Determination of Bisphenol A in Beverage Samples Using Ultrasonic- Extraction and Atomic Absorption Spectrometry

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Abstract: In this work, a simple and versatile ultrasound-assisted extraction (UAE) procedure, which provides high separation efficiency for bisphenol A (BPA), was developed for its indirect determination in beverages in contact with plastic containers by flame atomic absorption spectrometry (FAAS). The method is based on charge transfer reaction, in which BPA reacts with Cu(II) in alkaline tartrate solutions of pH 8.0 to produce Cu(I), which reacts with ion-pairing reagent, Promethazine, being a phenothiazine derivative (PMZ), in the presence of cetyl trimethylammonium bromide (CTAB). For the indirect determination of BPA using FAAS, the change in signal of Cu(II) depending on BPA concentration was investigated in detail. At optimal conditions, the analytical features of the method were obtained as follows; linearity ranges of 1.5-100 µg L⁻¹ for direct aqueous calibration solutions and 3-125 µg L⁻¹ for matrix matched calibration solutions; the limits of detection and quantification of 0.47 and 1.56 µg L⁻¹; sensitivity enhancement and pre-concentration factors of 135 and 150, respectively. The method accuracy was validated by repeatability/reproducibility precision studies using standard addition method. As the last, the method was successfully applied for determination of BPA in selected samples. BPA as a food stimulant was detected in ranges of 2.70-3.80 µg L⁻¹ in waters and 3.10-5.40 µg L⁻¹ in milk products while its levels changed in ranges of 6.40-7.70 and 4.30-19.2 µg L⁻¹ in beverages with and without alcohol. These levels were highly lower than the specific migration limit set by European Union.

Keywords: Bisphenol A, beverage samples, ultrasonic extraction, atomic absorption spectrometry, Cu complex.


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INTRODUCTION

Over the past few decades, endocrine disruptors (EDCs) have become a central topic in the international discussion in environmental and food chemistry because of their potential negative effects on the endocrine systems (1). Among phenolic EDCs, 2,2-bis(4-hydroxyphenyl) propane (bisphenol A, BPA) is a principal component of both polycarbonate and epoxy resins, and is widely used in the manufacture of consumer goods and products, including food containers and utensils, baby bottles, and water supply pipes (2, 3). Thus, there is a major source of concern for regulatory agencies and scientists. Bisphenol A can easily migrate into the food samples from lacquer-coated cans and plastic products due to hydrolysis of the polymer during thermal treatment (4). Consequently, it can cause adverse health effects such as recurrent miscarriages, endometrial hyperplasia, and polycystic ovarian syndrome (5). The scientific panel on food additives, flavourings and processing aids in contact with food of the European Food Safety Authority (EFSA) reported its risk assessment for BPA in 2007 and calculated a total daily intake (TDI) for BPA of 0.05 mg (kg body weight)$^{-1}$day$^{-1}$ with a specific migration limit (SML) of 0.6 mg kg$^{-1}$ for foods and its use was prohibited in the manufacture of polycarbonate feeding bottles intended for babies younger than one year since the beginning of 2011 (6, 7). So, there is still a strong requirement for rapid, efficient and sensitive analytical methods for the assessment of low amount of BPA exposure to humans.

To date, a variety of detection techniques have been developed to determine BPA in various samples, including micellar liquid chromatography (MLC) (8), liquid chromatography–fluorescence detection (LC-FD) (9), liquid chromatography–tandem mass spectrometry (LC-MS/MS) (10), gas chromatography–mass spectrometry (GC-MS) (11), capillary electrophoresis (CE) (12) and capillary zone electrophoresis (CZE) (13) as well as enzyme-linked immunosorbent assay (ELISA) (14) and micellar sensitive electroanalytical techniques such as linear sweep voltammetry (LSW) (15), differential pulse polarography (DPP) (16) and square wave voltammetry (SWV) (17).

In addition, analysis of trace levels of BPA in water and beverage samples using flame atomic absorption spectrometry (FAAS) is limited not only due to insufficient sensitivity, but also by matrix interference. Thus, different extraction procedures, including electrophoretic methods, ultrasonic-assisted extraction (UAE) (18), soxhlet extraction (19), cloud point extraction (CPE) (20), dispersive liquid–liquid micro-extraction (DLLME) (21) and solid phase extraction (SPE) (22) are frequently necessary to improve the detection limit and the selectivity. Among these procedures, the UAE is a key-technology in achieving the objective of sustainable “green chemistry”. Using ultrasound energy, full
extractions can now be completed in minutes with good reproducibility, lower organic solvent requirement, simplifying manipulation and work-up, giving higher purity of the selected samples (23). Moreover, the UAE procedures, either off- or on-line, are considered superior to other procedures for their simple, good pre-concentration factor, little organic solvent requirement, versatile use and time effectively. The UAE is an extraction procedure based on the clouding phenomenon of surfactants, and often used to preconcentrate toxic and non-toxic metals and metalloids from various sample matrices (24). Biodegradable surfactants like Tergitol TMN-6, Tergitol 15-S-7 and Tergitol 15-S-9 are used in CPE for extracting some polycyclic aromatic hydrocarbons (PAH) from real samples (25). Therefore, Tergitol 15-S-7 is expected to have many advantages in CPE combined with ultrasound energy of bisphenol A as a contaminant migrated from PC and PVC plastics into the beverage and foodstuffs (26).

Our research group considers the possibility of implementation of the UAE in combination with flame atomic absorption spectrometry (FAAS) in trace BPA analysis, and develop a new method for the determination of trace BPA in plastic bottle packaging beverage samples. For this purpose, (RS)-N,N-dimethyl-1-(10H-phenothiazin-10-yl)propan-2-amine, (Promethazine, PMZ) was selected as chelating reagent in the presence of Cu(II), tergitol 15-S-7 (extracting agent) at pH 8.0. To the best of our knowledge, there are no applications in the literature of FAAS for BPA determination from the prepared samples by ultrasonic-assisted extraction. Especially, the use of ultrasonic effect in sample preparation has provided features like low organic solvent usage and less extraction duration. The experimental parameters affecting the efficiency of UAE procedure and FAAS determination were systematically investigated. The precision and accuracy were confirmed by repeatability/reproducibility and recovery tests, respectively, and the method was then applied to the determination of BPA in the selected samples with satisfactory results.

MATERIALS AND METHODS

Apparatus

All measurements for the indirect determination of BPA were carried out using an atomic absorption spectrometer (AAS-6300 model, Shimadzu, Kyoto, Japan) equipped with a deuterium background correction and hollow cathode lamp of copper as the radiation source. The sequential device was used with following parameters: wavelength, 324.8 nm; lamp current, 3.0 mA; spectral bandwidth, 0.5 nm; burner height, 6.0 mm; acetylene and air flow rates, 1.8 L min⁻¹ and 15.0 L min⁻¹, respectively. A centrifuge (model Universal Hettich model, London, England) was used for complete phase separation. An ultrasound assisted water bath (UCP-10 model, Seoul, Korea) was used to fasten the extraction
process and digestion of the samples. A pH-meter (Selecta 2001 model, North America) was used for pH adjustment.

Chemicals and reagents
In this study, all chemical reagents used are at least analytical grade. The water utilized in throughout the experiment was high purity deionized water (18.2 MΩ cm), which obtained from a Labconco (Kansas City, USA) water purification system. Unless otherwise stated, the chemicals were purchased from Sigma (St. Louis, MO. USA) and Merck (Darmstadt, Germany). A stock solution of bisphenol A of 1000 mg L\(^{-1}\) (Sigma), was prepared in methanol and kept at 4 °C in the dark. A stock solution of Cu(II), 1000 mg L\(^{-1}\), was prepared by dissolving appropriate amounts of Cu(NO\(_3\))\(_2\) (Merck) in water. Working solutions were prepared daily by serial dilution of the stock solutions. A 1×10\(^{-4}\) mol L\(^{-1}\) solution of PMZ as ion-pairing reagent was prepared daily by dissolving an appropriate amount of solid in water. A 3×10\(^{-3}\) mol L\(^{-1}\) of the cationic surfactant solution, cetyl trimethyl ammonium bromide (CTAB, Sigma-Aldrich), was prepared by dissolving its suitable amount with the water. The non-ionic surfactant, a 10.0% (w/v) Tergitol 15-S-7 solution (Sigma-Aldrich), was prepared by dissolving in a mixture of water and methanol (9:1, v/v, Merck). The phosphate buffer solution of pH 8.0 (KH\(_2\)PO\(_4\)/NaOH, 0.2 mol L\(^{-1}\)) containing 5 mmol L\(^{-1}\) sodium potassium tartrate to prevent precipitation of Cu(II) ions and to improve signal reproducibility in alkaline conditions near to neutral was used to control the pH of the solutions. Because of the ubiquity of BPA, to avoid its contamination, no alkylphenol polyethoxylates detergents or plastics were used. Before starting the experiment, all the glassware was baked for 6 h at 400 °C and then washed five times with high purity deionized water and dried.

Sampling, sample pre-treatment
To demonstrate the applicability and reliability of the proposed method for beverages in contact with plastic containers, two groups, including alcoholic beverages (beer and wine) and non-alcoholic beverages (cherry juice, apricot juice, grape juice, water and milk samples) were bought from local supermarkets in Sivas, Turkey, and prepared for determination of BPA using the method. The selected samples, which last consumption date is less than one month, were purchased. The samples were stored as subsamples vacuum-packed in plastic bags at -10 °C until analysis.

Sample pre-treatment is very important step to separate the analyte from the matrix, because they may react with other chemical reagents with the analyte during the experimental process.
An aliquot (30 mL) of the water samples were firstly filtered through a cellulose membrane filter (Millipore) of pore size 0.45 mm. The samples were used without any pre-treatment before determination, and then the pH value was adjusted to 7.0 with 0.01–0.1 mol L\(^{-1}\) HCl and/or NaOH, and subjected to the extraction process.

Beverage samples were subjected to UAE prior to analysis. The samples were sequentially pre-treated as follows: (Step 1) an aliquot (200 mL) of the samples were transferred into a beaker and degassed in an ultrasonic bath. (Step 2) 20 mL of dichloromethane was added to the samples. (Step 3) The mixture was then shaken vigorously for 5 min at 3200 rpm using vortex device. (Step 4) The dichloromethane layer was transferred to a flask, and the extractant was evaporated to dryness under reduced pressure at 40 °C. (Step 5) The residue was dissolved in 5 mL of ethanol and filtered. (Step 6) The final solution was completed to 100 mL with water (27).

The milk samples (5 g or 10 mL) were diluted with 30 mL of water/methanol (5:1, v/v) to destabilize milk’s emulsion and to reduce viscosity of samples after efficiently shaking or vortexing for 2 min, and then sonicated in an ultrasonic bath for 10 min approximately at 30 °C until a homogeneous clear solution is obtained. The protein, casein, and fat were removed from the sample matrix by adding 5.0 mL of 2.5% (w/v) trichloroacetic acid (TCA) solution to the homogenized milk samples. After centrifugation for 10 min at 4000 rpm, the slurries were collected, and the precipitated protein and fat was rinsed five times by 3.0 mL of methanol to maximize extraction of bisphenol A. The eluate was then diluted to 100 mL with water and filtered using the membrane filters (28).

The same procedures were used for the blank solutions to determine the contamination of the reagents used. All experimental procedures were performed in triplicate.

**Ultrasonic extraction procedure**

The UAE procedure for separation and preconcentration of BPA was checked with model solutions. Firstly, 25.0 mL portion of a solutions containing of BPA in the range of 2–100 μg L\(^{-1}\), 0.2 mol L\(^{-1}\) of phosphate buffer containing 5 mmol L\(^{-1}\) tartrate (pH, 8.0), 75 μmol L\(^{-1}\) of PMZ, 125 μmol L\(^{-1}\) of Cu(II), 200 μmol L\(^{-1}\) of CTAB and 3.5 mmol L\(^{-1}\) of non-ionic surfactant, Tergitol 15-S-7 as extractant were added to 50 mL graduate tubes. To facilitate the charge transfer sensitized complex formation and mass transfer at micellar interface, the solution was shaken vigorously for one minute at 3200 rpm using vortex mixer, and were then filled with water up to the mark. After accomplishing the complexation, the mixture was left to stand in an ultrasonic bath at 55 °C for 5 min. in order to provide the cloud event of non-ionic surfactant. After the turbid solution is obtained, separation of the
phases was provided by centrifugation for 10 min at 4000 rpm. The aqueous phase was carefully removed with a Pasteur pipette, and the surfactant-rich phase was diluted to 1.0 mL with methanol to reduce its viscosity. After UAE, the diluted phase was introduced into nebuliser of FAAS for indirect analysis of BPA.

RESULTS AND DISCUSSION

The various chemicals used in indirect determination of BPA in presence of Cu(II) by FAAS, and the possible chemical equations participated in pre-concentration procedure are as follows:
The chemicals used in preconcentration

<table>
<thead>
<tr>
<th>Bisphenol A, BPA</th>
<th>Promethazine, PMZ</th>
<th>Tergitol 15-S-7</th>
<th>Cetyltrimethylammonium bromide, CTAB</th>
<th>Tartaric acid, H$_2$L</th>
<th>Copper(II) nitrate</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Bisphenol A, BPA" /></td>
<td><img src="image2" alt="Promethazine, PMZ" /></td>
<td><img src="image3" alt="Tergitol 15-S-7" /></td>
<td><img src="image4" alt="Cetyltrimethylammonium bromide, CTAB" /></td>
<td><img src="image5" alt="Tartaric acid, H$_2$L" /></td>
<td><img src="image6" alt="Copper(II) nitrate" /></td>
</tr>
</tbody>
</table>
\[ \text{Cu}^{2+} + 2\text{L}^{2-} + 2\text{H}_2\text{O} \rightarrow \text{Cu(OH)}_2\text{L}^{2+} + 2\text{OH}^- \], stable anionic chelate complex formation (Eq. 1)

\[ \text{Cu(OH)}_2\text{L}^{2+} + \text{PMZH}^+ \rightarrow \text{Extractable ion-pairing adduct complex} \] (Eq. 2)

Extractable ion-pairing complex $\rightarrow$ PMZ$^+$ radical + Cu(OH)L$^{2-}$ + L$^{2-}$ + H$_2$O, charge transfer (Eq. 3a)

PMZ$^+$. radical + Bisphenol A $\rightarrow$ PMZ$^{2+}$ + Bisphenolate radical, RO$^-$. (Eq. 3b)

PMZ$^{2+}$ + H$_2$O $\rightarrow$ PMZ=O + 2H$^+$ further sulfoxide formation or (Eq. 4)

PMZ$^{2+}$ + Cu(OH)L$^{2-}$ + L$^{2-}$ + H$_2$O $\rightarrow$ PMZ$^+$ + Cu(OH)$_2$L$^{2-}$ + H$^+$ or (Eq. 5a)

PMZ$^+$ + Cu(OH)$_2$L$^{2-}$ $\rightarrow$ Cu(OH)(PMZ)$^+$ + 2L$^{2-}$ + H$_2$O (Eq. 5b)

**Optimization of the UAE procedure**

To demonstrate efficient extraction of BPA, the extraction system must be optimized. The optimization involved testing different conditions such as pH, concentration of surfactant, ion-pairing reagent concentration, metal concentration, sample volume, temperature, time of ultrasonication, and interference effects from other matrix components. The variables were optimized by setting all variables to be constant and optimizing one each time. The BPA concentration was fixed at level of 25 µg L$^{-1}$ during optimization studies.

**Effect of pH**

In the extraction procedure, pH of the aqueous solution one of the main factors for metal chelates formation and the subsequent extraction. BPA is also a weakly acidic compound (pK$_a$ 9.7), and high pH can cause the ionization of compound(s) under test conditions (15). The effect of sample pH on the analytical signal from 6.5 to 10.5 was studied for measurement of Cu-complex, which is linearly related to bisphenol A concentration. The results obtained are given in Fig. 1(a). As it can be observed, there was a significant increase in analytical signal from pH 6.5 to pH 8.0, while the analytical signal decreased when increasing the pH. The cause of decrease can greatly be dissociation of BPA to phenolate anions with negative charge, due to have a pK$_a$ value ranging from 9.6 to 11.3. Another cause can be deprotonation of PMZ with a pK$_a$ value of 9.1 (3). Therefore, the pH of the solutions was adjusted to 8.0 using phosphate buffer solution (0.2 mol L$^{-1}$) containing tartrate ions at level of 5 mmol L$^{-1}$, aiming the more efficient charge transfer complexation between ion-pairing reagent, PMZH$^+$ and stable copper-tartrate complex, [Cu(OH)$_2$L$^2$]$^{2-}$ in presence of BPA in relation of the hydrolysis of the copper. Also, the effect of 0.2 mol L$^{-1}$ buffer volume at pH 8.0 was investigated in range of 0.5-5.0 mL, and a buffer volume of 2.5 mL was found to be enough, so as to give maximum, reproducible and stable analytical signal.
Effect of ion-pairing reagent concentration

Among chemical variables, ion-pairing reagent concentration is a critical parameter for formation of an ion-pairing complex also to compensate for any interactions with interfering ions that may exist in the sample. Thus, the ion-pairing reagent must provide the following features. (I) The ion-pair complex formed should be sufficiently hydrophobic, (II) have a high partition coefficient, and (III) form the stable complex quickly and quantitatively with minimum excess of reagent. As a result of the prior studies in literature, we selected PMZ, a phenothiazine derivative as ion-pairing reagent in order to obtain efficient separation and pre-concentration of BPA from sample matrix. Also, the $pK_a$ value of this ion-pairing reagent, which can easily be oxidized chemically or electrochemically, is 9.1 (29). It is a versatile chelating ligand, which can be relatively able to form stable metal complexes (30) and form aggregates in a micelle-like manner with the value of $N_{agg}$ (aggregation number) of the order of 6 to 15 depending on concentration, pH, and temperature (31).

The effect of PMZ concentration on the analytical signal is evaluated in the range of 10-125 µmol L$^{-1}$. As it can be observed (Fig. 1(b)), the analytical signals are enhanced remarkably depending on PMZ concentration. The analytical signal reaches to maximum when the PMZ concentration is 75 µmol L$^{-1}$. When the concentration continues to increase until 100 µmol L$^{-1}$, the analytic signal of BPA slightly decreased, later become flat. This is the main reason; the excess of PMZ, which is in equilibrium of protonated and deprotonated forms, PMZH$^+$ and PMZ, is presumably trapped in the micelles. A 75 µmol L$^{-1}$ of PMZ solution was therefore selected for successful extraction in subsequent experiments.

Effect of metal concentration

In order to be able to perform an indirect analysis with FAAS, the amount of analyte must be associated with a signal of metal. To accomplish this, preliminary study has been done with different metal ions (iron, nickel, cobalt and copper) at equal amounts in the presence of BPA and PMZ. The best sensitivity and stable signal is obtained when Cu(II) is used. This state can be explained with the fact that Cu(II) ions form a stable anionic complex, $[\text{Cu(OH)}_2\text{L}_2]^{4-}$ with tartrate ion in presence of BPA as a reducing species and PMZ, an ion-pairing reagent, which can be easily oxidized in redox environment and form dimer and further aggregates by pH dependent charge transfer. Also, it is implied in literature that Cu$^{2+}$ ions form a cationic complex with PMZ at pH 5.0, and anionic complex with tartrate ions with stability constants of log $\beta$: 20.7 and 17.3 in form of Cu(OH)$_2$L$^2$+ above pH 5.7 (30, 32). In this context, the effect of Cu(II) concentration was investigated in the range of 25-300 µmol L$^{-1}$. The results obtained in triplicate (Fig. 1(c)) showed that the analytical signal increases significantly with the Cu(II) concentration up to 125 µmol L$^{-1}$, at higher
concentrations there was no significant change. Therefore, a 125 µmol L\(^{-1}\) Cu(II) concentration was selected for successful extraction in subsequent experiments.

**Effect of auxiliary ligand concentration**

The UA-CPE efficiency depends on the formation of hydrophobic ion-pairing complex and the mass transference between the phases. To create a stable ion-association between cationic PMZH\(^+\) and anionic copper complex, Cu(OH)\(_2\)L\(_2\)\(^{4-}\) formed after pH sensitive charge transfer process between BPA and Cu(II). Therefore, the cationic surfactant, CTAB, was adopted and used as the sensitivity enhancer or counter ion in order to be able to detect BPA at sub-µg kg\(^{-1}\) or µg L\(^{-1}\) levels. It is indicated that CTAB below and above its critical micelle concentration (CMC) is used effectively to enhance the sensitivity and signal reproducibility of the electroanalytical techniques such as differential pulse voltammetry, linear sweep voltammetry and square wave voltammetry in determination of phenol and BPA as contaminant in waters and foodstuffs stored in plastic container (33, 34). The effect of CTAB concentration was investigated in the range 0.0–400 µmol L\(^{-1}\). According to the results shown in Fig. 1(d), the analytical signal increased by increasing CTAB concentration up to 400 µmol L\(^{-1}\) and decreased at higher concentrations. Excessive amount of CTAB is passed to surfactant rich phase. Therefore, a 200 µmol L\(^{-1}\) of CTAB concentration was selected for successful extraction in subsequent experiments.

**Effect of extracting agent concentration**

In the extraction process, one of the most important parameters is the type and concentration of the extracting agent. It is preferred that the extracting non-ionic surfactants to be used in experiments have properties such as cheap, eco-friendly, effective separation and commercial availability as well as being biodegradable and not absorbing and fluorescing in the UV region, in which the BPA is generally detected by LC, CE and/or CZE with detection of UV and fluorescence. Also, the surfactant concentration must be sufficient above the CMC to guarantee a quantitative extraction. In addition to all these, the volume ratio of the phases should be investigated, because an increase in surfactant concentration can decrease the analytical signal depending on dilution of the extract in the surfactant-rich phase volume. For all these reasons, the effect of type and concentration of the extracting non-ionic surfactant was investigated.

As can be seen in Fig. 1(e), the best results were obtained when using Tergitol 15-S-7. At lower concentrations, the phase separation was difficult due to the low-volume rich phase and the inadequacy of the assemblies to entrap the hydrophobic complexes quantitatively. Analytical signal was also increased in concentration range of 1.0-4.0 mmol L\(^{-1}\), and the highest signal was obtained at 3.5 mmol L\(^{-1}\) with a higher concentration of 40-fold, in which
its CMC value is 0.092 mmol L\(^{-1}\). Therefore, it was selected for successful extraction in subsequent experiments.

**Effect of sample volume**

Since amount of BPA is low into real samples, the effect of sample volume was also tested in range of 10-250 mL with a constant amount of BPA. The results, as can be seen in Fig. 1(f), demonstrated that quantitative analytical signal for sample volumes was obtained in range of 50-150 mL. Above 150 mL, the analytical signal decreased slightly. After UAE, the surfactant-rich phase was completed to 1 mL with methanol, so the pre-concentration factor was 150.

**Effects of equilibrium temperature and time**

Optimal equilibration temperature is necessary for the completion of the ion-pairing complex formation and efficient phase separation. This hydrophobic complex, which is bound to core and interface of the micelles by polar ethoxylate groups of micelles and hydrophobic interactions, is extracted to the surfactant-rich phase, and this event can be achieved when the equilibration temperature is above the cloud point temperature (CPT) of a non-ionic surfactant. Therefore, the equilibration temperature was investigated in the range of 25-70 \(^\circ\)C. From studies, when the temperature increased in the range from 25 to 55 \(^\circ\)C, the analytical signals increased correspondingly for BPA. At higher temperatures, the analytical signal is decreased. This is because the resulting ion-pair complex is reversibly dispersed to the solution depending on the temperature. Thus, 55 \(^\circ\)C was chosen as the optimal equilibration temperature for successful extraction in subsequent experiments.

In the extraction process, the sonication time is one of the prime factors influencing the BPA extraction and mass transfer into surfactant-rich phase. Sonication caused an increase in the mass transfer, and a decrease in reaction time. To minimize the time required for extraction, sonication time was investigated in range of 1-15 min and the results are shown in Fig. 1(g). Based on the results, the best analytical signal was obtained at 10 min. Thus, 10 min was chosen as the optimal sonication time for successful extraction in subsequent experiments.

**Effect of solvent**

After centrifugation for 5 min at 4000 rpm, the obtained surfactant rich phase is low volume and high viscous. Therefore, analysis with FAAS cannot be done. To overcome this problem, this phase must be diluted with a suitable solvent. The results indicated that methanol was
a suitable diluting solvent and also a suitable matrix for indirect determination of BPA using FAAS.

(a)

(b)

(c)

(d)
The BPA exists along with different interfering species in selected samples. This event may be attributed to the extraction step, because high selectivity with careful utility of FAAS can be provided. Thus, the effects of some foreign ions were investigated by conducting UAE experiments using solutions containing 20 μg L\(^{-1}\) of BPA in the presence of different mass ratios of foreign ions under the extraction conditions. Tolerable limit is considered as the interfering agent level that is not significantly affect the preconcentration via UAE and subsequent determination of BPA by FAAS as determinate error smaller than ±5.0%. The results showed that at least 10000 μg L\(^{-1}\) of Na\(^+\), Ca\(^{2+}\), NH\(^{4+}\), SO\(_4^{2-}\), NO\(_3^{-}\), Fe\(^{3+}\), Al\(^{3+}\), and Cl\(^{-}\), 5000 μg L\(^{-1}\) of Mg\(^{2+}\) and PO\(_4^{3-}\), 1000 μg L\(^{-1}\) of 2-chlorobenzaldehyde, bromobenzaldehyde, phenol, and 4-nitrophenol and 750 μg L\(^{-1}\) of 2,4-dinitrophenol had no remarkable interferences with the determination of BPA. 2-aminophenol, acetaldehyde and
acetate could be tolerated up to 500 μg L\(^{-1}\) and nonylphenol and octylphenol could be tolerated up to 250 μg L\(^{-1}\). As can be understood from the results, the tolerance limits of the foreign ions have a good tolerance to matrix interference. Therefore, this method could be applied to successfully for the extraction of BPA in selected sample matrices.

Analytical figures of merit

Under the optimal conditions, calibration graph was performed using standard addition calibrations prepared following the UAE procedure. Table 1 shows the characteristic performances of the proposed method. The calibration graph prepared from the aqueous standards was linear in the range of 1.5-100 μg L\(^{-1}\) with a good correlation coefficient (r) of 0.9943. The limit of detection (LOD), defined as the concentration that gives a signal equivalent to three times the standard deviation of 10 replicate measurements of the procedural blank sample (blank digest preconcentrated by the UAE procedure), was 0.47 μg L\(^{-1}\). For the precision of the method, the relative standard deviations (RSD) of the five independent replicate measurements for 20 and 50 μg L\(^{-1}\) of BPA are lower than 3.5%. The percent recoveries obtained were in range of 95.8-103.9% for the spiked BPA concentration of 20 and 50 μg L\(^{-1}\) to the selected samples. As mentioned previously, the amount of BPA in 150 mL of sample volume was determined after extraction process by 1.0 mL of surfactant-rich phase, therefore the pre-concentration factor for this method is 150. Sensitivity enhancement factor, which calculated from the ratio of the slopes of the calibration curves obtained with and without pre-concentration, was 135.

Due to lack of a certified reference material (CRM), which is suitable to sample matrix for evaluation of the accuracy, the matrix-matched solutions were also prepared by externally adding eight pointed standard solutions of BPA ranging from 5 to 125 μg L\(^{-1}\) to blank sample matrix. The milk powder that does not contain the analyte was used as blank sample for calibration of “matrix-matched”. The further pre-treatment of the spiked samples was carried out according to the two different extraction approaches described in sample preparation section. After stepwise dilution from stock solution, the eight concentration levels of concentrations for bisphenol A were 5, 10, 15, 25, 75, 100 and 125 μg L\(^{-1}\). In a similar way, the externally spiked samples under optimal conditions were submitted to UAE procedure, and then each point was detected three times by FAAS. From regression analysis, the calibration data obtained for matrix-matched solutions and the analytical features of the method based on these data are represented in Table 1. Additionally, the recovery study for five replicate measurements of 20 and 50 μg L\(^{-1}\) of the BPA was conducted and found to be in range of 93.5-106.2% with lower RSD than 4.2%.
**Table 1** The analytical features of the proposed method for BPA

<table>
<thead>
<tr>
<th>Parameters</th>
<th>By direct calibration solutions</th>
<th>By matrix matched calibration solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear range (µg L⁻¹)</td>
<td>1.5-100</td>
<td>3-125</td>
</tr>
<tr>
<td>Regression equation</td>
<td>$A = 4.2 \times 10^{-3} \text{[BPA µg L}^{-1}] + 1.7 \times 10^{-4}$</td>
<td>$A = 3.7 \times 10^{-3} \text{[BPA, µg L}^{-1}] + 2.2 \times 10^{-4}$</td>
</tr>
<tr>
<td>Correlation coefficient, $r$</td>
<td>0.9943</td>
<td>0.9956</td>
</tr>
<tr>
<td>Limit of detection (3σ/m) (µg L⁻¹)</td>
<td>0.47</td>
<td>0.95</td>
</tr>
<tr>
<td>Limit of quantification (10σ/m) (µg L⁻¹)</td>
<td>1.56</td>
<td>3.20</td>
</tr>
<tr>
<td>Recovery (%)</td>
<td>95.8-103.9</td>
<td>93.5–106.2</td>
</tr>
<tr>
<td>Reproducibility (%)</td>
<td>3.4</td>
<td>4.1</td>
</tr>
<tr>
<td>Repeatability (%)</td>
<td>3.2</td>
<td>3.9</td>
</tr>
<tr>
<td>Sensitivity enhancement factor (EF)</td>
<td>135</td>
<td>115</td>
</tr>
<tr>
<td>Pre-concentration factor (PF)</td>
<td>150</td>
<td>150</td>
</tr>
</tbody>
</table>
The accuracy and precision

The accuracy was tested through the recovery tests from spiked samples. The work was carried out in the following way, analytical recovery was checked for 20 and 50 μg L\(^{-1}\) of BPA, after spiking different aliquots of the selected samples. Each concentration level was repeated in five times by the UAE procedure, and each extract was determined through FAAS. The analytical recoveries obtained were in range of 95.0-103.8% for spiked concentrations. The more detailed results are given in Table 2 (a-c).

The precision was tested by studying the parameters of the repeatability and reproducibility. For the repeatability precision, using selected two of the beverages and milk samples fortified with BPA at the concentrations of 10, 25 and 50 μg L\(^{-1}\) were calculated by analysing (five replicate). Then, the mean BPA concentrations were determined as 10.5, 24.3, 50.7 μg L\(^{-1}\) and with associated RSD values of 3.4%, 3.1% and 2.9%, respectively. Regarding the reproducibility precision, the same three concentrations were calculated for five consecutive days, providing mean BPA concentrations of 10.2, 25.8, 51.1 μg L\(^{-1}\) and associated RSD values of 3.2%, 2.8% and 2.7%, respectively. When looking at the results obtained, we can conclude that the method offers good precision.

Analytical applications

Different beverage samples in contact with plastic containers were analyzed using the proposed method in order to prove its suitability for the routine control and selective extraction of BPA. The results were given in Table 2(a-c) as well as the recoveries obtained after spiking the samples with 20 μg L\(^{-1}\) and 50 μg L\(^{-1}\) of BPA. The recovery% was calculated by using the equation: Recovery% =100\(\frac{(C_S-C_0)}{m}\) where \(C_S\) is the amount of total BPA in sample after spiking, \(C_0\) is the amount of BPA in original sample and \(m\) is the amount of BPA spiked at known levels. The spiked recoveries of the method for analysis of BPA in water samples were found in range of 97.2-103.1%, with the RSD varying from 2.2 to 3.0%. For the beverage and milk samples, the spiked recoveries were found in range of 95.0-103.5% and 95.3-103.8%, respectively. The RSDs were lower than 3.9%. In the study, the lowest amount of BPA (2.70±0.07 μg L\(^{-1}\)) was found in water samples and the highest amount of BPA in grape juice (19.2±0.5 μg L\(^{-1}\)). This is the main reason; the acidic medium may accelerate the leaching of BPA into the sample. Thus, the method is capable of the determination of BPA in these sample matrices. In addition, BPA concentrations in range of 2.7 - 3.8 μg L\(^{-1}\) (bottled drinking water), 3.1-5.4 μg L\(^{-1}\) (milk samples) to 4.3-19.2 μg L\(^{-1}\) (beverages with and without alcohol) were significantly lower than specific migration limit (SML) of 600 μg kg\(^{-1}\) set by the EC Directive for BPA in foods or food simulants, so as not to lead any safety risk on humans consuming the samples.
Table 2(a) The analysis results of determination of BPA in spiked water samples using the proposed method (n: 5).

<table>
<thead>
<tr>
<th>Added (µg L(^{-1}))</th>
<th>Commercial drinking water(^1)</th>
<th>Commercial drinking water(^2)</th>
<th>Commercial drinking water(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Found (µg L(^{-1}))</td>
<td>Recovery (%)</td>
<td>RSD (%)</td>
</tr>
<tr>
<td>0</td>
<td>2.70±0.07</td>
<td>- 2.5</td>
<td>3.80±0.1</td>
</tr>
<tr>
<td>20</td>
<td>22.1±0.5</td>
<td>97.2</td>
<td>2.4</td>
</tr>
<tr>
<td>50</td>
<td>51.7±1.2</td>
<td>98.1</td>
<td>2.2</td>
</tr>
</tbody>
</table>

\(^1,\(^2,\(^3\) Represents the waters in different brands.

Table 2(b) The analysis results of determination of BPA in spiked milk samples using the proposed method (n: 5).

<table>
<thead>
<tr>
<th>Added (µg L(^{-1}))</th>
<th>Whole milk</th>
<th>Semi-skimmed milk</th>
<th>Milkshake</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Found (µg L(^{-1}))</td>
<td>Recovery (%)</td>
<td>RSD (%)</td>
</tr>
<tr>
<td>0</td>
<td>4.8±0.1</td>
<td>- 2.9</td>
<td>3.1±0.1</td>
</tr>
<tr>
<td>20</td>
<td>24.1±0.6</td>
<td>96.3</td>
<td>2.7</td>
</tr>
<tr>
<td>50</td>
<td>53.6±1.3</td>
<td>97.5</td>
<td>2.5</td>
</tr>
</tbody>
</table>
Table 2(c) The analysis results of determination of BPA in spiked beverage samples using the proposed method (n: 5).

<table>
<thead>
<tr>
<th>Sample group</th>
<th>Sample type</th>
<th>Added (µg L(^{-1}))</th>
<th>Found (µg L(^{-1}))</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcoholic beverages</td>
<td>Beer</td>
<td>-</td>
<td>6.4±0.2</td>
<td>-</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>25.4±0.8</td>
<td>95.0</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>54.8±1.5</td>
<td>96.7</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>Wine</td>
<td>7.7±0.3</td>
<td>-</td>
<td>96.7</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>28.4±1.1</td>
<td>103.5</td>
<td>3.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>58.8±2.0</td>
<td>102.1</td>
<td>3.4</td>
</tr>
<tr>
<td>Cherry juice</td>
<td>-</td>
<td>8.5±0.3</td>
<td>-</td>
<td>97.4</td>
<td>3.0</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>28.0±0.8</td>
<td>102.4</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>57.8±1.7</td>
<td>98.6</td>
<td>2.9</td>
<td></td>
</tr>
<tr>
<td>Non-alcoholic beverages</td>
<td>Apricot juice</td>
<td>-</td>
<td>4.3±0.1</td>
<td>-</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>24.8±0.5</td>
<td>102.4</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>54.9±1.2</td>
<td>101.2</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>Grape juice</td>
<td>-</td>
<td>19.2±0.5</td>
<td>-</td>
<td>96.9</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>38.6±0.9</td>
<td>97.8</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>68.1±1.6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Comparison with literature

In order to indicate positive aspects of the proposed method, a comparison between the figures of merit of the proposed method and some of the recently published methods for extraction and determination of BPA is given in Table 3 in terms of some optimization parameters. As can be seen from the data, the pre-concentration factor of the proposed method is relatively higher and, consequently, its detection limit is either lower or comparable to the more sensitive method of chromatographic techniques except for LC-MS-MS and GC-MS with pre-concentration and in situ derivatization. However, these techniques have lower recovery and poor precision than those of our method especially at low concentrations. Additionally, they are complex and expensive, and need expert user in his/her area and tedious and time consuming separation and/or pre-concentration steps before detection with UV, fluorescence and mass spectrometry. The combination of UAE with FAAS as element-selective detection tool enables accurate and reliable determination of BPA in ranges of 1.5-100 and 3-125 µg L\(^{-1}\) by calibration curves prepared from aqueous standards and matrix-matched standards with detection limits of 0.47 and 0.95 µg L\(^{-1}\), respectively. Also, the extraction time and the intra-day/inter-day precision of by the method as RSD% are far better than most of the other reported methods. In addition, the UAE procedure has some advantages including green chemistry solvents, simplicity, rapidity and low contamination risk for the analysis.
### Table 3 Comparison of proposed method with those of other methods.

<table>
<thead>
<tr>
<th>Sample matrix</th>
<th>Extraction process</th>
<th>Detection technique</th>
<th>$^a$LR ($\mu$g L$^{-1}$)</th>
<th>LOD ($\mu$g L$^{-1}$)</th>
<th>RSD (%)</th>
<th>Recovery (%)</th>
<th>$^b$EF or $^c$PF</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waters and urine</td>
<td>$^1$MISPE</td>
<td>$^2$CE–UV</td>
<td>3-500</td>
<td>0.3</td>
<td>$\leq$7.2%</td>
<td>95.2-105.4%</td>
<td>50</td>
<td>(3)</td>
</tr>
<tr>
<td>Urine samples</td>
<td>$^3$CME</td>
<td>$^4$LC–FD</td>
<td>0.4-149</td>
<td>0.197</td>
<td>4.5%</td>
<td>88-95%</td>
<td>38</td>
<td>(8)</td>
</tr>
<tr>
<td>Soils</td>
<td>$^5$UAE</td>
<td>$^6$GC–MS</td>
<td>5–300</td>
<td>0.03 ng g$^{-1}$</td>
<td>9.6%</td>
<td>88.1-107.7%</td>
<td>-</td>
<td>(18)</td>
</tr>
<tr>
<td>Waters</td>
<td>$^7$CPE</td>
<td>$^8$LC–UV</td>
<td>1-100</td>
<td>0.34</td>
<td>-</td>
<td>90-108.6%</td>
<td>50</td>
<td>(20)</td>
</tr>
<tr>
<td>Waters</td>
<td>$^9$SPE</td>
<td>$^{10}$LC–MS/MS</td>
<td>0.02-0.2</td>
<td>0.057</td>
<td>$\leq$13%</td>
<td>85-100%</td>
<td>-</td>
<td>(35)</td>
</tr>
<tr>
<td>Waters</td>
<td>$^{11}$DLLME</td>
<td>$^{12}$HPLC–UV</td>
<td>0.5–100</td>
<td>0.07</td>
<td>6.0%</td>
<td>93.4–98.2%</td>
<td>80</td>
<td>(36)</td>
</tr>
<tr>
<td>Serum</td>
<td>-</td>
<td>$^{13}$ELISA</td>
<td>0.3–100</td>
<td>0.3</td>
<td>13.6%</td>
<td>81.9–97.4%</td>
<td>-</td>
<td>(37)</td>
</tr>
<tr>
<td>Leachate</td>
<td>$^{14}$SPME</td>
<td>HPLC–UV</td>
<td>12.8–192</td>
<td>3.25</td>
<td>4.4%</td>
<td>94.5–103.3%</td>
<td>-</td>
<td>(38)</td>
</tr>
<tr>
<td>Soft drinks and powdered infant formula</td>
<td>$^{15}$LPME</td>
<td>GC–MS</td>
<td>1–1000</td>
<td>0.005</td>
<td>15%</td>
<td>82–111%, 68-</td>
<td>-</td>
<td>(39)</td>
</tr>
</tbody>
</table>

Water, urine, plasma and saliva samples  $^{16}$SBSE GC–MS with and without derivatization  0.02-10, 2–100 0.005, 0.5 3.8-9.6% 95.2-104.6% - (40)

Beverages and Waters  $^{17}$UA–CPE FAAS  1.5-100, 3-125 0.47, 0.95 $\leq$3.9% 95.8-103.9% 135, 150, Present method

$^a$Linear range, $^b$Enhancement factor, $^c$Preconcentration factor  $^1$Molecularly imprinted solid-phase extraction; $^2$Capillary electrophoresis–UV method; $^3$Coacervative microextraction; $^4$Liquid chromatography–fluorescence detection; $^5$Ultrasonic assisted extraction; $^6$Gas chromatography–mass spectrometry; $^7$Cloud-point extraction; $^8$Liquid chromatography; $^9$Solid-phase extraction; $^{10}$Liquid chromatography–tandem mass spectrometry; $^{11}$Dispersive liquid–liquid microextraction; $^{12}$High-performance liquid chromatography; $^{13}$Enzyme-linked immunosorbent assay; $^{14}$Solid phase microextraction; $^{15}$Liquid phase microextraction; $^{16}$Stir bar sorptive extraction.
CONCLUSIONS

In the present study, a simple, rapid, inexpensive, and sensitive analytical method, based on highly effective coupling of UAE with highly selective FAAS was proposed to determine BPA in different beverage samples. This study is the first method reported for indirect determination of BPA using FAAS. Especially, the use of ultrasound energy in sample preparation has provided features like low organic solvent usage, and less extraction duration. Other advantages can be pointed out like the low limits of detection, good precision and good selectivity, wide linear working range, high analytical signal, as well as low reagent consumption, high pre-concentration and sensitivity enhancement factors. The quantitation or determination limit of the proposed method is about 1.6 µg L\(^{-1}\), so it can be used for the routine control of BPA in different beverage samples below the current specific migration limit (SML) of 600 µg L\(^{-1}\) set by the EU Commission. As a result, the effectiveness and efficiency of the proposed method was successfully demonstrated for BPA determination and applied to different beverage samples.

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REFERENCES


