



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 $\mu\text{g L}^{-1}$ for direct aqueous calibration solutions and 3-125 $\mu\text{g L}^{-1}$ for matrix matched calibration solutions; the limits of detection and quantification of 0.47 and 1.56 $\mu\text{g L}^{-1}$; 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 $\mu\text{g L}^{-1}$ in waters and 3.10-5.40 $\mu\text{g L}^{-1}$ in milk products while its levels changed in ranges of 6.40-7.70 and 4.30-19.2 $\mu\text{g L}^{-1}$ 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.

Submitted: January 28, 2017. **Revised:** April 22, 2017. **Accepted:** May 04, 2017.

Cite this: Yildirim E, Altunay N, Gürkan R. Determination of Bisphenol A in Beverage Samples Using Ultrasonic- Extraction and Atomic Absorption Spectrometry. JOTCSA. 2017;4(2):607-30.

DOI: 10.18596/jotcsa.288389.

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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 M Ω 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(NO₃)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 (KH₂PO₄/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 μm . 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 $\mu\text{g L}^{-1}$, 0.2 mol L^{-1} of phosphate buffer containing 5 mmol L^{-1} tartrate (pH, 8.0), 75 $\mu\text{mol L}^{-1}$ of PMZ, 125 $\mu\text{mol L}^{-1}$ of Cu(II), 200 $\mu\text{mol L}^{-1}$ 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

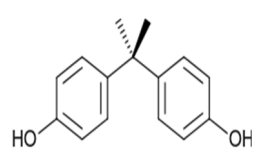
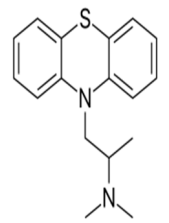
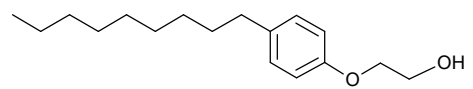
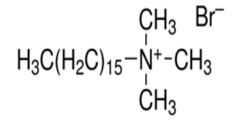
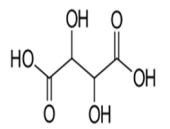
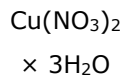
phases was provided by centrifugation for 10 min at 4000 rpm. The aqueous phase was carefully removed with a Pasteur pipette, and the surfactant-rich phase was diluted to 1.0 mL with methanol to reduce its viscosity. After UAE, the diluted phase was introduced into nebuliser of FAAS for indirect analysis of BPA.

RESULTS AND DISCUSSION

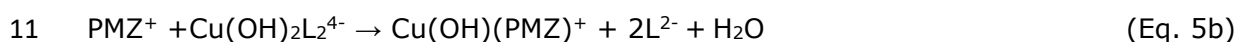
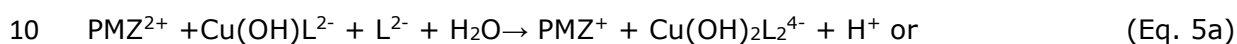
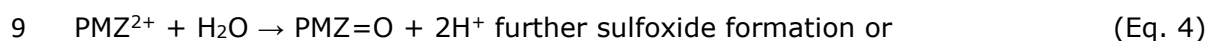
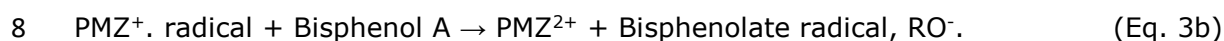
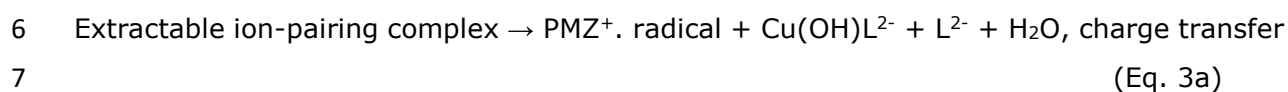
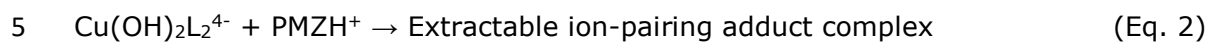
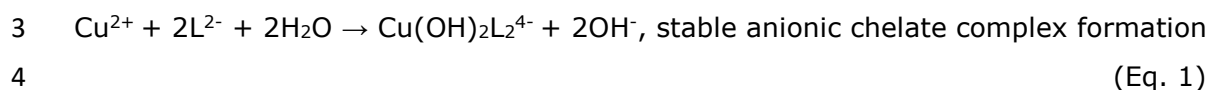
The various chemicals used in indirect determination of BPA in presence of Cu(II) by FAAS, and the possible chemical equations participated in pre-concentration procedure are as follows:

1

The chemicals used in preconcentration

Bisphenol A, BPA	Promethazine, PMZ	Tergitol 15-S-7	Cetyltrimethylammonium bromide, CTAB	Tartaric acid, H ₂ L	Copper(II) nitrate
					

2



12

13 Optimization of the UAE procedure

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

20

21 Effect of pH

22 In the extraction procedure, pH of the aqueous solution one of the main factors for metal
23 chelates formation and the subsequent extraction. BPA is also a weakly acidic compound
24 (pK_a 9.7), and high pH can cause the ionization of compound(s) under test conditions (15).
25 The effect of sample pH on the analytical signal from 6.5 to 10.5 was studied for
26 measurement of Cu-complex, which is linearly related to bisphenol A concentration. The
27 results obtained are given in Fig. 1(a). As it can be observed, there was a significant
28 increase in analytical signal from pH 6.5 to pH 8.0, while the analytical signal decreased
29 when increasing the pH. The cause of decrease can greatly be dissociation of BPA to
30 phenolate anions with negative charge, due to have a pK_a value ranging from 9.6 to 11.3.
31 Another cause can be deprotonation of PMZ with a pK_a value of 9.1 (3). Therefore, the pH
32 of the solutions was adjusted to 8.0 using phosphate buffer solution (0.2 mol L^{-1}) containing
33 tartrate ions at level of 5 mmol L^{-1} , aiming the more efficient charge transfer complexation
34 between ion-pairing reagent, PMZH^+ and stable copper-tartrate complex, $[\text{Cu}(\text{OH})_2\text{L}_2]^{4-}$ in
35 presence of BPA in relation of the hydrolysis of the copper. Also, the effect of 0.2 mol L^{-1}
36 buffer volume at pH 8.0 was investigated in range of 0.5-5.0 mL, and a buffer volume of
37 2.5 mL was found to be enough, so as to give maximum, reproducible and stable analytical
38 signal.

39

40

41 *Effect of ion-pairing reagent concentration*

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

54
55 The effect of PMZ concentration on the analytical signal is evaluated in the range of 10-
56 125 $\mu\text{mol L}^{-1}$. As it can be observed (Fig. 1(b)), the analytical signals are enhanced
57 remarkably depending on PMZ concentration. The analytical signal reaches to maximum
58 when the PMZ concentration is 75 $\mu\text{mol L}^{-1}$. When the concentration continues to increase
59 until 100 $\mu\text{mol L}^{-1}$, the analytic signal of BPA slightly decreased, later become flat. This is
60 the main reason; the excess of PMZ, which is in equilibrium of protonated and deprotonated
61 forms, PMZH^+ and PMZ , is presumably trapped in the micelles. A 75 $\mu\text{mol L}^{-1}$ of PMZ solution
62 was therefore selected for successful extraction in subsequent experiments.

63 64 *Effect of metal concentration*

65 In order to be able to perform an indirect analysis with FAAS, the amount of analyte must
66 be associated with a signal of metal. To accomplish this, preliminary study has been done
67 with different metal ions (iron, nickel, cobalt and copper) at equal amounts in the presence
68 of BPA and PMZ. The best sensitivity and stable signal is obtained when Cu(II) is used.
69 This state can be explained with the fact that Cu(II) ions form a stable anionic complex,
70 $[\text{Cu(OH)}_2\text{L}_2]^{4-}$ with tartrate ion in presence of BPA as a reducing species and PMZ, an ion-
71 pairing reagent, which can be easily oxidized in redox environment and form dimer and
72 further aggregates by pH dependent charge transfer. Also, it is implied in literature that
73 Cu^{2+} ions form a cationic complex with PMZ at pH 5.0, and anionic complex with tartrate
74 ions with stability constants of $\log \beta$: 20.7 and 17.3 in form of $\text{Cu(OH)}_2\text{L}_2^{4-}$ above pH 5.7
75 (30, 32). In this context, the effect of Cu(II) concentration was investigated in the range
76 of 25-300 $\mu\text{mol L}^{-1}$. The results obtained in triplicate (Fig. 1(c)) showed that the analytical
77 signal increases significantly with the Cu(II) concentration up to 125 $\mu\text{mol L}^{-1}$, at higher

78 concentrations there was no significant change. Therefore, a 125 $\mu\text{mol L}^{-1}$ Cu(II)
79 concentration was selected for successful extraction in subsequent experiments.

80

81 *Effect of auxiliary ligand concentration*

82 The UA-CPE efficiency depends on the formation of hydrophobic ion-pairing complex and
83 the mass transference between the phases. To create a stable ion-association between
84 cationic PMZH⁺ and anionic copper complex, Cu(OH)₂L₂⁴⁻ formed after pH sensitive charge
85 transfer process between BPA and Cu(II). Therefore, the cationic surfactant, CTAB, was
86 adopted and used as the sensitivity enhancer or counter ion in order to be able to detect
87 BPA at sub- $\mu\text{g kg}^{-1}$ or $\mu\text{g L}^{-1}$ levels. It is indicated that CTAB below and above its critical
88 micelle concentration (CMC) is used effectively to enhance the sensitivity and signal
89 reproducibility of the electroanalytical techniques such as differential pulse voltammetry,
90 linear sweep voltammetry and square wave voltammetry in determination of phenol and
91 BPA as contaminant in waters and foodstuffs stored in plastic container (33, 34). The effect
92 of CTAB concentration was investigated in the range 0.0–400 $\mu\text{mol L}^{-1}$. According to the
93 results shown in Fig. 1(d), the analytical signal increased by increasing CTAB concentration
94 up to 400 $\mu\text{mol L}^{-1}$ and decreased at higher concentrations. Excessive amount of CTAB is
95 passed to surfactant rich phase. Therefore, a 200 $\mu\text{mol L}^{-1}$ of CTAB concentration was
96 selected for successful extraction in subsequent experiments.

97

98 *Effect of extracting agent concentration*

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

110

111 As can be seen in Fig. 1(e), the best results were obtained when using Tergitol 15-S-7. At
112 lower concentrations, the phase separation was difficult due to the low-volume rich phase
113 and the inadequacy of the assemblies to entrap the hydrophobic complexes quantitatively.
114 Analytical signal was also increased in concentration range of 1.0-4.0 mmol L^{-1} , and the
115 highest signal was obtained at 3.5 mmol L^{-1} with a higher concentration of 40-fold, in which

116 its CMC value is 0.092 mmol L⁻¹. Therefore, it was selected for successful extraction in
117 subsequent experiments.

118

119 *Effect of sample volume*

120 Since amount of BPA is low into real samples, the effect of sample volume was also tested
121 in range of 10-250 mL with a constant amount of BPA. The results, as can be seen in Fig.
122 1(f), demonstrated that quantitative analytical signal for sample volumes was obtained in
123 range of 50-150 mL. Above 150 mL, the analytical signal decreased slightly. After UAE, the
124 surfactant-rich phase was completed to 1 mL with methanol, so the pre-concentration
125 factor was 150.

126

127 *Effects of equilibrium temperature and time*

128 Optimal equilibration temperature is necessary for the completion of the ion-pairing
129 complex formation and efficient phase separation. This hydrophobic complex, which is
130 bound to core and interface of the micelles by polar ethoxylate groups of micelles and
131 hydrophobic interactions, is extracted to the surfactant-rich phase, and this event can be
132 achieved when the equilibration temperature is above the cloud point temperature (CPT)
133 of a non-ionic surfactant. Therefore, the equilibration temperature was investigated in the
134 range of 25-70 °C. From studies, when the temperature increased in the range from 25 to
135 55 °C, the analytical signals increased correspondingly for BPA. At higher temperatures,
136 the analytical signal is decreased. This is because the resulting ion-pair complex is
137 reversibly dispersed to the solution depending on the temperature. Thus, 55 °C was chosen
138 as the optimal equilibration temperature for successful extraction in subsequent
139 experiments

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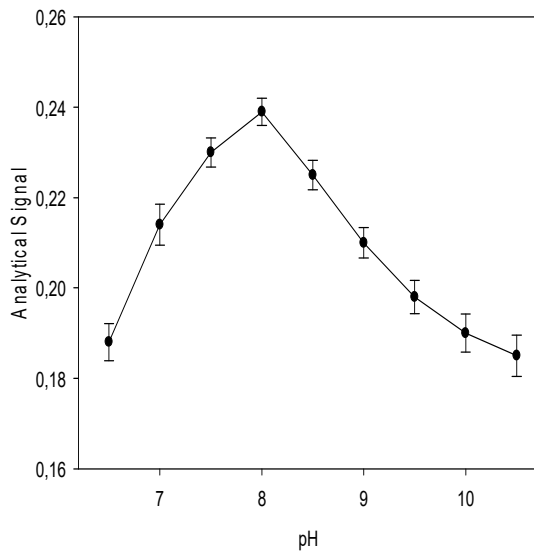
141 In the extraction process, the sonication time is one of the prime factors influencing the
142 BPA extraction and mass transfer into surfactant-rich phase. Sonication caused an increase
143 in the mass transfer, and a decrease in reaction time. To minimize the time required for
144 extraction, sonication time was investigated in range of 1-15 min and the results are shown
145 in Fig. 1(g). Based on the results, the best analytical signal was obtained at 10 min. Thus,
146 10 min was chosen as the optimal sonication time for successful extraction in subsequent
147 experiments.

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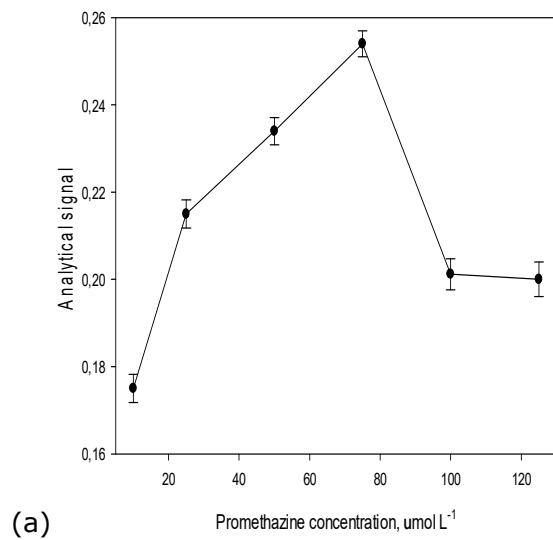
149 *Effect of solvent*

150 After centrifugation for 5 min at 4000 rpm, the obtained surfactant rich phase is low volume
151 and high viscous. Therefore, analysis with FAAS cannot be done. To overcome this problem,
152 this phase must be diluted with a suitable solvent. The results indicated that methanol was

153 a suitable diluting solvent and also a suitable matrix for indirect determination of BPA using
 154 FAAS.

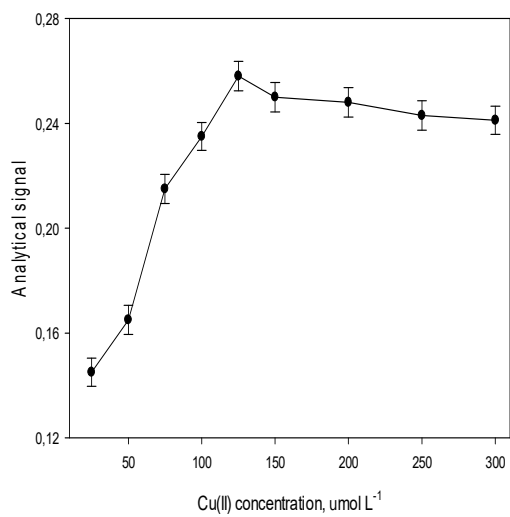


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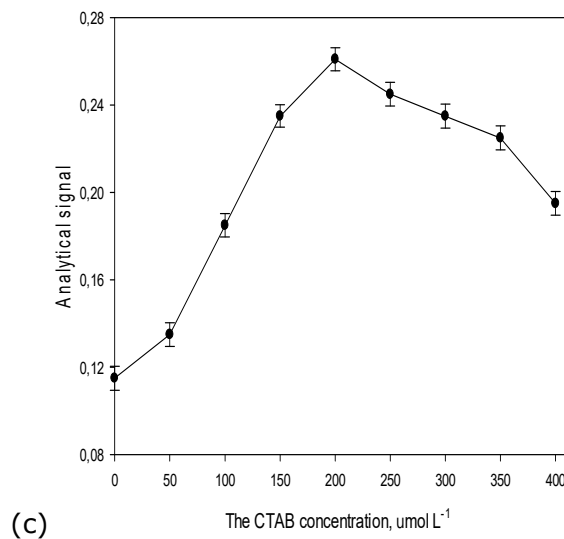


(a)

(b)

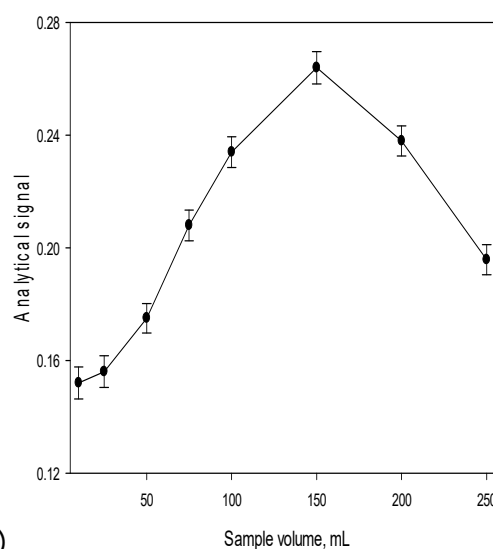
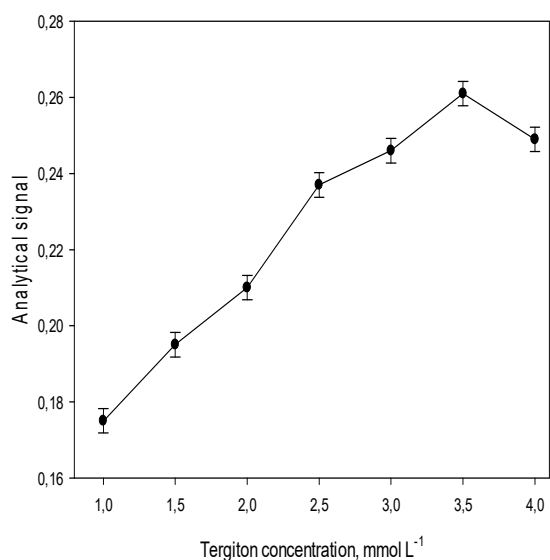


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(c)

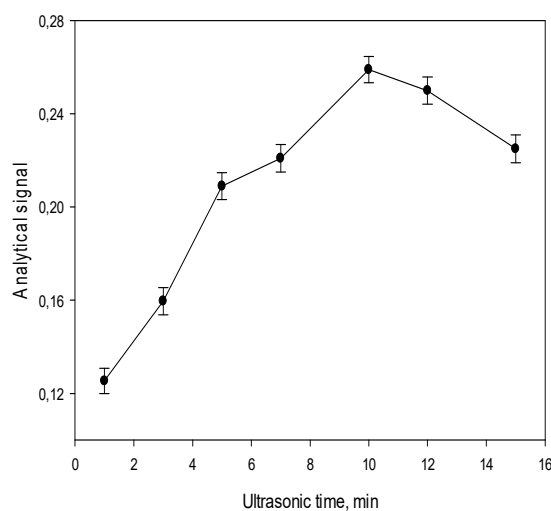
(d)



157

(e)

(f)



158

(g)

159 **Figure 1 (a-g).** Optimization of parameters affecting UAE for triplicate measurements of
 160 25 $\mu\text{g L}^{-1}$ BPA.

161 *The matrix effect*

162 The BPA exists along with different interfering species in selected samples. This event may
 163 be attributed to the extraction step, because high selectivity with careful utility of FAAS
 164 can be provided. Thus, the effects of some foreign ions were investigated by conducting
 165 UAE experiments using solutions containing 20 $\mu\text{g L}^{-1}$ of BPA in the presence of different
 166 mass ratios of foreign ions under the extraction conditions. Tolerable limit is considered as
 167 the interfering agent level that is not significantly affect the preconcentration via UAE and
 168 subsequent determination of BPA by FAAS as determinate error smaller than $\pm 5.0\%$. The
 169 results showed that at least 10000 $\mu\text{g L}^{-1}$ of Na^+ , Ca^{2+} , NH_4^+ , SO_4^{2-} , NO_3^- , Fe^{3+} , Al^{3+} , and
 170 Cl^- , 5000 $\mu\text{g L}^{-1}$ of Mg^{2+} and PO_4^{3-} , 1000 $\mu\text{g L}^{-1}$ of 2-chlorobenzaldehyde,
 171 bromobenzaldehyde, phenol, and 4-nitrophenol and 750 $\mu\text{g L}^{-1}$ of 2,4-dinitrophenol had no
 172 remarkable interferences with the determination of BPA. 2-aminophenol, acetaldehyde and

173 acetate could be tolerated up to $500 \mu\text{g L}^{-1}$ and nonylphenol and octylphenol could be
174 tolerated up to $250 \mu\text{g L}^{-1}$. As can be understood from the results, the tolerance limits of
175 the foreign ions have a good tolerance to matrix interference. Therefore, this method could
176 be applied to successfully for the extraction of BPA in selected sample matrices.

177

178 *Analytical figures of merit*

179 Under the optimal conditions, calibration graph was performed using standard addition
180 calibrations prepared following the UAE procedure. Table 1 shows the characteristic
181 performances of the proposed method. The calibration graph prepared from the aqueous
182 standards was linear in the range of $1.5\text{-}100 \mu\text{g L}^{-1}$ with a good correlation coefficient (r)
183 of 0.9943. The limit of detection (LOD), defined as the concentration that gives a signal
184 equivalent to three times the standard deviation of 10 replicate measurements of the
185 procedural blank sample (blank digest preconcentrated by the UAE procedure), was 0.47
186 $\mu\text{g L}^{-1}$. For the precision of the method, the relative standard deviations (RSD) of the five
187 independent replicate measurements for 20 and $50 \mu\text{g L}^{-1}$ of BPA are lower than 3.5%. The
188 percent recoveries obtained were in range of 95.8-103.9% for the spiked BPA
189 concentration of 20 and $50 \mu\text{g L}^{-1}$ to the selected samples. As mentioned previously, the
190 amount of BPA in 150 mL of sample volume was determined after extraction process by
191 1.0 mL of surfactant-rich phase, therefore the pre-concentration factor for this method is
192 150. Sensitivity enhancement factor, which calculated from the ratio of the slopes of the
193 calibration curves obtained with and without pre-concentration, was 135.

194

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

209

Table 1 The analytical features of the proposed method for BPA

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

210

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 $\mu\text{g L}^{-1}$ 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 $\mu\text{g L}^{-1}$ were calculated by analysing (five replicate). Then, the mean BPA concentrations were determined as 10.5, 24.3, 50.7 $\mu\text{g L}^{-1}$ 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 $\mu\text{g L}^{-1}$ 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 $\mu\text{g L}^{-1}$ and 50 $\mu\text{g L}^{-1}$ of BPA. The recovery% was calculated by using the equation: $\text{Recovery\%} = 100 (C_s - C_0) / m$ 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 ($2.70 \pm 0.07 \mu\text{g L}^{-1}$) was found in water samples and the highest amount of BPA in grape juice ($19.2 \pm 0.5 \mu\text{g L}^{-1}$). 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 $\mu\text{g L}^{-1}$ (bottled drinking water), 3.1-5.4 $\mu\text{g L}^{-1}$ (milk samples) to 4.3-19.2 $\mu\text{g L}^{-1}$ (beverages with and without alcohol) were significantly lower than specific migration limit (SML) of 600 $\mu\text{g kg}^{-1}$ 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).

Added ($\mu\text{g L}^{-1}$)	Commercial drinking water ¹			Commercial drinking water ²			Commercial drinking water ³		
	Found ($\mu\text{g L}^{-1}$)	Recovery (%)	RSD (%)	Found ($\mu\text{g L}^{-1}$)	Recovery (%)	RSD (%)	Found ($\mu\text{g L}^{-1}$)	Recovery (%)	RSD (%)
-	2.70±0.07	-	2.5	3.80±0.1	-	3.0	3.10±0.08	-	2.7
20	22.1±0.5	97.2	2.4	24.4±0.7	103.1	2.7	22.8±0.6	98.5	2.5
50	51.7±1.2	98.1	2.2	54.7±1.4	101.8	2.6	52.6±1.3	99.0	2.4

^{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).

Added ($\mu\text{g L}^{-1}$)	Whole milk			Semi-skimmed milk			Milkshake		
	Found ($\mu\text{g L}^{-1}$)	Recovery (%)	RSD (%)	Found ($\mu\text{g L}^{-1}$)	Recovery (%)	RSD (%)	Found ($\mu\text{g L}^{-1}$)	Recovery (%)	RSD (%)
-	4.8±0.1	-	2.9	3.1±0.1	-	3.3	5.4±0.2	-	3.5
20	24.1±0.6	96.3	2.7	22.2±0.7	95.3	3.1	26.2±0.8	103.8	3.2
50	53.6±1.3	97.5	2.5	51.3±1.4	96.4	2.8	56.6±1.7	102.3	3.0

1 **Table 2(c)** The analysis results of determination of BPA in spiked beverage samples
 2 using the proposed method (n: 5).

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

3

4 *Comparison with literature*

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

23

Table 3 Comparison of proposed method with those of other methods.

Sample matrix	Extraction process	Detection technique	^a LR ($\mu\text{g L}^{-1}$)	LOD ($\mu\text{g L}^{-1}$)	RSD (%)	Recovery (%)	^b EF or ^c PF	References
Waters and urine	¹ MISPE	² CE-UV	3-500	0.3	$\leq 7.2\%$	95.2-105.4%	50	(3)
Urine samples	³ CME	⁴ LC-FD	0.4-149	0.197	4.5%	88-95%	38	(8)
Soils	⁵ UAE	⁶ GC-MS	5-300	0.03 ng g ⁻¹	9.6%	88.1-107.7%	-	(18)
Waters	⁷ CPE	⁸ LC-UV	1-100	0.34	-	90-108.6%	50	(20)
Waters	⁹ SPE	¹⁰ LC-MS/MS	0.02-0.2	0.057	$\leq 13\%$	85-100%	-	(35)
Waters	¹¹ DLLME	¹² HPLC-UV	0.5-100	0.07	6.0%	93.4-98.2%	80	(36)
Serum	-	¹³ ELISA	0.3-100	0.3	13.6%	81.9-97.4%	-	(37)
Leachate	¹⁴ SPME	HPLC-UV	12.8-192	3.25	4.4%	94.5-103.3%	-	(38)
Soft drinks and powdered infant formula	¹⁵ LPME	GC-MS	1-1000	0.005	15%	82-111%, 68-114%	-	(39)
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	UA-CPE	FAAS	1.5-100, 3-125	0.47, 0.95	$\leq 3.9\%$	95.8-103.9%	135, 150	Present method

24 ^aLinear range, ^bEnhancement factor, ^cPreconcentration factor ¹Molecularly imprinted solid-phase extraction; ²Capillary electrophoresis-UV
25 method; ³Coacervative microextraction; ⁴Liquid chromatography-fluorescence detection; ⁵Ultrasonic assisted extraction; ⁶Gas
26 chromatography-mass spectrometry; ⁷Cloud-point extraction; ⁸Liquid chromatography; ⁹Solid-phase extraction; ¹⁰Liquid chromatography-
27 tandem mass spectrometry; ¹¹Dispersive liquid-liquid microextraction; ¹²High-performance liquid chromatography; ¹³Enzyme-linked
28 immunosorbent assay; ¹⁴Solid phase microextraction; ¹⁵Liquid phase microextraction; ¹⁶Stir bar sorptive extraction.

29 CONCLUSIONS

30
31 In the present study, a simple, rapid, inexpensive, and sensitive analytical method, based
32 on highly effective coupling of UAE with highly selective FAAS was proposed to determine
33 BPA in different beverage samples. This study is the first method reported for indirect
34 determination of BPA using FAAS. Especially, the use of ultrasound energy in sample
35 preparation has provided features like low organic solvent usage, and less extraction
36 duration. Other advantages can be pointed out like the low limits of detection, good
37 precision and good selectivity, wide linear working range, high analytical signal, as well as
38 low reagent consumption, high pre-concentration and sensitivity enhancement factors. The
39 quantitation or determination limit of the proposed method is about $1.6 \mu\text{g L}^{-1}$, so it can
40 be used for the routine control of BPA in different beverage samples below the current
41 specific migration limit (SML) of $600 \mu\text{g L}^{-1}$ set by the EU Commission. As a result, the
42 effectiveness and efficiency of the proposed method was successfully demonstrated for
43 BPA determination and applied to different beverage samples.

44

45 ACKNOWLEDGMENTS

46

47 The current study was financially supported by Cumhuriyet University Scientific Research
48 Projects Commission as the research projects with the F-503 code.

49

50 REFERENCES

51

52 1. Braunrath, R, Podlipna, D, Padlesak, S, Cichna-Markl M. Determination of bisphenol A in canned
53 foods by immunoaffinity chromatography, HPLC, and fluorescence detection. *Journal of agricultural
54 and food chemistry*. 2005; 53(23): 8911-8917.

55

56 2. Calafat AM, Kuklennyik Z, Reidy JA, Caudill SP, Ekong J, Needham LL. Urinary concentrations of
57 bisphenol A and 4-nonylphenol in a human reference population. *Environmental health
58 perspectives*. 2005; 391-395.

59

60 3. Mei S, Wu D, Jiang M, Lu B, Lim JM, Zhou YK, Lee YI. Determination of trace bisphenol A in
61 complex samples using selective molecularly imprinted solid-phase extraction coupled with
62 capillary electrophoresis. *Microchemical Journal*. 2011; 98(1): 150-155.

63

64 4. Lang IA, Galloway TS, Scarlett A, Henley WE, Depledge M, Wallace RB, Melzer D. Association of
65 urinary bisphenol A concentration with medical disorders and laboratory abnormalities in adults.
66 *Jama*. 2008; 300(11): 1303-1310.

67

- 68 5. Najafi M, Khalilzadeh MA, Karimi-Maleh H. A new strategy for determination of bisphenol A in the
69 presence of Sudan I using a ZnO/CNTs/ionic liquid paste electrode in food samples. Food
70 chemistry. 2014; 158: 125-131.
71
- 72 6. Christian LJ, Mortensen A. European Food Safety Authority (EFSA), 2007.
73
- 74 7. Commission Directive 2011/8/EU of 28 January 2011 amending Directive 2002/72/EC as regards
75 the restriction of use of bisphenol A in plastic infant feeding bottles. Off J Eur Union. L26/11
76
- 77 8. García-Prieto A, Lunar ML, Rubio S, Pérez-Bendito D. Determination of urinary bisphenol A by
78 coacervativemicroextraction and liquid chromatography–fluorescence detection. Analytical
79 acta. 2008; 630(1): 19-27.
80
- 81 9. Alabi A, Caballero-Casero N, Rubio S. Quick and simple sample treatment for multiresidue
82 analysis of bisphenols, bisphenol diglycidyl ethers and their derivatives in canned food prior to
83 liquid chromatography and fluorescence detection. Journal of Chromatography A. 2014; 1336: 23-
84 33.
85
- 86 10. Wilczewska K, Namieśnik J, Wasik A. Troubleshooting of the determination of bisphenol A at
87 ultra-trace levels by liquid chromatography and tandem mass spectrometry. Analytical and
88 bioanalytical chemistry. 2016; 408(3): 1009-1013.
89
- 90 11. Deceuninck Y, Bichon E, Marchand P, Boquien CY, Legrand A, Boscher C, Le Bizec B.
91 Determination of bisphenol A and related substitutes/analogues in human breast milk using gas
92 chromatography-tandem mass spectrometry. Analytical and bioanalytical chemistry. 2015; 407(9):
93 2485-2497.
94
- 95 12. Zhang X, Zhu D, Huang C, Sun Y, Lee YI. Sensitive detection of bisphenol A in complex
96 samples by in-column molecularly imprinted solid-phase extraction coupled with capillary
97 electrophoresis. Microchemical Journal. 2015; 121: 1-5.
98
- 99 13. Zhong S, Tan SN, Ge L, Wang W, Chen, J. Determination of bisphenol A and naphthols in river
100 water samples by capillary zone electrophoresis after cloud point extraction. Talanta. 2011; 85(1):
101 488-492.
102
- 103 14. De Meulenaer B, Baert K, Lanckriet H, Van Hoed V, Huyghebaert A. Development of an
104 enzyme-linked immunosorbent assay for bisphenol A using chicken immunoglobulins. Journal of
105 agricultural and food chemistry. 2002; 50(19): 5273-5282.
106
- 107 15. Li J, Kuang D, Feng Y, Zhang F, Liu M. Voltammetric determination of bisphenol A in food
108 package by a glassy carbon electrode modified with carboxylated multi-walled carbon nanotubes.
109 Microchimica Acta. 2011; 172(3-4): 379-386.
110

- 111 16. Chen X, Ren T, Ma M, Wang Z, Zhan G, Li C. Voltammetric sensing of bisphenol A based on a
112 single-walled carbon nanotubes/poly {3-butyl-1-[3-(N-pyrrolyl) propyl] imidazolium ionic liquid}
113 composite film modified electrode. *Electrochimica Acta*. 2013; 111: 49-56.
114
- 115 17. Karimi-Maleh H, Sanati AL, Gupta VK, Yoosefian M, Asif M, Bahari A. A voltammetric biosensor
116 based on ionic liquid/NiO nanoparticle modified carbon paste electrode for the determination of
117 nicotinamide adenine dinucleotide (NADH). *Sensors and Actuators B: Chemical*. 2014; 204: 647-
118 654.
119
- 120 18. Sánchez-Brunete C, Miguel E, Tadeo JL. Determination of tetrabromobisphenol-A,
121 tetrachlorobisphenol-A and bisphenol-A in soil by ultrasonic assisted extraction and gas
122 chromatography–mass spectrometry. *Journal of Chromatography A*. 2009; 1216(29):5497-5503.
123
- 124 19. O’Mahony J, Moloney M, McCormack M, Nicholls IA, Mizaikoff B, Danaher M. Design and
125 implementation of an imprinted material for the extraction of the endocrine disruptor bisphenol A
126 from milk. *Journal of Chromatography B*. 2013; 931, 164-169.
127
- 128 20. Yi-Jun YU, Guan-Yong SU, Michael HW, Paul KS, Hong-Xia YU. Cloud point extraction of
129 bisphenol A from water utilizing cationic surfactant aliquat 336. *Chinese Journal of Analytical
130 Chemistry*. 2009; 37(12): 1717-1721.
131
- 132 21. Cunha SC, Cunha C, Ferreira AR, Fernandes JO. Determination of bisphenol A and bisphenol B
133 in canned seafood combining QuEChERS extraction with dispersive liquid–liquid
134 microextraction followed by gas chromatography–mass spectrometry. *Analytical and bioanalytical
135 chemistry*. 2012; 404(8): 2453-2463.
136
- 137 22. Hu X, Wu X, Yang F, Wang Q, He C, Liu S. Novel surface dummy molecularly imprinted silica as
138 sorbent for solid-phase extraction of bisphenol A from water samples. *Talanta*. 2016; 148: 29-36.
139
- 140 23. Chemat F, Rombaut N, Sicaire AG, Meullemiestre A, Fabiano-Tixier AS, Abert-Vian M.
141 Ultrasound assisted extraction of food and natural products. Mechanisms, techniques,
142 combinations, protocols and applications. A review. *Ultrasonics Sonochemistry*. 2017; 34:540-560.
143
- 144 24. Altunay N, Gürkan, R. An Inexpensive and Sensitive Method for Speciative Determination of
145 Sn (IV), Sn (II), and Total Sn as Sn (IV) in Selected Beverages by Micellar Improved
146 Spectrophotometry. *Food Analytical Methods*. 2015; 8(4): 994-1004.
147
- 148 25. Li JL, Chen BH. Equilibrium partition of polycyclic aromatic hydrocarbons in a cloud-point
149 extraction process. *Journal of colloid and interface science*. 2003; 263(2):625-632.
150
- 151 26. Bai D, Li J, Chen SB, Chen BH. A novel cloud-point extraction process for preconcentrating
152 selected polycyclic aromatic hydrocarbons in aqueous solution. *Environmental science &
153 technology*. 2001; 35(19): 3936-3940.

- 154
155 27. Deng P, Xu Z, Kuang Y. Electrochemical determination of bisphenol A in plastic bottled drinking
156 water and canned beverages using a molecularly imprinted chitosan-graphene composite film
157 modified electrode. *Food chemistry*. 2014; 157: 490-497.
158
- 159 28. Liu X, Ji Y, Zhang H, Liu M. Elimination of matrix effects in the determination of bisphenol A in
160 milk by solid-phase microextraction-high-performance liquid chromatography. *Food Additives and*
161 *Contaminants*. 2008; 25(6); 772-778.
162
- 163 29. Katzung, B. G., Masters, S. B., & Trevor, A. J. (Eds.). (2004). *Basic & clinical*
164 *pharmacology* (Vol. 8). New York, NY, USA:: Lange Medical Books/McGraw-Hill.
165
- 166 30. BagheriGhomi A, Mazinani F. Spectrophotometric Study of Stability Constants of Metal
167 Complexes of Promethazine at Different Temperatures. *Physical Chemistry & Electrochemistry*.
168 2013; 2(1): 13-19.
169
- 170 31. Kabir-ud-Din, Rub, MA, Alam MS. Micellization and Clouding Phenomenon of Phenothiazine
171 Drug Promethazine Hydrochloride: Effect of NaCl and Urea Addition. *Journal of Dispersion Science*
172 *and Technology*. 2010; 31(9): 1182-1187.
173
- 174 32. Ballesteros J C, Chainet E., Ozil, P., Meas, Y., & Trejo, G. (2011). Electrodeposition of Copper
175 from Non-Cyanide Alkaline Solution Containing Tartrate. *Int. J. Electrochem. Sci*, 6, 2632-2651.
176
- 177 33. Huang W, Zhou D, Liu X, Zheng X. Electrochemical determination of phenol using CTAB-
178 functionalized montmorillonite electrode. *Environmental technology*. 2009; 30(7):701-706.
179
- 180 34. Kim BK, Kim JY, Kim DH, Choi HN, Lee WY. Electrochemical determination of bisphenol A at
181 carbon nanotube-doped titania-nafion composite modified electrode. *Bulletin of the Korean*
182 *Chemical Society*. 2013; 34(4): 1065-1069.
183
- 184 35. Gallart-Ayala H, Moyano E, Galceran MT. On-line solid phase extraction fast liquid
185 chromatography-tandem mass spectrometry for the analysis of bisphenol A and its chlorinated
186 derivatives in water samples. *Journal of Chromatography A*. 2010; 1217(21):3511-3518.
187
- 188 36. Rezaee M, Yamini Y, Shariati S, Esrafil A, Shamsipur M. Dispersive liquid-liquid
189 microextraction combined with high-performance liquid chromatography-UV detection as a very
190 simple, rapid and sensitive method for the determination of bisphenol A in water samples. *Journal*
191 *of Chromatography A*. 2009; 1216(9): 1511-1514.
192
- 193 37. Ohkuma H, Abe K, Ito M, Kokado A, Kambegawa A, Maeda M. Development of a highly
194 sensitive enzyme-linked immunosorbent assay for bisphenol A in serum. *Analyst*. 2002; 127(1):
195 93-97.

- 196
197 38. Deng L, Liu Y X, Chen PY, Wang L, Deng NS. Determination of trace bisphenol A in leachate by
198 solid phase microextraction coupled with high performance liquid chromatography. Analytical
199 letters. 2006; 39(2): 395-404.
200
201 39. Cunha SC, Almeida C, Mendes E, Fernandes JO. Simultaneous determination of bisphenol A
202 and bisphenol B in beverages and powdered infant formula by dispersive liquid-liquid micro-
203 extraction and heart-cutting multidimensional gas chromatography-mass spectrometry. Food
204 Additives and Contaminants. 2011; 28(4): 513-526.
205
206 40. Kawaguchi M, Inoue K, Yoshimura M, Ito R, Sakui N, Okanouchi N, Nakazawa H. Determination
207 of bisphenol A in river water and body fluid samples by stir bar sorptive extraction with in situ
208 derivatization and thermal desorption-gas chromatography-mass spectrometry. Journal of
209 Chromatography B. 2004; 805(1): 41-48.
210