

One-Step Derivatization and Preconcentration Microextraction Technique for Determination of Bisphenol A in Beverage Samples by Gas Chromatography–Mass Spectrometry

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ABSTRACT: A simple technique based on ultrasound-assisted emulsification microextraction in situ derivatization (USAEME-*ISD*) is proposed for the one-step derivatization, extraction, and preconcentration of bisphenol A (BPA) in beverage samples prior to gas chromatography–mass spectrometry (GC-MS) analysis. BPA was in situ derivatized with acetic anhydride and simultaneously extracted and preconcentrated by using USAEME. Variables affecting the extraction efficiency of BPA were evaluated. Under optimal experimental conditions, the detection limit (LOD) was 38 ng L⁻¹ with a relative standard deviation (RSD) value of 11.6%. The linear working range was 100–1250 ng L⁻¹, and the coefficient of estimation (*r*²) of the calibration curve was ≥0.9971. The robustness of the proposed methodology was probed by developing a recovery study at two concentrations (125 and 500 ng L⁻¹) over different beverage samples. This study led to a satisfactory result achieving recoveries of ≥82%, which showed acceptable robustness for determination of nanograms per liter of BPA in samples of food safety interest.

KEYWORDS: bisphenol A, ultrasound-assisted emulsification microextraction, in situ derivatization, food analysis beverages samples, gas chromatography–mass spectrometry

INTRODUCTION

BPA is a chemical used in the synthesis of polycarbonate plastics and epoxy resins of food containers.^{1,2} The reaction synthesis is incomplete; therefore, the remaining BPA nonassociated with the polymer may leach from the polymeric product into the surrounding environment and, thus, contaminate it.^{1,3} More than 8 billion pounds of BPA are produced each year, making it one of the world's most heavily used chemicals. BPA is used in baby bottles, drinking bottles, food storage containers, polyvinyl chloride, stretch films, paper, cardboard, medical equipment, and the epoxy resins lining most metallic food and beverage cans. BPA is considered to be an endocrine-disrupting chemical, and animal studies have linked low-level exposure to altered development of the male and female reproductive tract and brain as well as cancers of the mammary gland and prostate.^{4–11} These findings have raised public concern over their human health effects and make their routine monitoring in food samples still necessary today.¹² Many analytical techniques for the determination of BPA in food matrices have been reported. They are based on chromatographic methods, including gas chromatography (GC) and liquid chromatography (LC).^{13–16} GC is preferred because many plastic-additive chemicals, such as phthalates, with the potential to interfere in the detection of BPA may leach from the plastics to the water.¹⁷ A GC-MS method is highly selective, preventing such interference. However, GC-MS analysis reveals that low-volatile polar compounds, such as phenolic compounds, exhibit low sensitivity and peak tailing. Therefore, the determination of BPA by GC-MS requires a derivatization step, which generally is tedious and time-consuming and,

besides, can lead to analyte losses. To avoid these disadvantages, in situ derivatization was proposed, which is based on the addition of a reagent into the sample prior to extraction and determination. One of the alternative reactions to derivatize the active hydroxyl hydrogen is the acetylation by using acetic anhydride. By means of it, phenols are turned into their corresponding less polar acetate derivatives.^{18,19} The main advantages of this technique are direct application in aqueous medium and fast kinetics. In this way, the derivatization is achieved quickly, making it possible to include it within the extraction process of the analytes.

Sample preparation is an important stage in the analytical process when trace analyte determination is needed.²⁰ Moreover, the concern for analysis at low concentrations in complex matrices requires the elimination of interferences and the reduction of final extract volumes to attain higher preconcentration of target analytes. In recent years, microextraction techniques have gained interest in the analytical chemistry field, representing an important development in the sample preparation area. Microextraction techniques resulted in a more efficient analyte enrichment, faster sample preparation, and lower solvent consumption. Furthermore, they are simple, inexpensive, environmentally friendly, and compatible with many analytical instruments.^{21–23} The miniaturization of liquid–liquid (L-L) extraction has

Received: December 1, 2010

Revised: March 2, 2011

Accepted: March 7, 2011

Published: March 07, 2011

received major attention, and newer L-L microextraction procedures have been reported. Solvent microextraction was first introduced by Jeannot and Cantwell,²⁴ and to date several modifications of the original technique have been introduced.^{21,22,25–27}

Recently, a novel type of liquid–liquid microextraction, called ultrasound-assisted emulsification microextraction (USAEME), has been developed.²⁸ It is based on the application of ultrasonic (US) radiation as an efficient tool to facilitate the emulsification phenomenon and accelerate the mass-transfer process between two immiscible phases.²⁹ The application of US produces smaller droplets of organic solvent in the aqueous bulk than vigorous stirring.²³ This significantly enlarges the contact surface of the organic solvent with the aqueous bulk, favoring the mass-transfer process of analytes into the organic phase.²⁹ Combining the benefit of microextraction and ultrasound radiation has made it possible to establish an efficient preconcentration technique for determining analytes at trace concentration levels. In this way, USAEME has been reported for the extraction of synthetic musk fragrances, phthalate esters, lindane, polybrominated diphenyl ethers, trichloroanisole, pesticides, and other analytes in liquid samples prior to their determination by several detection techniques.^{28,30–32}

The aim of this work was to develop an ultrasound-assisted emulsification microextraction in situ derivatization (USAEME-ISD) for the one-step derivatization, extraction, and preconcentration of BPA in beverage samples prior to its determination by GC-MS. To our knowledge this paper describes the first application of USAEME for the determination of BPA in food samples. Several factors including extraction solvent type and its volume, derivatization reagent volume, extraction temperature and time, and effect of matrix modifiers such as buffers and ionic strength were studied and optimized over the relative response of the proposed technique. The method performance was evaluated in terms of limits of detection (LODs), repeatability, and linear working range. Moreover, the USAEME-ISD-GC-MS methodology was applied for the analysis of different beverage samples, and the robustness of the methodology was evaluated in terms of recovery factors (RF%).

MATERIALS AND METHODS

Reagents. The standard of BPA (99%, solid crystal form) was purchased from Sigma-Aldrich (Steinheim, Germany). Stock solution was prepared in methanol at a concentration of 250 mg L⁻¹ and stored in brown bottles at -20 °C. Working standard solutions were prepared weekly in methanol and stored at 4 °C.

Methanol and trichloroethene were purchased from Merck (Darmstadt, Germany), and carbon tetrachloride was purchased from Sigma-Aldrich. Chloroform was purchased from Mallinckrodt Baker Inc. (Phillipsburg, NJ). Sodium chloride, hydrochloric acid, ammonia solution (28–30%), sodium tetraborate, and sodium hydroxide were all purchased from Merck. Sodium carbonate and potassium carbonate were purchased from Sigma-Aldrich, and sodium phosphate dibasic and 2-amino-2-hydroxymethylpropane-1,3-diol (Tris buffer) were purchased from Mallinckrodt Baker. Sodium hydroxide, sodium tetraborate, sodium carbonate, potassium carbonate, ammonia, sodium phosphate dibasic, and tris were used to prepare the buffer solutions. The final concentration of each buffer was 1 mol L⁻¹. Ultrapure water (18 MΩ cm) was obtained from a Milli-Q water purification system (Millipore, Paris, France). All reagents were of analytical grade or above.

Beverage Samples. Three different liquid food samples were purchased in different supermarkets of Mendoza city, Mendoza, Argentina. The samples corresponded to beverages stored in cans internally

protected with epoxy lacquers and were a cola, an energy drink, and tonic water. Samples were stored at room temperature before analysis and analyzed just after opening. Before extraction, carbonated drink samples were degassed in an ultrasonic bath for 30 min. All samples were analyzed in triplicate with the proposed USAEME-ISD-GC-MS methodology.

GC-MS Analysis. GC-MS analyses were performed on a Varian 3900 gas chromatograph equipped with Varian Saturn 2000 ion trap mass detector (Varian, Walnut Creek, CA). The system was operated by Saturn GC-MS WorkStation v6.4.1 software. The GC column used was a VF-5 ms (25 m × 0.25 mm, 0.25 μm film thickness; Varian, Lake Forest, CA). The oven temperature program was as follows: 100 °C; ramping at 10 °C min⁻¹ to 270 °C; ramping at 30 °C min⁻¹ to a final temperature of 300 °C; and held for 2 min. Helium (purity = 99.999%) was used as a carrier gas at 1.0 mL min⁻¹ flow rate. The injector temperature was set at 280 °C, and the injections were performed in the splitless mode. The mass spectrometer was operated in electron impact ionization mode at -70 eV. The trap, manifold, and transfer line temperatures were set at 220, 120, and 280 °C, respectively. Samples were analyzed in selected ion storage (SIS) mode. Peak identification was based on the retention time, base peak, and isotopic pattern of the BPA-diacetate. Specific ions were selected from BPA-diacetate mass spectra (Figure 3), and the resulting base ion was the quantitative ion (underlined *m/z*). Quantification of BPA was carried out by using *m/z* 213. Confirmation ions were 228, 255, and 119 with ion ratios for each confirmation ion of 0.12, 0.10, and 0.55, respectively.

A 40 kHz and 600W US-bath with temperature control (Test Lab, Buenos Aires, Argentina) was used for assisting the emulsification process of the microextraction technique. The volume of the extraction phase was measured using a 250 μL Hamilton syringe (Reno, NV). Injections into the GC-MS were made by using a 5 μL Hamilton syringe.

USAEME-ISD Procedure. Five milliliters of beverage sample was placed into a 15 mL glass centrifuge tube. Five hundred microliters of 1 mol L⁻¹ ammonia buffer and 30 μL of acetic anhydride were subsequently added. Then, 100 μL of chloroform as extraction solvent was added and mixed by hand-shaking. The resulting emulsion was sonicated for 5 min at 55 °C. During the sonication process, a cloudy state was observed due to the dispersion of fine chloroform droplets into the sample. The emulsion was disrupted by centrifuging during 5 min at 3500 rpm (1852.2g). After that, the extraction solvent remained at the bottom of the conical tube with a volume of 30 ± 1.5 μL. Five microliters of the resulting organic phase was removed from the bottom of the centrifuge tube, and 1 μL was injected into the GC-MS.

RESULTS AND DISCUSSION

The efficiency of the USAEME-ISD technique could be affected by several parameters, including type and volume of extraction solvent, volume of acetic anhydride, and pH and type of buffer, as well as extraction temperature and time. The study and optimization of the mentioned variables were performed by modifying one at a time while keeping the remaining constant. A 5 mL sample containing 1.25 μg L⁻¹ of BPA was used to perform the assays, which were done in triplicate. The relative response was the parameter used to evaluate the influence of those variables on the performance of USAEME-ISD for the derivatization, extraction, and preconcentration of BPA. Considering a specific variable studied within a working range and the analytical signal (chromatographic peak area) resulting from those assays, the relative response was calculated as the ratio between the analytical signal resulting for any assayed value of the studied variable and the maximum analytical signal achieved within the working range.

Extraction Solvent Type and Volume. Extraction solvent is crucial for developing an efficient USAEME-ISD technique because its physicochemical properties govern the emulsification phenomenon and affect the extraction efficiency of the technique. It has to be water immiscible to efficiently form an emulsion in sample bulk and easily separate after centrifugation. USAEME can be performed with solvents with densities higher and lower than that of water. Furthermore, it is desired that the resulting organic phase remains at the bottom of the centrifuge tube after phase separation to facilitate taking it for analysis. In addition, the analytes' affinity for it has to be high, and it has to be compatible with the analytical instrumentation to be used. With these considerations in mind, three organic solvents with different polarities and water solubilities, including carbon tetrachloride, chloroform, and trichloroethene, were examined. To perform the assay, 100 μL of each solvent was added to 5 mL of beverage sample and extracted as described above. All of these solvents were able to form an emulsion during sonication, leading to a biphasic system after centrifugation. The results revealed that the relative recovery for chloroform was the higher one. The achieved results could be affected by the emulsification efficiency of the solvents in the aqueous bulk due to the vapor pressure and surface tension of the studied solvents. The vapor pressures for the studied solvents are carbon tetrachloride, 12.2; chloroform, 21.2; and trichloroethene, 7.7 kPa. US-assisted emulsification is based on the cavitation effect. It is based on the implosion bubbles generated by the cavitation phenomenon, which produces intensive shock waves in the surrounding liquid and high-velocity liquid jets. Such microjets can cause droplet disruption in the vicinity of collapsing bubbles and thus improve emulsification by generating smaller droplet size of the dispersed phase right after disruption.²⁹ The cavitation induction is diminished in a low vapor pressure solvent because fewer vapors would be able to enter into the bubbles. On the other hand, higher cavitation at lower acoustic energy produces vapor-filled bubbles, which are better supported in a more volatile solvent.^{33,34} When these bubbles are broken apart, smaller droplets are formed, favoring emulsion formation and increasing the extraction efficiency of the technique. Additionally, cavitation requires the formation of a liquid–gas interface. Thus, it could be expected that the use of a solvent of low surface energy per unit area would lower the cavitation threshold and increase the emulsification.³⁴ This is in agreement with lowering the order of surface tension for the studied solvents (chloroform < trichloroethene < carbon tetrachloride). Therefore, chloroform was selected as the extraction solvent for further studies.

The volume of extraction solvent was studied within a range of 70–150 μL . The aim of this study was to determine the minimum volume of extraction solvent necessary to achieve the highest extraction efficiency and enrichment factor (EF) for the proposed microextraction technique. By using solvent aliquots of <80 μL , the biphasic system was not achieved after centrifugation, and a viscous phase containing some matrix components was observed. When the chloroform volume was increased, an optimum phase separation was observed. As can be seen from Figure 1, the highest relative response was achieved when 100 μL of chloroform was used. When lower volumes (80–90 μL) were used, lower relative responses were observed because the volume of chloroform was insufficient to quantitatively extract the BPA. Aliquots of >100 μL led to a decrease in the relative response of the analytes due to an increment in the extraction phase volume and the consequent dilution effect of the

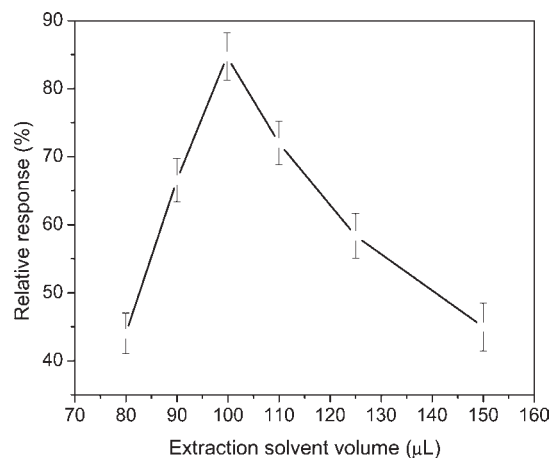


Figure 1. Effect of extraction solvent volume on the relative response of BPA. Extraction conditions: sample volume, 5 mL; 200 μL of NaOH 0.1 mol L^{-1} ; acetic anhydride, 25 μL ; extraction solvent, chloroform; extraction time, 5 min; extraction temperature, 25 $^{\circ}\text{C}$; centrifugation time, 3 min; BPA concentration, 1.25 ng mL^{-1} .

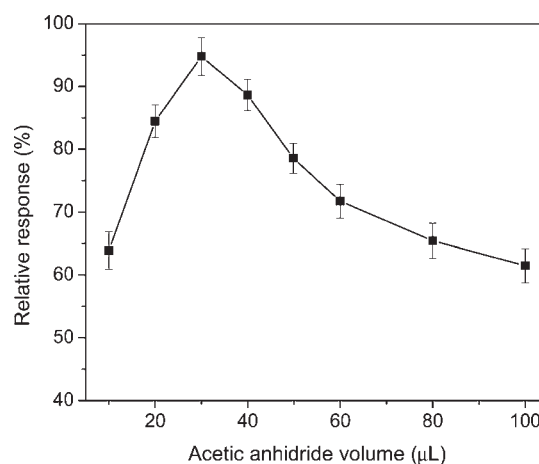


Figure 2. Effect of acetic anhydride volume on the relative response of BPA. Extraction conditions were as described in Figure 1.

analyte in this phase. According to the mentioned results, 100 μL of chloroform was selected to develop further studies.

In Situ Derivatization Reaction. Polar compounds such as BPA exhibit low sensitivity and peak tailing when analyzed by GC-MS techniques. Therefore, to overcome this problem, the determination of BPA by GC-MS requires a derivatization step. It leads to sharper peaks and, consequently, to higher sensitivity for the determination of BPA. In situ derivatization has been proposed to simultaneously perform derivatization and sample preparation steps, improving relative recovery and sensitivity of analysis. Furthermore, these approaches avoid the application of additional steps to derivatize the analytes, increasing the reproducibility and sample throughput of the methodology. Acetic anhydride has been previously reported for BPA in situ derivatization in aqueous media to form BPA-diacetates.^{2,35,36} The volume of acetic anhydride necessary to achieve a quantitative reaction was studied within a volume range of 10–100 μL , and the results are shown in Figure 2. As can be seen, the best relative response was obtained for 30 μL of acetic anhydride; lower

volumes led to poor response due nonquantitative BPA derivatization. Larger volumes showed lower responses, which could be due to an increment in solution acidity caused by the acetic anhydride hydrolysis.³⁶ Thus, 30 μL of acetic anhydride was selected as the optimum for further studies.

The effect of the sample pH was investigated within the pH range of 2–14 by adjusting it through the addition of hydrochloric acid or sodium hydroxide solutions. The best results were obtained at pH 10, which was in agreement with previous reports for BPA in situ derivatization reaction.^{35,36} This could be due to an alkaline pH preserving the BPA in neutral form, favoring its reaction with acetic anhydride. Therefore, the buffer effect was studied by adding 500 μL of different types of buffers of pH 10 including sodium hydroxide, sodium tetraborate, sodium carbonate, potassium carbonate, ammonia, sodium phosphate dibasic, and tris. Sodium phosphate dibasic, sodium tetraborate, and tris reported the smallest relative responses ($\leq 54\%$) for the derivatized analyte. The remaining buffers showed relative responses of $>69\%$, and ammonia buffer reported the highest value (93%). Thus, the samples were adjusted at pH 10 prior to extraction by adding 500 μL of 1.0 mol L^{-1} ammonia buffer solution.

Extraction Temperature and Time. Extraction temperature is also an important parameter that affects the extraction efficiency of the USAEME-ISD, because it affects several variables including the kinetics of the in situ derivatization reaction and the mass-transfer process. Additionally, it concerns the analyte and organic solvent solubility in aqueous sample as well as the emulsification phenomenon due to a variation in the viscosity and superficial strength of the fluids. On the other hand, by raising the temperature, the solvent vapor pressure is increased and a larger number of bubbles is generated, affecting, thus, the cavitation phenomenon resulting from US radiation.^{33,34} Taking into account these considerations, extraction temperature was found to be of interest in the range of 15–60 °C. Higher temperatures were omitted because at >60 °C chloroform was completely dissolved in aqueous bulk and emulsion conditions were not achieved. The results revealed that by increasing the extraction temperature, the relative response of the derivatized analyte increased, reaching the maximum value at 50 °C, after which it remained invariant. In view of the results, an extraction temperature of 55 °C was selected as working condition.

Extraction time is another important variable that affects the emulsification and mass-transfer phenomena. In addition, derivatization reactions are greatly affected by US waves and, thus, the US exposure time is significant. US is able to reveal subtle interactions and particular effects of entropic and enthalpy origin. Pressure waves associated with the propagation of the acoustic waves or essentially the shock waves generated during the cavitation bubble's collapse can affect reactions in the medium.^{37,38} To determine the influence of the extraction time, it was varied within the range of 1–15 min. The results showed that by increasing the extraction time, the relative response increased, reaching the maximum value at 5 min, after which it remained invariant. Consequently, a 5 min extraction time was selected as optimum for both extraction and in situ derivatization of BPA.

Ionic Strength. The ionic strength affects the activity coefficients of the analytes in the aqueous phase and, in this way, can modify the extraction efficiency of BPA. On the other hand, as the ionic strength of the medium increases, the viscosity and density increase, diminishing, thus, the efficiency of the mass-transfer process and, consequently, the extraction efficiency of

the technique.^{23,28} In addition, the ultrasound waves could be absorbed and dispersed as calorific energy in the viscous medium resulting from salt addition to the medium. This fact withdraws the cavitation process reducing the emulsification phenomenon.³³ In view of these considerations, the salting-out study was carried out by adding different volumes of sodium chloride (6.15 mol L^{-1}) to the extraction system within the range of 0–1.4 mL. The results showed that as the NaCl volume was increased, lower relative responses were observed. Additionally, an increment in the resulting extraction phase volume was observed. Furthermore, it was difficult to achieve a homogeneous emulsion, and the sample solution was observed scarcely emulsified. The observed results are in agreement with previously reported USAEME applications.^{28,29} Therefore, further experiments were performed without addition of NaCl to the samples.

USAEME-ISD Analytical Performance, Comparison with the Other Microextraction Techniques, and Application to Beverage Samples. EF was calculated as the ratio between the initial beverage sample volume and the resulting chloroform volume after the USAEME technique, considering the obtained efficiencies with respect to a standard of BPA. In this way, the final EF was 53. The calibration curves were made under optimized conditions with a tonic water beverage spiked at different concentrations of BPA, and the slope of this calibration curve was compared with the slope of the other samples (cola and energy drink beverages). The sensitivity was the same for the different matrix-matched calibration curves, and all of them showed decreased sensitivity with respect to the pure solvent calibration curve. In this sense, matrix-matched calibration was selected as the recommended method for quantification purposes. The LOD of BPA was calculated as 3 times the S/N of a sample spiked at 125 ng L^{-1} . The resulting LOD for BPA was 38 ng L^{-1} . The precision of USAEME-ISD-GC-MS was evaluated over five replicates spiked at 125 ng L^{-1} , with a resulting RSD of 11.6%. The calibration curve linearity was investigated within the concentration range of 100–1250 ng L^{-1} , and it showed a satisfactory linearity with a coefficient of estimation (r^2) of 0.9971.

The analytical performance of USAEME-ISD-GC-MS for BPA determination in beverage samples is comparable with that of other liquid–liquid microextraction techniques previously reported for water samples such as DLLME-GC-MS³⁶ and DLLME-HPLC-UV.³⁹ The LODs of SPME-GC-MS,¹³ LPME-GC-MS,³⁵ and SBSE-GC-MS¹⁵ were lower than that of USAEME-ISD-GC-MS, but all of their extraction times were >50 min. Therefore, the extraction equilibrium is achieved within a few minutes in the proposed microextraction technique. In this sense, USAEME-ISD-GC-MS increases the sample throughput of the analytical methodology in comparison with other nonequilibrium extraction techniques.

To validate the analytical methodology, a recovery study of BPA at two different concentrations (125 and 500 ng L^{-1}) was carried out over the real beverage samples. The analyzed samples included a cola, tonic water, and an energy drink stored in cans protected with epoxy lacquers. The samples were collected, degassed, and immediately analyzed as described above. Sample and relative recovery results were carried out in triplicate. This study led to a satisfactory robustness, achieving recoveries of $\geq 82\%$ (Table 1). As was mentioned previously, several differences were observed between the matrices of each sample and, thus, quantification could be performed by matrix-matched calibration curve. The BPA concentration in the analyzed samples was below the detection limit of the proposed methodology.

Figure 3 shows the chromatograms of a cola sample analyzed with the proposed USAEME-GC-MS/MS and BPA-diacetate mass spectra.

The proposed analytical approach based on USAEME-ISD is simple and rapid for the one-step derivatization, extraction, and preconcentration of BPA from beverage samples of interest prior

Table 1. Relative Recovery Study of BPA in Different Beverage Samples^a

sample	base	125 ng L ⁻¹ spiked		500 ng L ⁻¹ spiked	
		found ^b (ng L ⁻¹)	relative recovery ^c (%)	found ^b (ng L ⁻¹)	relative recovery ^c (%)
cola	nd ^d	103 ± 11	82	446 ± 51	89
tonic water	nd	116 ± 13	93	476 ± 55	95
energy drink	nd	107 ± 12	86	455 ± 53	91

^a Extraction conditions were as described under USAEME-ISD Procedure. ^b Results expressed as average concentration ± standard deviation; *n* = 3; ng L⁻¹. ^c [(Found - base)/added] × 100. ^d nd, not detectable

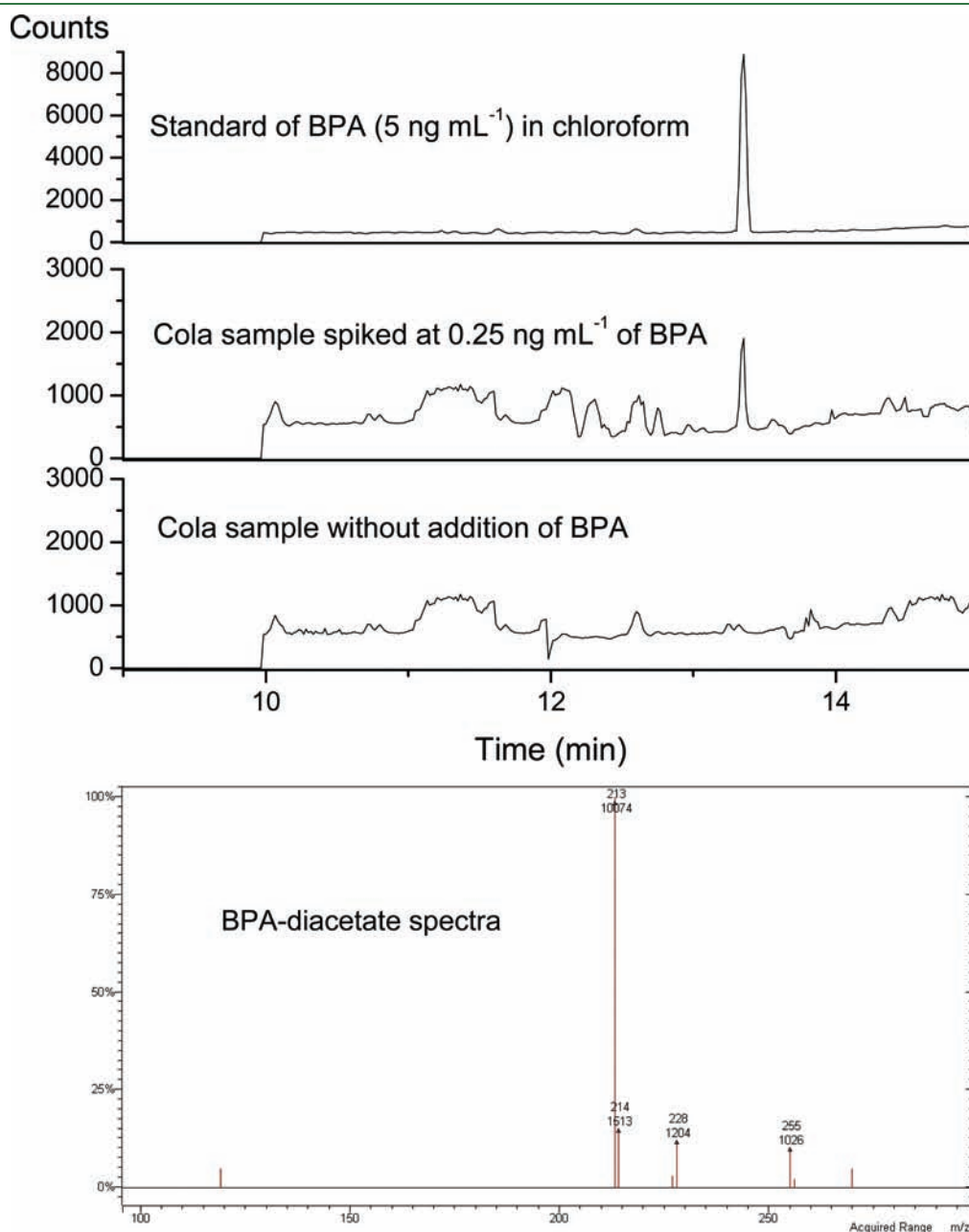


Figure 3. Analysis of cola sample with the proposed USAEME-ISD-GC-MS methodology and BPA-diacetate mass spectra. EIC for *m/z* 213.

to determination by GC-MS. In situ derivatization was successfully used in the USAEME extraction/preconcentration step, reducing the overall extraction time. This fact contributed to increase the reproducibility and sample throughput of the analytical methodology. In comparison to the time-consuming and cumbersome SPE technique,¹² the proposed approach had definite advantages in terms of lower input cost, analysis time, solvent consumption, and LODs. In addition, simple and inexpensive equipment is required. Finally, the analytical performance of USAEME-ISD-GC-MS verifies its suitability for the determination of BPA at low nanograms per liter level with an acceptable precision in food samples of interest.

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Funding Sources

This work was supported by Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Agencia Nacional de Promoción Científica y Tecnológica (FONCYT) (PICT-BID), Universidad Nacional de Cuyo, and Universidad Nacional del Litoral.

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