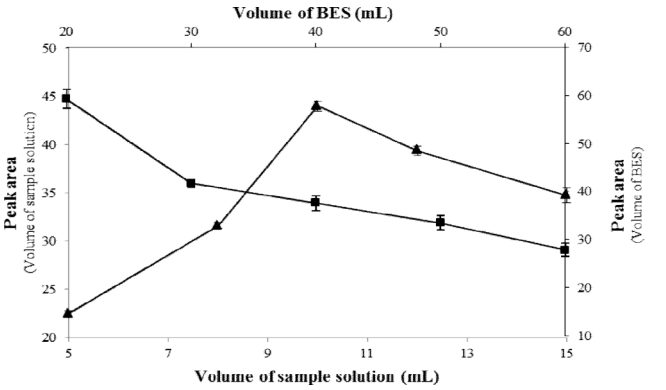

FIGURE 4 Effect of the volume of sample (▲) and back-extraction (■) solutions on extraction efficiency. Samples spiked to 10 μg L^{-1} of BPA. Extraction conditions: different volumes of aqueous sample; extracted with 90 μL 1-UN in 1.5 mL of acetone; extraction time: 1 min; no salt addition; BES: different volumes of 0.10 mol L^{-1} NaOH.

results in DLLME-SFO depending on the studied analyte(s).[37] The effect of increasing the ionic strength of the sample solution on the extraction efficiency of BPA was evaluated by the addition of NaCl (0–0.4 mol L^{-1}) into the sample solution. It was observed that extraction efficiency decreased with increasing salt content (data not shown). Hence, further extractions were performed without salt addition.

Effect of Extraction Time

In DLLME–SFO, extraction time is defined as the time interval between the injection of the mixture of disperser and extraction solvents and the time at which the sample is centrifuged[33] which corresponded to the time of vortex mixing in this study. The effect of extraction time on the extraction efficiency was examined in the range of 0–5 min under constant experimental conditions. The results obtained showed that the extraction time did not have any significant influence on the signal of BPA (data not shown). This was due to the fact that in DLLME after formation of the cloudy solution, the surface area between extraction solvent and aqueous sample is infinitely large. Thereby, transition of the analyte from the aqueous sample into the extraction solvent is considerably fast. In fact, independence on time is one of the great advantages of DLLME. In this method, the time-consuming steps were centrifugation of the sample solution and solidification of 1-UN, which were about 5 min each.

Analytical Performance and Figures of Merit

Limits of detection (LOD) of the target analyte generated by DLLME-SFO combined with counter-EOF NSM under optimized conditions

TABLE 1 Figures of Merit of DLLME-SFO with Co-EOF NSM

	Linear equation	Linear range ($\mu g\ L^{-1}$)	R^2	RSD (%)[a] ($n=5$) Intra-day	RSD (%)[a] ($n=5$) Inter-day	LOD ($\mu g\ L^{-1}$)	IF[b]
Water	$y=8.4075x+3.2701$	2.5–100	0.9992	0.5	1.2	0.8	1250
Urine	$y=3.1873x+10.867$	10.0–100	0.9989	0.9	1.9	2.5	430

[a]Data were calculated based on extraction of $20\ \mu g\ L^{-1}$ BPA.
[b]Overall improvement factor (Ratio of LOD in conventional CZE to that with DLLME-SFO combined with counter-EOF NSM).

in water and urine matrices are listed in Table 1. LOD [calculated based on a signal-to-noise (S/N) ratio of 3; N: noise of the baseline calculated for eleven noise peaks chosen at different places of the baseline void of analytical peaks] obtained using CZE was $1.0\ mg\ L^{-1}$. Applying counter-EOF NSM produced an LOD ($145\ \mu g\ L^{-1}$) that was lower by 6.9 times as compared to CZE. In addition, application of DLLME-SFO improved the CE sensitivity further by 181 times in water matrix and 58 times in urine matrix giving rise to LODs of $0.8\ \mu g\ L^{-1}$ and $2.5\ \mu g\ L^{-1}$ for BPA in water and urine, respectively (Table 1). Thus, overall improvement factors of CE sensitivity for the determination of BPA (Ratio of LOD in conventional CZE to that with DLLME-SFO combined with counter-EOF NSM) were 430 and 1250 in water and urine, respectively. Representative electropherograms of extracts of tap water and urine after extraction by DLLME-SFO method under optimum extraction and stacking conditions are provided in Figure 5.

Regression data and linearity of the calibration plots were investigated over a concentration range of 2.5–100 and 10–100 $\mu g\ L^{-1}$ for water and urine, respectively. As shown in Table 1, BPA exhibited good linearity with a coefficient of determination greater than 0.9989. Reproducibility of the proposed method was determined by intra-day and inter-day precision. As shown in Table 1, intra-day and inter-day ($n=5$) precisions (RSD) for $20\ \mu g\ L^{-1}$ BPA were equal to or less than 0.9% and 1.9%, respectively.

Analysis of Real Water and Urine Samples

In order to examine the possibility of matrix effects and investigate the applicability of the method to the analyses of real samples, the proposed method was used to determine BPA in three water samples, that is, tap, bottled and spring water as well as urine. Water and urine samples were spiked with the target compound at three concentration levels. The results are summarized in Table 2. Relative recoveries (RR) in water matrix were in the range of 92.4–104%. RRs in urine matrix were calculated using matrix-matched calibration and they were in the range of 99.5–100.3%.

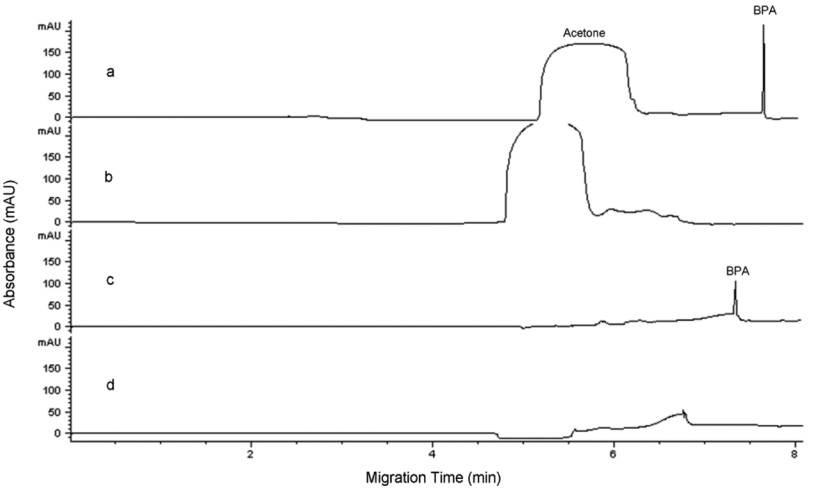

FIGURE 5 Electropherograms of extracts of tap water and urine after extraction by DLLME-SFO method under optimum conditions. (a) tap water spiked with BPA to 20 μg L^{-1}, (b) blank tap water (c) urine spiked with BPA to 20 μg L^{-1} and (d) blank urine. Electrophoretic conditions: separation temperature: 30°C; separation voltage: 20 kV; BGE: 25 mM borate buffer containing 5.0% methanol (pH* 9.3); sample injection mode: pressure, 50 mbar, 50 s.

TABLE 2 Relative Recoveries of BPA from Water and Urine Samples Spiked with the Target Analyte

Sample	C_{Added} (μg L^{-1})	C_{Found} (μg L^{-1})	RR[a]	RSD (%)
Tap water	–	n.d.[b]	–	–
	20.0	20.8 ± 0.1	104.0	0.5
	40.0	40.8 ± 0.3	102.0	0.7
	70.0	69.6 ± 0.4	99.4	0.6
Bottled water	–	n.d.[b]	–	–
	20.0	18.9 ± 0.2	94.5	1.1
	40.0	37.2 ± 0.3	93.0	0.8
	70.0	64.7 ± 0.5	92.4	0.8
Spring water	–	2.7 ± 0.5	–	–
	20.0	19.1 ± 0.2	95.5	1.0
	40.0	37.4 ± 0.4	93.5	1.1
	70.0	64.9 ± 0.5	92.7	0.8
Urine[c]	–	9.2 ± 0.4	–	–
	20.0	19.9 ± 0.2	99.5	1.0
	40.0	40.1 ± 0.5	100.3	1.2
	70.0	69.9 ± 1.1	99.9	1.6

[a]Relative recovery, percentage value obtained considering extraction yields in deionized water as 100%.

[b]Not detected.

[c]Relative recovery, percentage value obtained considering extraction yields from matrix-matched calibration.

Comparison with Other Preconcentration Methods

The developed DLLME-SFO-CE method was compared with other preconcentration methods used for the determination of BPA in terms of LOD, linearity, RSD%, volume of extraction solvent and extraction time for ionic liquid-dispersive liquid phase microextraction (IL-DLPME), liquid–liquid–liquid microextraction (LLLME), SDME, DLLME, and solid-phase microextraction (SPME). The results given in Table 3 show that this method is most importantly much faster than the other extraction methods. With the exception of DLLME which is also very fast (extraction time is less than 3 min), extraction times for IL-DLPME, LLLME, SDME, and SPME ranged from 20 to 60 min, without equilibrium being reached in most cases.[38] As no specific holder is required for supporting the organic microdrop like in SDME, DLLME-SFO is much more robust. Also, this method had the lowest RSD among the other methods. This method provided an acceptable LOD ($0.8\,\mu g\,L^{-1}$) and a good linear range ($2.5–100\,\mu g\,L^{-1}$) without using derivatization reagents, which may complicate the extraction process and extend the extraction time, or applying more sensitive detectors such as MS which are expensive and are not affordable by many laboratories. In contrast to IL-DLPME, LLLME, SDME, and SPME, extraction time had no influence on the DLLME-SFO efficiency. In addition to other advantages of the proposed method, it is simple, rapid, inexpensive, and easy to apply.

CONLUSION

In this study, a novel combination of DLLME-SFO and counter-EOF NSM in CE was successfully carried out for preconcentration and determination of BPA in different water and human urine samples. Factors

TABLE 3 Comparison of the Proposed Method with Other Methods for Extraction and Determination of BPA

Preconcentration Method		Detection System	LOD ($\mu g\,L^{-1}$)	Linear Range ($\mu g\,L^{-1}$)	RSD (%)	V_{ES}[a] (μL)	$t_{extraction}$ (min)	Ref.
IL-DLPME[b]		HPLC-FLD[c]	0.15	1.0–100	3.4	65	20	[39]
LLLME		HPLC-FLD	0.014	0.1–200	4.7	15	50	[40]
SDME		HPLC-UV	4	15–125	4.1	2.5	60	[41]
DLLME		HPLC-UV	0.07	0.5–100	6.0	142	<3	[38]
SPME		GC-MS	0.04	0.027–195	10.0	–	60	[42]
DLLME-SFO-co-EOF NSM	Water	CE-UV	0.8	2.5–100	1.2	90	2	This study
	Urine		2.5	10–100	1.9			

[a]Volume of extraction solvent.
[b]Ionic liquid-dispersive liquid phase microextraction.
[c]Fluorescence detection.

affecting the microextraction efficiency were systematically investigated and optimized. Under optimum conditions, this method gave an LOD at the ng L^{-1} level due to the high improvement factor obtained. Compared to CZE, the proposed method provided high sensitivity, with a lower LOD by up to 1250 times. Highly reproducible and interference-free electropherograms were obtained in the analysis of water and urine samples, indicating that the developed method has potential applicability in the determination of this target analyte in genuine samples. Although recoveries were not very high in urine samples, good relative recoveries ($\geq$99.5%) were achieved with matrix-matched standards. Due to its simplicity, low cost, low volume of organic solvent requirement, high improvement factors, and compatibility with CE, the proposed method can be extended for preconcentration and determination of a variety of organic compounds in these matrices.

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