

# Application of ultrasonic-assisted cloud point extraction/ flame atomic absorption spectrometry (UA-CPE/FAAS) for preconcentration and determination of low levels of antimony in some beverage samples

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**Abstract** Due to be able to migrate or leach from food packaging materials into the foods and/or beverages, development of a new, sensitive and selective analytical methods for low levels of antimony as a food contaminant is of great importance in terms of food safety. In this context, an ultrasonic-assisted cloud point extraction method was developed for the preconcentration and determination of antimony as Sb(III) using 4-(2-thiazolylazo)resorcinol (TAR) and 2-(2-thiazolylazo)-p-cresol (TAC) as chelating agents and sodium dodecyl sulfate as signal enhancing agent at pH 6.0 and mediated by nonionic surfactant, *t*-octylphenoxy-polyethoxyethanol by flame atomic absorption spectrometry. Using the optimized conditions, the calibration curves obtained from Sb(III) with TAR and TAC were linear in the concentration ranges of 0.5–180 and 1–180  $\mu\text{g L}^{-1}$  with detection limits of 0.13 and 0.28  $\mu\text{g L}^{-1}$ , respectively. The precision (as relative standard deviations, RSDs) was lower than 3.9 % (25 and 100  $\mu\text{g L}^{-1}$ ,  $n$ : 6). The method accuracy was validated by the analysis of two standard reference materials. The results obtained were statistically in a good agreement with the certified values at 95 % confidence limit. The method has successfully been applied to the determination of Sb(III) and total Sb in selected beverages, milk and fruit-flavored milk products before and after pre-reduction of Sb(V) to Sb(III) with a mixture of KI/ascorbic acid in acidic media. The Sb(V) contents of samples were quantitatively calculated from analytical difference between total Sb and Sb(III) levels.

**Keywords** Antimony · Chelating agents · SDS/Tween 80 · UA-CPE · FAAS · Beverages/milk products

## Abbreviations

UA-CPE	Ultrasonic-assisted cloud point extraction
TAR	4-(2-Thiazolylazo)resorcinol
TAC	2-(2-Thiazolylazo)-p-cresol
SDS	Sodium dodecyl sulfate
Tween 80	<i>t</i> -Octylphenoxy-polyethoxyethanol
FAAS	Flame atomic absorption spectrometry
SRMs	Standard reference materials
RSDs	Relative standard deviations
SML	Specific migration limit
PET	Polyethylene terephthalate
HG-ICP-AES	Hydride generation inductively coupled plasma atomic emission spectrometry
MIP-AES	Microwave-induced plasma atomic emission spectrometry
ETV-ICP-AES	Electrothermal vaporization inductively coupled plasma atomic emission spectrometry
ICP-MS	Inductively coupled plasma mass spectrometry
GF-AAS	Graphite furnace atomic absorption spectrometry
XRF	X-ray fluorescence spectrometry
HG-ICP-OES	Hydride generation inductively coupled plasma optical emission spectroscopy
ET-AAS	Electrothermal atomic absorption spectrometry
LPME	Liquid phase microextraction
FI-SPE	Flow injection solid phase extraction
LPS-ME	Liquid phase semi-microextraction
SDME	Single-drop microextraction
HS-SDME	Head space single-drop microextraction

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VASEME-SFO	Vortex-assisted surfactant-enhanced emulsification microextraction based on solidification of floating organic drop
DLLME	Dispersive liquid–liquid microextraction
MME	Micelle-mediated extraction
CPE	Cloud point extraction
CPT	Cloud point temperature
CMC	Critical micelle concentration
LODs	Limits of detection
LC	Liquid chromatography

## Introduction

Antimony is a metalloid, which has received relatively little environmental attention. This is due to the fact that antimony is recognized as a non-essential element for life and also because its content in most environmental matrices is very low, implying the use of very sensitive analytical techniques for its determination. Owing to its use especially as a catalyst and flame retardant in the fabrication of polyethylene terephthalate (PET), antimony is invariably found in plastic containers made of this material used for water and fruit juices, and consequently fruit juices have been found to contain antimony levels greater than the EU limit,  $5 \mu\text{g L}^{-1}$  Sb [1–3]. In this context, the European Commission [4] set a specific migration limit (SML) of  $40 \mu\text{g kg}^{-1}$  for migration of Sb from food contact materials into beverages and foods. Long-term exposure to elevated Sb can lead to increased blood cholesterol and decreased blood sugar [5]. For these reasons, the monitoring of antimony species in foods and beverages is of great importance in terms of food safety, and the methods used for their determination and even speciation analysis should be fast, simple, low cost, sensitive, selective, accurate and reproducible.

Many analytical techniques such as hydride generation inductively coupled plasma atomic emission spectrometry (HG-ICP-AES) [6], microwave-induced plasma atomic emission spectrometry (MIP-AES) [7], flame atomic absorption spectrometry (FAAS) [8], electrothermal vaporization inductively coupled plasma atomic emission spectrometry (ETV-ICP-AES) [9], inductively coupled plasma mass spectrometry (ICP-MS) [10], graphite furnace atomic absorption spectrometry (GF-AAS) [11], X-ray fluorescence spectrometry (XRF) [12], hydride generation inductively coupled plasma optical emission spectroscopy (HG-ICP-OES) [13] and electrothermal atomic absorption spectrometry (ET-AAS) [14] were used for determination of inorganic Sb species (Sb(III), Sb(V) and total Sb) in food and beverages until now. Among these techniques, flame AAS may be considered as a relatively easy to use, high precision, inexpensive, well-established and well-known detection technique with high sample throughput,

which is widely used for the determination of trace metal ions. However, direct determination of antimony as a metalloid at trace levels by FAAS does not present the sensitivity requirements, and more importantly, it is affected by matrix interference. Also, it needs higher amount of samples when compared to hydride generation or electrothermal AAS. Graphite furnace or electrothermal methods of analysis on the other hand are slower and less precise; however, these are more sensitive and need much smaller samples. Thus, prior separation and preconcentration of trace amounts of antimony from the sample matrix are frequently necessary to improve the detection limit and the selectivity. These factors make several procedures developed for the separation and preconcentration of antimony from food and beverage matrices, including multivariate optimization [15], on nanometer-sized  $\text{TiO}_2$  [16], liquid phase microextraction (LPME) [12], flow injection solid phase extraction (FI-SPE) [17], liquid phase semi-microextraction (LPS-ME) [18], single-drop microextraction (SDME) [19], head space single-drop microextraction (HS-SDME) [20], vortex-assisted surfactant-enhanced emulsification microextraction based on solidification of floating organic drop (VASEME-SFO) [21], dispersive liquid–liquid microextraction (DLLME) [22, 23] and micelle-mediated extraction (MME) or cloud point extraction (CPE) [8, 24–28] have been applied prior to analysis. The CPE can solve these problems and lead to a higher confidence level and easy determination of the trace elements.

The conventional CPE is one of analytical tools often used for the separation and preconcentration of trace metal ions. The principle of conventional CPE is well known, and its application area is continuously extended with new approaches such as induction with ultrasonic, microwave, vortex, pH, temperature and salt effect for fast and efficient mass transfer from aqueous bulk phase to micellar phase greatly to reduce process time, reagent consumption and waste generation [29]. This method is simple preconcentration tool with low cost, eco-friendly, high capacity for preconcentration of wide variety of analytes, and high preconcentration factor with good recoveries [30]. In view of these advantages, ultrasonic-assisted cloud point extraction (UA-CPE) have been efficiently coupled to atomic spectroscopic [9, 13, 14, 26, 31, 32] and molecular spectroscopic [24, 27, 28] techniques to determine trace amounts of antimony in different sample matrices.

In the existing study, a new UA-CPE method is proposed for the determination of low levels of antimony in selected beverage samples. 4-(2-Thiazolylazo)resorcinol (TAR) and 2-(2-thiazolylazo)-p-cresol (TAC) in parallel were successfully used as selective chelating agents for Sb(III) as analyte in presence of sodium dodecyl sulfate (SDS) as both an auxiliary ligand and sensitivity improvement agent at pH 6.0. So, development of an accurate and reliable analytical

method is great of importance for the selective determination of Sb(III) as neutral species,  $\text{SbO}(\text{OH})$  or  $\text{Sb}(\text{OH})_3$  in presence of excess Sb(V), which is available in form of  $\text{Sb}(\text{OH})_6^-$  or  $\text{SbO}(\text{OH})_4^-$  at pH 6.0, in different food and beverage matrices. The proposed method can determine low levels of antimony by using conventional FAAS after a preconcentration step with UA-CPE, and it can be considered as an alternative to expensive and tedious analytical techniques such as ICP-MS, ICP-OES with and without hydride generation, HG-AAS and GF-AAS.

## Materials and methods

### Instrumentation

The atomic absorption spectrophotometer (Shimadzu AA-6300, Kyoto, Japan) equipped with  $\text{D}_2$ -background correction, an antimony hollow cathode lamp, an air-acetylene flame atomizers were used for the determination of antimony in selected beverages and foods. The wavelength, lamp current, spectral bandwidth, burner height, air and acetylene flow rates for antimony were 217.6 nm, 13 mA, 0.7 nm, 1.6 cm, 2.0 L  $\text{min}^{-1}$  and 15.0 L  $\text{min}^{-1}$ , respectively. The nebulizer flow rate and burner height were adjusted in order to obtain the maximum absorbance signal by aspirating a solution containing antimony in methanol containing 0.1 mol  $\text{L}^{-1}$  nitric acid. 50 mL calibrated centrifuge tubes in the centrifuge (Hettich Universal-320, England) were used to accelerate the phase separation. An ultrasonic cleaner (Jeio-Tech UCP-10 model, Seoul, Korea) was used to maintain the temperature and to induce complex formation with sonication in UA-CPE experiments. The pH measurements were carried out using a pH-2005 digital pH meter equipped with a glass-calomel electrode (pH-2005, JP Selecta, Barcelona, Spain). Eppendorf vary-pipettes (10–100 and 200–1000  $\mu\text{L}$ ) were used to deliver accurate volumes. A refrigerator was used to keep the biological and beverages samples fresh and cool till the analysis.

### Reagents and standard solutions

All chemicals used were of analytical reagent grade. All solutions were prepared with deionized water (18.2 M $\Omega$  cm) obtained from a Labcanco (Kansas City, USA) water purification system. Stock solutions were prepared from appropriate amounts of its oxide and chlorides,  $\text{Sb}_2\text{O}_3$  and  $\text{SbCl}_5$  (E. Merck, Darmstadt, Germany) as 1000 mg  $\text{L}^{-1}$  in deionized water and diluted daily for obtaining working solutions prior to use. The standard Sb(III) and Sb(V) solutions used for calibration were prepared before use by dilution of the stock solutions with deionized water. The solutions of nonionic surfactant,

5.0 % (v/v) (Tween 80) (Sigma, St. Louis, MO, USA) was prepared by dissolving in water without further purification. The  $1.0 \times 10^{-3}$  mol  $\text{L}^{-1}$  TAR and TAC (Sigma) solutions as chelating agents were prepared by dissolving their appropriate amounts (Sigma) in ethanol and diluting to 1 L with water. The 0.1 % (w/v) ionic surfactant solution (SDS) was prepared by dissolving appropriate amount (Sigma) in water. A 0.04 mol  $\text{L}^{-1}$  of Britton-Robinson (BR) buffer was used to keep pH of the solutions in range of 3.0–8.0. The buffer consists of a mixture of 0.04 mol  $\text{L}^{-1}$   $\text{H}_3\text{PO}_4$  (Merck), 0.04 mol  $\text{L}^{-1}$   $\text{H}_3\text{BO}_3$  (Merck) and 0.04 mol  $\text{L}^{-1}$   $\text{CH}_3\text{COOH}$  (Merck) that has been titrated to the desired pH with 0.2 mol  $\text{L}^{-1}$  NaOH. Laboratory glasswares were kept overnight in 10 % nitric acid solution. Before use, the glassware was rinsed with deionized water and dried in dust-free environment. The following standard reference materials (SRMs) supplied from NIST (Gaithersburg, USA) were used for method validation: SRM 1573a tomato leaves and SRM 1548a typical diet.

### Sampling, sample preparation

All of samples selected for analysis were supplied from local markets in Sivas, Turkey. Initially, all of the glassware and other mineralization containers used were acid-washed to avoid possible contamination. A 0.1 % (v/v) antifoam solution was added to the beer samples to prevent foaming, and they were degassed for 15 min using an ultrasonic bath. Potassium metabisulfite (8 mg  $\text{L}^{-1}$ , 1 mL) was added to 100 mL of beer to destroy any protein. The solution was maintained at room temperature for 15 min, then pre-sonicated for 5 min and analyzed immediately. Wine samples (50 mL) were de-alcoholized at 80 °C using a reduced pressure evaporator until the total volume was approximately a quarter of the initial volume. These were kept cool, filtered into a volumetric flask of 50 mL and the final volume was adjusted with deionized water.

A microwave oven was used for digestion of samples and reduction of Sb(V) to Sb(III) by adopting suitable working conditions. Particularly, the digestion procedure was varied according to the nature and type of sample matrix prior to antimony analysis by means of CPE/FAAS system. The semi-solid and liquid samples (0.1–1.0 g of milk and milk products, 3 mL of beverages with and without alcohol) were dissolved in 5 mL of a mixture of  $\text{HNO}_3$  (68 %, w/w) and  $\text{H}_2\text{O}_2$  (30 % (w/w) (3:2, v/v). Especially, the liquid beverages without alcohol after centrifugation independently were evaluated in terms of Sb content in sampling step. Then, the following digestion program was applied: 5 min at 250 W, 15 min at 450 W and finally 5 min at 650 W. After microwave digestion, samples were cooled and transferred to calibrated volumetric flasks of 50 mL for subsequent analysis. At the end of this process, completely

clear solutions were obtained. It is worth underlining that milk products should be diluted as little as possible to avoid diluting the low Sb contents.

For reduction of Sb(V) to Sb(III) in the digested and diluted samples at suitable ratios, the 7.0 mL of mixture of  $1.0 \times 10^{-3}$  mol L<sup>-1</sup> KI and  $1.0 \times 10^{-2}$  mol L<sup>-1</sup> ascorbic acid in acidic media (7.0 mL, 5.0 mol L<sup>-1</sup> HCl) was added to each, and the samples processed in a microwave oven with the following program: 2 min at 250 W, 5 min at 450 W, 2 min at 650 W and finally 3 min at 900 W. After reduction of Sb(V) to Sb(III), total Sb analysis was also performed by using FAAS after UA-CPE under the optimized reagent conditions, in which the free Sb(III) analysis directly is performed without pre-reduction after digestion of samples under microwave power. The Sb(V) contents of samples were determined from the analytical difference between total Sb and free Sb(III) contents. A blank analysis was carried out following the same procedure without the beverage and milk samples. Also, two different SRMs were analyzed in order to test the accuracy of the method. The SRMs were subjected to the same microwave digestion program with beverage samples.

### The UA-CPE procedure

For the CPE, aliquots of the cold solution containing the analytes in the range of 5.0–180 µg L<sup>-1</sup> for Sb(III), 0.7 mL of  $1.0 \times 10^{-3}$  mol L<sup>-1</sup> of TAR or TAC, 0.5 mL of 0.1 % (w/v) SDS, 0.1 mL of 5.0 % (v/v) Tween 80 and 0.5 mL of 0.01 mol L<sup>-1</sup> Na<sub>2</sub>SO<sub>4</sub> were buffered to pH 6.0 using 0.1 mL of 0.04 mol L<sup>-1</sup> BR buffer, and kept in ultrasonic bath (operating with ultrasound frequency of 40 kHz at 300 W) for 15 min at 65 °C efficiently to start the process of extraction and preconcentration of analyte in the surfactant-rich phase. Then, the resultant turbid solution was cooled for 5 min in refrigerator to facilitate phase separation. The solution was centrifuged for 10 min at 3000 rpm. Surfactant-rich phase was separated from aqueous phase by a simple decantation. The surfactant-rich phase was dissolved with 2.0 mL of 1.0 mol L<sup>-1</sup> HNO<sub>3</sub> in methanol to decrease the viscosity. The concentrations of analyte, Sb(III) in the final solutions were monitored by FAAS based on excitation with hollow cathode lamp at 217.6 nm in air/acetylene flame for Sb(III).

## Results and discussion

### The general aspects

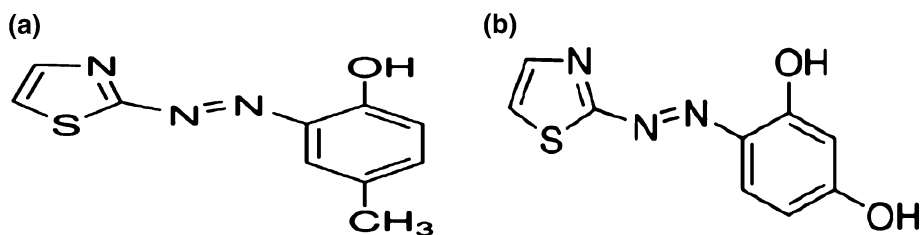
The 4-(2-thiazolylazo)-resorcinol (TAR) with pK<sub>a</sub> values of 0.5, 6.54 and 11.8 and TAC with pK<sub>a</sub> values of 0.50 and 8.34, which are selected as chelating agents in the present study, are the chelating reagents forming neutral or ionic stable chelates with high molar absorptivity with many metal ions depending on pH of environment [33–35]. The open molecular structures of the selected chelating agents for Sb(III) are represented in Scheme 1.

It was observed in literature that TAR gave a well-defined absorption band at 481 nm with a red shift of 38 nm as a result of complexation in presence of Sb(III) at pH range of 5.0–6.0, while it showed a absorption peak at 443 nm in visible region. In the relevant study, it has been explained that TAR acts as a bidentate ligand, and coordinates antimony via azo and hydroxyl groups. It is also implied that this characteristic absorption peak linearly increased with increasing antimony concentration [36]. It is expected that TAC having a similar structure selectively will bind Sb(III), so as to give a stable complex (in form of neutral SbOL structure at mole ratio of 1:1), depending on pH of aqueous micellar media. From prior studies conducted in lights of all these literature information, it has been observed that both TAR and TAC selectively bind Sb(III) with a significant sensitivity difference in presence of excess Sb(V) at pH 6.0. Hence, all the measurements were carried out for Sb(III) selected as analyte at pH 6.0. The various analytical variables affecting on UA-CPE efficiency were extensively investigated in order to achieve the highest calibration sensitivity.

### Optimization of variables affecting complex formation and UA-CPE efficiency

The various analytical variables were optimized by using model solutions containing 50 µg L<sup>-1</sup> Sb(III) in order to obtain the maximum extraction efficiency. Each point in optimization step was replicated three times, and the results were given with their average value plus standard deviations as error bars. So, parameters like the effect of pH, concentrations of ligand and surfactants, ionic strength,

**Scheme 1** The open molecular structures of **a** TAC, 2-(2-thiazolylazo)-p-cresol, **b** TAR, 4-(2-thiazolylazo)resorcinol



diluent agent for surfactant-rich phase, and incubation conditions were studied in detail. All the other parameters were kept constant, while a parameter was optimizing.

### Effect of pH

As can be seen in Fig. 1, the maximum efficiency was achieved at pH 6.0 in pH range of 3.0–8.0 for Sb(III). In this pH range, Sb(V) is predominantly in form of  $\text{Sb}(\text{OH})_6^-$  or  $\text{SbO}(\text{OH})_4^-$  with a  $\text{pK}_a$  value of 2.72, in which Sb(III) is present in form of neutral  $\text{Sb}(\text{OH})_3$  or  $\text{SbO}(\text{OH})$  with a  $\text{pK}_a$  value of 11.9 [37, 38]. From prior studies, it has not been considered as analyte in terms of speciation analysis for further studies since Sb(V) gives a stable complex with both chelating agents, TAR and TAC. Moreover, in this pH range, a significant signal increase was not given in presence of Sb(III) at equal concentrations of  $50 \mu\text{g L}^{-1}$ . Therefore, a pH of 6.0 was selected as optimal pH for subsequent studies. At lower pHs than 6.0, a weak complexation and uptake occurs, which is explained by competition between Sb(III) and proton ions. On the other hand, the active sites of ligands such as azo- and phenol groups for  $\text{Sb}(\text{OH})_3$  or  $\text{SbO}(\text{OH})$  are used by proton ions and consequently extraction efficiency is decreased at low pHs. The extraction efficiency gradually increased with increasing pH and reached a maximum with increasing sensitivity at around pH 6.0 (Fig. 1a).

The decrease in signal at higher pHs than 6.0 may be due to strongly electrostatic repulsion between phenolate active sites of ligands and  $\text{Sb}(\text{OH})_4^-$  ions produced as a consequence of hydrolysis of  $\text{Sb}(\text{OH})_3$  [37, 38]. After an optimal pH selected as 6.0 (with BR buffer) due to give the maximum sensitivity, the buffer volume was also studied

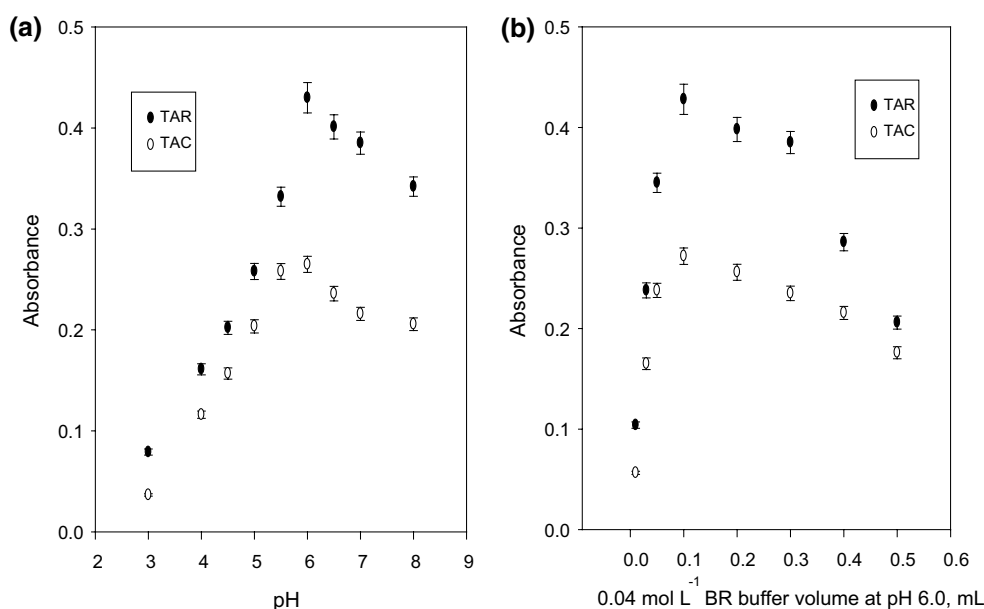
in concentration range of 0.01–0.5 mL of  $0.04 \text{ mol L}^{-1}$  in Fig. 1b, and the maximum analytical signal was obtained by using 0.1 mL of  $0.04 \text{ mol L}^{-1}$  BR buffer solution in the final volume of 50.0 mL.

### Effect of chelating ligands and sensitivity enhancement agent concentrations

TAR and TAC were chosen as a selective chelating ligands because Sb(III) predominantly present in the form of neutral  $\text{SbO}(\text{OH})$  or  $\text{Sb}(\text{OH})_3$  at pH 6.0. Therefore, the selected ligands can form the stable hydrophobic chelate complexes by condensation, in structure  $\text{Sb} = \text{OL}$  with one mole water loss, in which ligands act such as bidentate, in presence of SDS as a sensitivity enhancement agent. Then, these neutral complexes can be extracted to micelles of nonionic surfactant, Tween 80 in presence of SDS and  $\text{Na}_2\text{SO}_4$  as salting-out agent by UA-CPE. The effect of each ligand volume on the extraction of Sb(III) selected as analyte was studied in the range of 0.05–1.5 mL of  $1.0 \times 10^{-3} \text{ mol L}^{-1}$ , and the results are shown in Fig. 2. As can be seen, the absorbance increased with increasing ligand volume for both ligand and reached to a maximum value at 0.7 mL. Therefore, a chelating ligand volume of 0.7 mL at  $1.0 \times 10^{-3} \text{ mol L}^{-1}$  was selected as optimal value for further studies.

The variation in analytical signal as a function of ionic surfactant concentration (SDS), which is chosen as sensitivity enhancement agent, was monitored. The dependence of UA-CPE to ionic surfactant volume at fixed concentration of 0.1 % (w/v) was studied in the range of 0.01–1.00 mL in Fig. 3a. The best analytical signal was obtained with SDS volume of 0.1 mL. At higher volumes, absorbance gradually decreased. The reason of this decrease may

**Fig. 1** Effect of **a** pH and **b**  $0.04 \text{ mol L}^{-1}$  BR buffer volume at pH 6.0 on analytical signal. Optimal conditions: 0.7 mL of  $1.0 \times 10^{-3} \text{ mol L}^{-1}$  TAR or TAC, 0.5 mL of 0.1 % (w/v) SDS, 0.1 mL of 5.0 % (v/v) Tween 80, 0.5 mL of  $0.01 \text{ mol L}^{-1} \text{ Na}_2\text{SO}_4$ , extraction time of 15 min at  $65^\circ\text{C}$  for three replicate measurements of  $50 \mu\text{g L}^{-1}$  Sb(III) ( $n: 3$ )

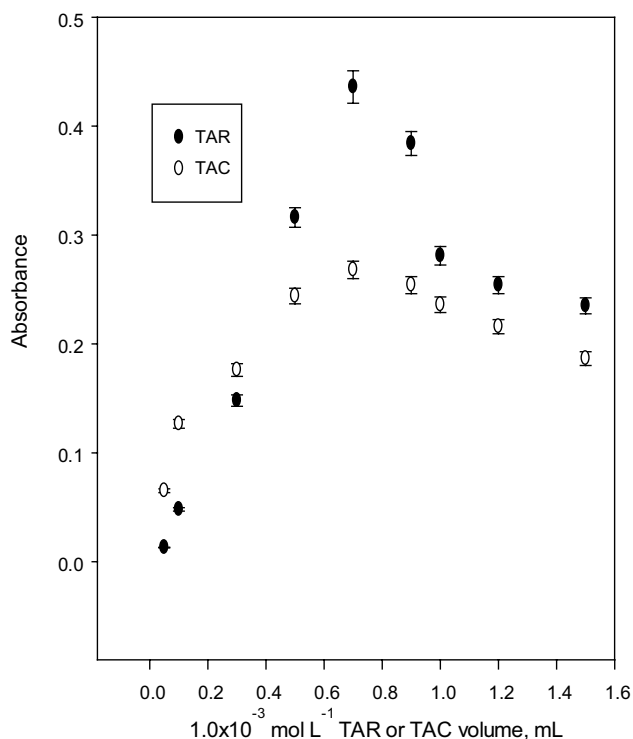


be increase in blank signal as a consequence of hydrophobic interactions between SDS and chelating ligands. Therefore, a reasonable SDS volume of 0.1 mL so as to make a

compromise on accuracy and precision was adopted as the optimal value for the subsequent studies.

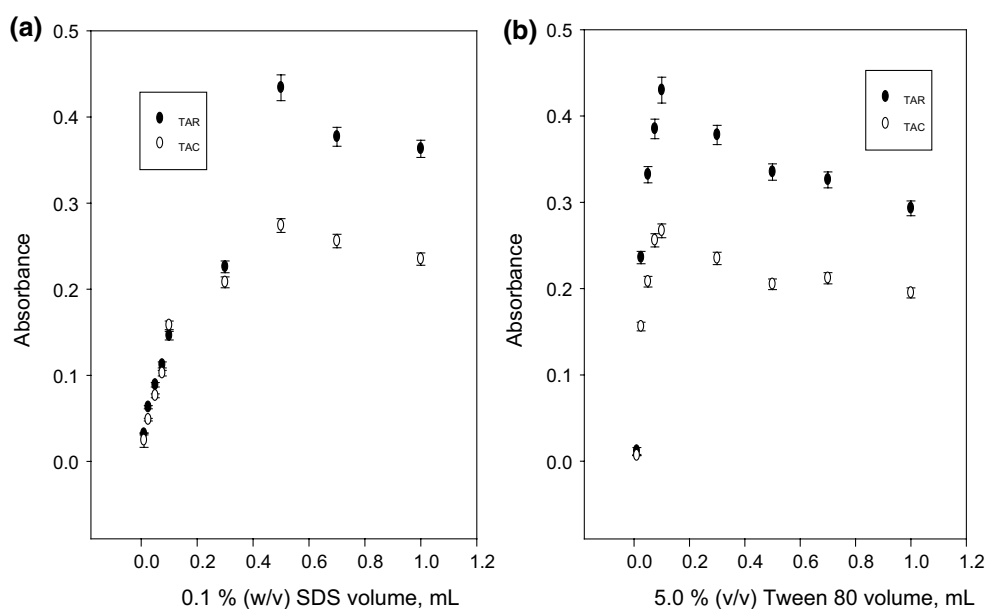
#### Effect of nonionic surfactant concentration

The hydrophobic surfactant, Tween 80, is one of the limited used surfactants in CPE of other inorganic species including Sb(III). The reason of its limited usage in analytical separations is the high cloud point temperature (CPT > 70 °C) in water. By using ionic surfactants (SDS) and inorganic electrolytes ( $\text{Na}_2\text{SO}_4$ ), its cloud point could be lowered to room temperature as a result of decrease in critical micelle concentration (CMC) with increasing salt and/or ionic surfactant concentrations [39–41]. It has been generally used to sensitize the complexation reaction or to separate the analyte phase without using organic solvent as a medium. The effect of surfactant concentration on extraction efficiency is a crucial parameter in CPE-based methods. If excess surfactant is used, analytical signal gets worse due to increase in final surfactant volume. On the contrary, if the surfactant concentration is lower from the optimal values, accuracy and reproducibility of analysis would be poorer. However, the signals get worse at higher concentrations due to increasing viscosity [6, 7]. The variation of the absorbance as a function of Tween 80 volume in the range of 0.01–1.0 mL of 5.0 % (v/v) is investigated in Fig. 3b. From results obtained, the maximum signal was obtained by 0.1 mL of 5.0 % (v/v) Tween 80 for both chelating agents. In range of 0.1–1.0 mL, absorbance gradually decreased. This is due to increasing phase volume ratio as a result of increase in volume of surfactant-rich phase. Therefore, a nonionic surfactant volume of 0.1 mL of 5.0 % (v/v) as extracting agent was chosen for subsequent studies in order to achieve high analytical signal



**Fig. 2** Effect of  $1.0 \times 10^{-3} \text{ mol L}^{-1}$  TAR and TAC volumes on analytical signal. Optimal conditions: 0.1 mL of  $0.04 \text{ mol L}^{-1}$  BR buffer, 0.5 mL of 0.1 % (w/v) SDS, 0.1 mL of 5.0 % (v/v) Tween 80, 0.5 mL of  $0.01 \text{ mol L}^{-1}$   $\text{Na}_2\text{SO}_4$ , extraction time of 15 min at  $65^\circ\text{C}$  for three replicate measurements of  $50 \mu\text{g L}^{-1}$  Sb(III) ( $n: 3$ ) at pH 6.0

**Fig. 3** Effect of **a** 0.1 % (w/v) SDS as both sensitivity enhancement agent and counter ion and **b** 5.0 % (v/v) Tween 80 volumes as extracting agent on analytical signal. Optimal conditions: 0.1 mL of  $0.04 \text{ mol L}^{-1}$  BR buffer, 0.7 mL of  $1.0 \times 10^{-3} \text{ mol L}^{-1}$  TAR or TAC, 0.5 mL of  $0.01 \text{ mol L}^{-1}$   $\text{Na}_2\text{SO}_4$ , extraction time of 15 min at  $65^\circ\text{C}$  for three replicate measurements of  $50 \mu\text{g L}^{-1}$  Sb(III) ( $n: 3$ ) at pH 6.0





and good preconcentration factor in conjunction with the possible maximum extraction efficiency.

#### *Effect of ionic strength*

The cloud point temperature is decreased in the presence of electrolytes, and this circumstance is caused to low extraction efficiency as a result of salting-out effect. The lower CPE efficiency is attributed to electrolytes promoting dehydration of the poly-(oxyethylene) chains. It is well known that the salting-out phenomenon is directly related to desorption of ions from the hydrophilic parts of the micelles, increasing interaction between micelles and consequently leading to the precipitation of surfactant molecules [8]. Based on this discussion, four different ionic salts (KCl, KNO<sub>3</sub>, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and Na<sub>2</sub>SO<sub>4</sub>) as salting-out agent were investigated in volume range of 0.05–1.0 mL of 0.01 mol L<sup>-1</sup>. The results clearly show that addition of salt in concentration of 0.5 mL of 0.01 mol L<sup>-1</sup> Na<sub>2</sub>SO<sub>4</sub> is the most appropriate for maximum extraction of the hydrophobic complexes. Therefore, a salting-out agent concentration of 0.5 mL of 0.01 mol L<sup>-1</sup> Na<sub>2</sub>SO<sub>4</sub> was selected as optimum for further studies.

#### *Effect of diluting agent for surfactant-rich phase*

The obtained surfactant-rich phase after UA-CPE is very viscous with an optimal value of 0.0106 % (w/v), in which CMC is 0.0016 % (w/v). In addition, the volume of the surfactant-rich phase after separation and preconcentration with UA-CPE is very small for detection with FAAS. The choosing of the most appropriate solvent is very important for maximum extraction efficiency. Two milliliters of various diluents such as, ethanol, methanol, acetonitrile, acetone, THF, 1.0 mol L<sup>-1</sup> HNO<sub>3</sub> in methanol and ethanol were added to surfactant-rich phase after phase separation. From the results obtained, the best analytical sensitivity and regression coefficient ( $r^2$ ) obtained from calibration curves based on three different concentrations of Sb (25, 50 and 100 μg L<sup>-1</sup>) and complete solubilization of surfactant-rich phase was provided in the presence of 1.0 mol L<sup>-1</sup> HNO<sub>3</sub> in methanol.

#### *Effect of incubation temperature and time*

In order to achieve easy phase separation and preconcentration as efficient as possible, optimal incubation time and equilibration temperature are necessary to complete reactions. It is desired to employ the shortest equilibration time and the lowest possible equilibration temperature as a compromise between completion of extraction and efficient separation of phases. The dependence of extraction efficiency upon temperature and time of incubation was studied in the range of 20–80 °C and 2–30 min, respectively. The results

showed that 65 °C as temperature and 15 min as incubation time are appropriate and enough for CPE experiments. In addition, centrifugation time and rates are very necessary to preconcentrate low levels of Sb(III) with high efficiency in a short time. Thus, under optimal conditions obtained, the effect of the centrifugation time and rate was studied in range of 2–20 min and 1000–4000 rpm, respectively. The results showed that centrifugation for 10 min at 3000 rpm and cooling for 5 min in refrigerator leads to the maximum recovery and sensitivity for both chelating agents.

#### **The analytical figures of merit**

From calibration studies conducted with a serial standard Sb(III) solutions ranging from 0.5 (or 1.0) to 180 μg L<sup>-1</sup> under the optimized conditions, in which each data point is five times replicated ( $n$ : 5), the calibrations curves obtained for TAR and TAC with changing sensitivity are as follows:

$$\text{Abs} = 1.76 \times 10^{-3} [\text{Sb(III)}, \mu\text{g L}^{-1}] + 0.0119,$$

$$r^2 : 0.9992 \text{ for TAR and}$$

$$\text{Abs} = 1.16 \times 10^{-3} [\text{Sb(III)}, \mu\text{g L}^{-1}] + 0.0356,$$

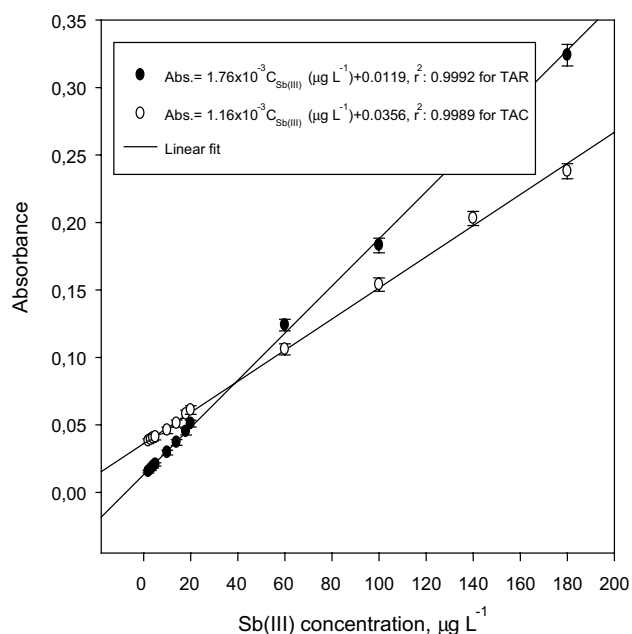
$$r^2 : 0.9989 \text{ for TAC}$$

The linear fit graphics are represented in Fig. 4.

The limits of detection that is statistically based on the calculation of LOD:  $3\sigma_{\text{blank}}/m$  where the  $\sigma_{\text{blank}}$  is the standard deviation of ten replicate blank measurements and  $m$  is the slope of the calibration curves obtained after preconcentration with UA-CPE were found to be 0.13 and 0.28 μg L<sup>-1</sup> for Sb(III) in presence of TAR and TAC with sensitivity enhancement factors of 85 and 60, respectively. Preconcentration factor is defined as the ratio of the analyte concentrations after and before UA-CPE. The precision of the proposed method was checked by the RSDs of six independent measurements taken in solutions containing all ions. The sensitivity enhancement factor is calculated as the ratio of slopes of the calibration curves obtained with without preconcentration by means of UA-CPE. The obtained results are consistent with a significant sensitivity difference for both chelating ligands. The precision as RSDs was lower than 3.9 % for five replicate measurements of two different concentration levels of 25 and 100 μg L<sup>-1</sup>. The analytical features of method are extensively indicated in Table 1.

#### **Matrix effect**

The matrix effect of different cations and anions on determination of 50 μg L<sup>-1</sup> Sb(III) were evaluated by the proposed UA-CPE method. In view of the high selectivity provided by FAAS, the interferences may be based on



**Fig. 4** The calibration curves obtained for detection of Sb(III) with TAR and TAC in linear working ranges of 0.5–180 or 1.0–180  $\mu\text{g L}^{-1}$ . Optimal conditions: 0.1 mL of 0.04 mol  $\text{L}^{-1}$  BR buffer, 0.7 mL of  $1.0 \times 10^{-3}$  mol  $\text{L}^{-1}$  TAR or TAC, 0.5 mL of 0.1 % (w/v) SDS, 0.1 mL of 5.0 % (v/v) Tween 80, 0.5 mL of 0.01 mol  $\text{L}^{-1}$   $\text{Na}_2\text{SO}_4$ , extraction time of 15 min at 65 °C for three replicate measurements of Sb(III) ( $n$ : 3) at pH 6.0

**Table 1** Analytical features of the proposed UA-CPE method

Analytical features	Analytical species detected by FAAS	
	Sb(III) with TAR	Sb(III) with TAC
Linear working range ( $\mu\text{g L}^{-1}$ )	0.5–180	1–180
Slope, $m$	$1.76 \times 10^{-3}$	$1.16 \times 10^{-3}$
Intercept, $b$	0.0119	0.0156
Regression coefficient, $r^2$	0.9992	0.9989
Recovery % (25, 75 and 150 $\mu\text{g L}^{-1}$ , $n$ : 5)	97.5–103	98.3–103
RSD % (25 and 100 $\mu\text{g L}^{-1}$ , $n$ : 5)	1.8–3.2	2.3–3.5
Limit of detection, LOD ( $\mu\text{g L}^{-1}$ , $n$ : 10)	0.13	0.28
Limit of quantification, LOQ ( $\mu\text{g L}^{-1}$ , $n$ : 10)	0.43	0.93
Preconcentration factor, PF <sup>a</sup>	45	40
Sensitivity enhancement factor, EF <sup>b</sup>	85	60

<sup>a</sup> Preconcentration factor is defined as the ratio of the analyte concentrations after and before separation preconcentration with UA-CPE

<sup>b</sup> Sensitivity enhancement factor is defined as the slope ratios of the calibration curves obtained after and before separation and preconcentration with UA-CPE

preconcentration step: cations may react with chelating ligands and anions may form complexes with Sb(III), and decrease extraction efficiency. The interferences induced by other cations, which readily complex with ligands, can be avoided by increasing the amount of each ligand dissolved in solution. An ion was considered as an interfering ion when it caused an error greater than  $\pm 5.0$  % in the determination step. From the results obtained at tolerance limit ranging from 5 to 250, it was observed that common cations ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{NH}_4^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Al}^{3+}$ ,  $\text{VO}^{2+}$ ,  $\text{MoO}_4^{2-}$ ) and anions ( $\text{F}^-$ ,  $\text{Cl}^-$ ,  $\text{Br}^-$ ,  $\text{I}^-$ ,  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{SCN}^-$ ,  $\text{H}_2\text{PO}_4^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{HCO}_3^-$ ) did not interfere to proposed method in determination of antimony; only,  $\text{Cu}^{2+}$ ,  $\text{Bi}^{3+}$ ,  $\text{Fe}^{3+}$  and  $\text{Al}^{3+}$  ions interfere with the proposed method at ratios of 10–35 fold. Before determination by means of FAAS, the interference of  $\text{Cu}^{2+}$  and  $\text{Bi}^{3+}$  ions can be improved up to a tolerance limit of 150 after pretreatment of the sample solution with 100  $\mu\text{L}$  of 0.01 mol  $\text{L}^{-1}$   $\text{S}_2\text{O}_3^{2-}$  while the interference of  $\text{Fe}^{3+}$  and  $\text{Al}^{3+}$  ions is largely improved up to a tolerance limit of 250-fold after pretreatment of sample solution with 100  $\mu\text{L}$  of 0.02 mol  $\text{L}^{-1}$   $\text{NH}_4\text{F}$ . Also, the possible interference of  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{Fe}^{3+}$  ions, which are present in great amounts in beverage and milk products, may greatly improve by pretreatment of the digested and diluted samples with a mixture of diluted ascorbic acid and EDTA solutions before UA-CPE. As a result, the effect of possible interferences appeared to be negligible, which evidently proved very good selectivity.

### Accuracy and analytical applications of the method

The accuracy of the proposed method was checked by analysis of two certified reference materials (SRMs). The certified plant and typical diet samples, SRM 1573a tomato leaves and SRM 1548a typical diet, were used for validation. The antimony contents of certified samples were determined by using proposed UA-CPE method. The results are indicated in Table 2.

It was observed that the results are in a highly good agreement with the certified values. It is clear that there is statistically not a significant difference between the certified values and the results found by the proposed method for five replicate measurements at probability of 0.05. In light of these observations, the results are satisfactory and reasonable, which indicate the capability of the present analytical system in the determination of antimony in real samples.

### Optimization of reduction conditions of Sb(V) to Sb(III) and determination of total Sb

Suitable aliquots of Sb(III) plus Sb(V) mixtures (preferably at ratios 1:4, 2:3, 3:2 4:1 for 150  $\mu\text{g L}^{-1}$  Sb(III), Sb(V))



**Table 2** The analytical results obtained from five replicate measurements for accuracy of the proposed UA-CPE method ( $n: 5$ )

SRMs	Certified value ( $\mu\text{g kg}^{-1}$ total Sb)	With TAC			With TAR			Student's $t$ test <sup>b</sup>
		Observed <sup>a</sup> ( $\mu\text{g kg}^{-1}$ )	Recovery %	RSD %	Observed <sup>a</sup> ( $\mu\text{g kg}^{-1}$ )	Recovery %	RSD %	
SRM 1573a Tomato leaves	63 ± 6	61.3 ± 2.5	97.3	4.1	60.8 ± 2.5	96.5	4.1	1.52, 1.97
SRM 1548a Typical diet	9	8.8 ± 0.4	97.8	4.5	8.7 ± 0.4	96.7	4.6	1.12, 1.68

<sup>a</sup> The average value plus standard deviation for five replicate measurements at probability level of 0.05

<sup>b</sup> The one paired critical  $t$  value is 2.78 for four degrees of freedom at probability level of 0.05

**Table 3** The results of binary mixtures containing Sb(III) and Sb(V) at known concentration ratios by the proposed UA-CPE method after pre-reduction with a mixture KI/ascorbic acid in HCl media under the optimized conditions ( $n: 5$ )

Sb(V)/Sb(III) Added ratio	Added Sb(V) ( $\mu\text{g L}^{-1}$ )	Added Sb(III) ( $\mu\text{g L}^{-1}$ )	Found Sb(III) <sup>a</sup> ( $\mu\text{g L}^{-1}$ )	RSD %	Recovery %	Total Sb <sup>b</sup> (Sb(III) plus Sb(V), $\mu\text{g L}^{-1}$ )	RSD %	Recovery %	Calculated Sb(V) <sup>c</sup> ( $\mu\text{g L}^{-1}$ )	Recovery %
–	–	150	146.5 ± 5.2	3.5	97.7	147.0 ± 5.0	3.4	98.0	–	–
1:4	30	120	118.3 ± 4.1	3.5	98.6	146.5 ± 5.3	3.6	97.7	28.2	94.0
2:3	60	90	87.4 ± 3.5	4.0	97.1	147.2 ± 5.0	3.4	98.1	59.8	99.7
3:2	90	60	58.1 ± 2.5	4.3	96.8	148.5 ± 5.6	3.8	99.0	90.4	100
4:1	120	30	28.6 ± 1.3	4.5	95.3	147.1 ± 5.2	3.5	98.1	118.5	98.7
–	150	–	–	–	–	147.5 ± 5.2	3.5	97.3	147.5	98.3

<sup>a</sup> The results indicate the Sb(III) values independently found by the present method without pre-reduction for five replicate measurements

<sup>b</sup> The results indicate the total Sb values independently found by the proposed method after pre-reduction with mixture of  $1.0 \times 10^{-3}$  mol L<sup>-1</sup> KI and  $1.0 \times 10^{-2}$  mol L<sup>-1</sup> ascorbic acid in acidic media (0.5 mL of 5.0 mol L<sup>-1</sup> HCl in final volume of 50 mL). The average values and their standard deviations of UA-CPE/FAAS measurements with five replicates at 95 % confidence level

<sup>c</sup> The results indicate the Sb(V) values directly found by calculating the differences between the levels of the Sb(III) and total Sb obtained for five replicate measurements with the present method

and their mixtures) were taken in a 50 mL of conical flask. Optimal 7.0 mL in volume range of 0–20 mL of mixture of  $1.0 \times 10^{-3}$  mol L<sup>-1</sup> KI and  $1.0 \times 10^{-2}$  mol L<sup>-1</sup> ascorbic acid solution in HCl media (with optimal 7.0 mL in volume range of 1–20 mL of 5 mol L<sup>-1</sup>) was added to samples for reduction of Sb(V) to Sb(III) at fixed concentration of 150  $\mu\text{g L}^{-1}$  for time interval of 15 min at room temperature. After reduction, the mixtures were analyzed by proposed method to determine total Sb levels. The same procedures were also conducted without reduction for the binary mixtures. The Sb(III) and total Sb contents were calculated by using a calibration curve. However, the amount of Sb(V) ions were calculated from the difference between the amounts of Sb(III) and total Sb before and after reduction due to give more accurate and precise results. The recovery studies of the speciation procedure were carried out by the analysis of spiked binary mixtures. The speciation results are extensively presented in Table 3.

It is clear that the recoveries for Sb(III) are quantitative in range of 95.3–98.6 % with a RSD ranging from 3.50 to 4.50 %, while the recoveries for total Sb are in range of 97.3–99.0 % with a RSD ranging from 3.40 to 3.80 %.

Similarly, the spiked beverage (apple juice) and milk samples (semi-skimmed milk) at known concentrations of Sb [as Sb(V) in presence of Sb(III)] were prepared and analyzed by the proposed method before and after pre-reduction, so that it will be used as references in the real cases in terms of matrix compatibility. From the results, it has been observed that Sb(III) and total Sb quantitatively are recovered with a higher recovery rate than 94 % in speciation analysis.

The feasibility of the method for the determination of antimony in different beverages, milk and fruit-flavored milk products was investigated. The method was successfully applied to different beverages and milk products with and without fruit flavor. The results obtained from new developed UA-CPE/FAAS method are presented in Table 4. The proposed method was validated by analysis of the spiked samples with known amounts of Sb(III) at two different concentration levels, 1.0 or 1.5  $\mu\text{g L}^{-1}$ . The recoveries from spiked solutions have been varied in the range of 95–98 % with RSDs of 2.6–5.2 and 2.7–4.9 % for both Sb(III) and total Sb in nonalcoholic and alcoholic beverages and milk products, respectively. Due to be more toxic of inorganic antimony species, especially Sb(III) than

**Table 4** The levels of Sb(III), Sb(V) and total Sb found by analysis of 3 mL of the selected beverages, and fruit-flavored milk products diluted at ratios ranging from 1:5 to 1:25 after microwave assisted digestion with a mixture of conc. HNO<sub>3</sub>/H<sub>2</sub>O<sub>2</sub> (3:2, v/v), respectively

Samples	Added ( $\mu\text{g L}^{-1}$ ) Sb(III)	Sb(III)			Total Sb			Sb(V)
		Found <sup>a</sup> ( $\mu\text{g L}^{-1}$ )	Recovery %	RSD %	Found <sup>b</sup> ( $\mu\text{g L}^{-1}$ )	Recovery %	RSD %	Found <sup>c</sup> ( $\mu\text{g L}^{-1}$ )
<i>Beverages without alcohol</i>								
Cherry juice	–	0.95 ± 0.05	–	5.2	3.45 ± 0.15	–	4.4	2.50
	1.5	2.40 ± 0.10	97.0	4.2	4.90 ± 0.20	97.0	4.1	–
Pomegranate juice	–	0.80 ± 0.04	–	5.0	2.50 ± 0.12	–	4.8	1.70
	1.5	2.25 ± 0.10	97.0	4.4	3.95 ± 0.15	97.0	3.8	–
Apple juice	–	1.20 ± 0.05	–	4.2	3.15 ± 0.12	–	3.8	1.95
	1.5	2.60 ± 0.10	93.0	3.8	4.60 ± 0.16	97.0	3.5	–
Lemon juice	–	1.30 ± 0.06	–	4.6	2.45 ± 0.12	–	4.9	1.15
	1.5	2.75 ± 0.10	97.0	3.6	3.90 ± 0.15	97.0	3.8	–
Mixed fruit juice	–	1.80 ± 0.06	–	3.3	3.40 ± 0.15	–	4.4	1.60
	1.5	3.25 ± 0.10	97.0	3.1	4.85 ± 0.16	97.0	3.3	–
<i>Beverages with alcohol</i>								
Doluca, Red grape wine	–	0.53 ± 0.02	–	3.8	3.45 ± 0.15	–	4.3	2.92
	1.0	1.50 ± 0.05	97.0	3.3	4.40 ± 0.15	95.0	3.4	–
Doluca, white grape wine	–	0.70 ± 0.03	–	4.3	5.30 ± 0.20	–	3.8	4.60
	1.0	1.68 ± 0.06	98.0	3.6	6.25 ± 0.50	95.0	3.8	–
Efes malt	–	0.45 ± 0.02	–	4.4	3.20 ± 0.13	–	4.1	2.75
Beer	1.0	1.40 ± 0.04	95.0	2.9	4.16 ± 0.15	96.0	3.6	–
Efes dark	–	1.35 ± 0.05	–	3.7	7.60 ± 0.30	–	3.9	6.25
Beer	1.0	2.30 ± 0.07	95.0	3.0	8.55 ± 0.30	95.0	3.5	–
<i>Milk and fruit-flavored milk products</i>								
Whole milk	–	0.51 ± 0.02	–	3.9	4.15 ± 0.15	–	3.6	3.64
	1.0	1.47 ± 0.04	96.0	2.7	5.12 ± 0.15	97.0	2.9	–
Semi-skimmed milk	–	0.45 ± 0.02	–	4.4	3.60 ± 0.13	–	3.6	3.15
	1.0	1.42 ± 0.04	97.0	2.8	4.58 ± 0.14	98.0	3.1	–
Normal yogurt	–	0.48 ± 0.02	–	4.2	3.50 ± 0.12	–	3.4	3.02
	1.0	1.45 ± 0.04	97.0	2.8	4.48 ± 0.13	98.0	2.9	–
Cherry-flavored yogurt	–	0.54 ± 0.02	–	3.7	3.80 ± 0.13	–	3.4	3.26
	1.0	1.52 ± 0.04	98.0	2.6	4.75 ± 0.14	95.0	2.9	–
Strawberry-flavored yogurt	–	0.90 ± 0.04	–	4.4	5.50 ± 0.15	–	2.7	4.60
	1.0	1.86 ± 0.06	96.0	3.2	6.47 ± 0.16	97.0	2.5	–
Blackberry-flavored yogurt	–	0.68 ± 0.03	–	4.4	4.15 ± 0.12	–	2.9	3.47
	1.0	1.65 ± 0.05	97.0	3.0	5.12 ± 0.13	97.0	2.5	–
Coca-flavored yogurt	–	0.70 ± 0.03	–	4.3	4.60 ± 0.15	–	3.6	3.90
	1.0	1.67 ± 0.05	97.0	3.0	5.57 ± 0.15	97.0	2.7	–

<sup>a</sup> The results indicate the Sb(III) values independently found by the present method without pre-reduction for five replicate measurements

<sup>b</sup> The results indicate the total antimony values independently found by the proposed method after pre-reduction with mixture of 10 % (w/v) KI and 20 % (w/v) ascorbic acid in acidic media (2 mL of 0.1 mol L<sup>-1</sup> HCl in final volume of 50 mL). The average values and their standard deviations of UA-CPE/FAAS measurements with five replicates at 95 % confidence level

<sup>c</sup> The results indicate the Sb(V) values directly found from the analytical differences between the levels of the Sb(III) and total Sb obtained for five replicate measurements with the proposed method

organic antimony species, especially milk-based beverages/foods for infants can be used as an indicator of environmental contamination with different food contact packaging materials such as PET for soft drink bottles and water bottles, and polypropylene (PP) for syrup bottles, yogurt

tubs, and bottle caps; which are widely used as a catalyst and flame retardant for beverages and especially mineral water. In the light of the average level of beverage consumption in the EU limit, 5  $\mu\text{g L}^{-1}$  Sb [3] and a SML of 40  $\mu\text{g kg}^{-1}$ , the levels of detected antimony in range of

**Table 5** Comparison of analytical features of the method with those of some procedures reported in literature

Analyte	Samples	Preconcentration technique	Detection method	Preconcentration factor (or enhancement factor)	Detection limit ( $\mu\text{g L}^{-1}$ )	Linear range ( $\mu\text{g L}^{-1}$ )	% RSD or recovery %	References
Sb(III), total Sb	Urine and different samples	Online CPE	ETV-ICP-OES	872	0.09	–	4.3	[9]
Sb(III), total Sb	Different sample matrices	SPE	GFAAS	50	0.02	–	7.0	[11]
Sb(III), total Sb	Different water, white wine and nut samples	CPE	HG-ICP-OES	–	0.09	–	–, 85–107	[13]
Sb(III), total Sb	Different food packaging materials	CPE	ETAAS	–	0.02	0.1–3	7.8	[14]
Sb(III)	Serum samples	Multivariate optimization with CCD	GFAAS	–	0.3	0–30	2.6–5.6	[15]
Sb(III), Sb(V)	Seawater, river water and hot spring water	Preconcentration on nanometer-sized $\text{TiO}_2$	FI-HGAAS	50	0.05, 0.06	0.1–30	2.1, 2.4	[16]
Sb(III), total Sb	Waters	HS-SDME	ETAAS	176	0.025	0–2	4.7	[20]
Sb(III), total Sb	Different water samples	VASEME-SFO	ETAAS	53	0.09	0.4–8	5.4	[21]
Sb(III), total Sb	Different water samples	DLLM	ETAAS	(115)	0.05	0.05–5	2.9–4.5	[22]
Sb(III), Sb(V)	Blood plasma and urine samples	MME or CPE	Spectrophotometry	20 (27.3, 21.9)	0.05, 0.08	0.2–20, 0.4–25	1.9–2.7	[24]
Sb(III), total Sb	Well and lake water	CPE	ETAAS	28	0.03	0.1–3.5	6.5	[26]
Sb(III), total Sb	Well and lake water	Online lab-in-syringe CPE	Spectrophotometry	28	1.8	5–50	1.6	[28]
Sb(III), total Sb	Water, vegetable and tea samples	SFODME	ETAAS	187.5, 437.5	0.005	0.06–0.45	3.5	[31]
Sb(III), total Sb	Water samples	UA-EME	FAAS	67	0.62	4.0–900	3.6	[32]
Sb(III), Total Sb	Milk-based products, beverages	UA-CPE	FAAS	45, 40 (85, 80)	0.13, 0.28	0.5–180, 1–180	1.8–3.5	This study

0.45–1.80  $\mu\text{g L}^{-1}$  for free Sb(II) and 3.45–7.60  $\mu\text{g L}^{-1}$  for total Sb in analyzed beverages do not pose a threat to human health except for a beer sample (Efes dark) with a value of 7.60  $\mu\text{g L}^{-1}$ .

### The comparison of the method with other methods reported in literature

According to our knowledge, the proposed method is the first approach for selective determination of Sb(III) selected as analyte including Sb(V) and total Sb by UA-CPE coupled to FAAS. TAR, which is a more sensitive and selective reagent for Sb(III), specially permits to detection of its more lower concentrations from those determined directly by FAAS as a result of synergistic effect of boric acid as buffer component at optimal concentration of 0.08  $\text{mmol L}^{-1}$  in mixed micellar media containing SDS and Tween 80 at pH 6.0.

As can be seen in Table 5, the proposed UA-CPE method gives lower or comparable LODs [with a detection limit of 0.13 and 0.28  $\mu\text{g L}^{-1}$  in a wide linear range of 0.5–180 and 1.0–180  $\mu\text{g L}^{-1}$  for Sb(III)] with TAR and TAC, respectively, minimum solvent consumption, good selectivity/precision [3.80 and 2.15 % for Sb(III)] and reasonable sensitivity enhancement factor [85 for Sb(III)] when considered those of the other analytical methods such as online lab-in-syringe CPE/spectrophotometry [28], CPE/FAAS [26], GFAAS after multivariate optimization [15], CPE/spectrophotometry [27], CPE/ETAAS [14], CPE/ETV-ICP-AES [9], GFAAS after preconcentration on tetraethylenepentamine-bonded silica gel column [11] and CPE/HG-ICP-OES [13], VASEME-SFO/ETAAS [21], FI-HGAAS after preconcentration on nanometer-sized  $\text{TiO}_2$  [16], MME/spectrophotometry [24], DLLME/ETAAS [23] and HS-SDME/ETAAS [20] for antimony. Moreover, the

more sensitive, but expensive and time-consuming techniques such as HG-ICP-OES, ETV-ICP-OES, ETAAS and/or GFAAS show poor precision and recovery than those of our newly developed method (9, 11, 13–15, 20–22, 26). In addition, the method can also determine the concentrations of total Sb after pre-reduction and Sb(V) from difference between total Sb and Sb(III) in presence of boric acid as synergistic buffer component without doing any tedious speciation procedure. Therefore, the application of the method to complex matrices is analytically facilitated comparing with hyphenated techniques LC/HG-AFS, LC/HG-ICP-OES and LC/HG-ICP-MS that require a laborious and time-consuming separation/speciation procedure.

## Conclusions

Antimony above  $13 \text{ mg L}^{-1}$  for air/C<sub>2</sub>H<sub>2</sub> flame can be identified directly with FAAS. Because the Sb levels in food and beverage matrices are highly below this amount, it usually needs a preconcentration step or more sensitive analytical instrumentation such as ETV-ICP OES, FI-VG-ICP-MS, HG-ICP-OES, and GF-AAS which are very expensive, time-consuming and needs experienced user in his/her area. In the present study, the UA-CPE combined with FAAS provides a simple, fast and efficient way for the determination of low levels of Sb in milk products and beverages with and without alcohol. Simplicity, low cost, good accuracy and precision, simple apparatus and minimum organic solvent consumption are the main advantages of the proposed method. Moreover, a low detection limit, high sensitivity improvement and preconcentration factors in a wide linear range were easily obtained by this method. The Sb(III), Sb(V) and total Sb prior to detection by FAAS can quantitatively be separated and preconcentrated from the complex matrices with help of TAR and/or TAC in presence of SDS and Na<sub>2</sub>SO<sub>4</sub> as salting-out agent by using Tween 80 as extracting agent at pH 6.0. In this context, due to higher sensitivity than TAC, especially TAR was selectively used for the first time for the determination of inorganic Sb species in milk products and alcoholic and nonalcoholic beverages.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Compliance with ethics requirements** This article does not contain any studies with human or animal subjects.

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