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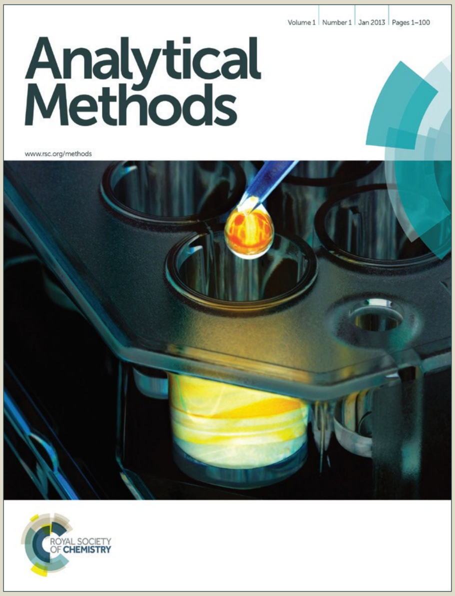

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A simple, inexpensive and convenient procedure for determination of inorganic Sb species in milk and beverage samples in PET containers by Flame Atomic Absorption Spectrometry

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Abstract

A simple and efficient Cloud Point Extraction (CPE) method has been proposed for separation and preconcentration of inorganic Sb species. The method is based on stable hydrophobic complex formation of Sb(III, V) with Pyronin B in presence of sodium dodecyl sulfate (SDS) as both auxiliary ligand and sensitivity improvement agent, and polyethylene glycol *tert*-octylphenyl ether (Triton X-114) as extracting agent at pH 2.0. The inorganic Sb species were detected and quantified by flame atomic absorption spectrometry (FAAS). Under the optimized conditions, the limits of detection of 1.68 and 4.28 μg L^{-1} for Sb(III) and Sb(V) along with sensitivity improvement factors of 117.6 and 83.4 respectively, were obtained. The method was successfully applied to a reliable determination of Sb(III, V) and total Sb in beverage and milk samples, and the recoveries in the range of 97.0–100.6 % were obtained from the spiked samples. The accuracy was checked by analysis of two certified reference materials (CRMs) with a matrix similar to that of the samples. The values obtained were in accordance with those reported for the certified values.

Keywords: Inorganic Sb Species, Beverages, Milk Samples, Flame Atomic Absorption Spectrometry, Food safety

Highlights

► A new CPE/FAAS method is proposed for detection of Sb(III, V) in milk and beverage samples.

► It is based on competitive reaction of Sb(III, V) with Pyronin B in presence of SDS.

► A speciative determination was also verified to be reliable by means of total Sb analysis.

► The sampling procedure under ultrasonic power is a simple, low-cost and eco-friendly.

1. Introduction

Antimony (Sb), which can have both acute and chronic toxicity effects, is an emerging contaminant regulated in drinking water in the United States, Canada, Europe and Japan at action levels ranging from 2 to 6_μg L^{-1}. [1] Sb contamination can result from metal smelting, petroleum refineries, but new studies have shown that it can also leach from polyethylene terephthalate (PET) plastic bottles due to the use of antimony trioxide as catalyst during PET manufacture. [2] Knowledge of the inorganic Sb speciation at trace levels in water, beverage and food samples is of significance because the bioavailability and the physiological and toxicological effects of antimony depend on its chemical form.[3] Thus, in order to obtain accurate and reliable information on the toxicity and biotransformation of antimony, it is necessary not only to determine the total amount of antimony but also to differentiate Sb species in food and beverages stored in PET containers.

Many scientific studies related to determination of Sb species in waters, soft drinks and beverages stored in PET containers using different detection tools were extensively conducted by Krachler and other authors.[4-10] When it is considered health risks associated with PET bottled mineral waters and beverages, the detailed quantitative information's about leaching of Sb from PET bottles into waters, beverages and foodstuffs depending on storage time and temperature, color of bottle, pH, type of beverage or food were extracted.

In this sense, several analytical methods such as flame atomic absorption spectrometry (FAAS),[11] electrothermal vaporization inductively coupled plasma optical emission spectrometry (ETV-ICP-OES),[12] hydride generation and inductively coupled plasma optical emission spectrometry (HG-ICP-OES),[13] inductively coupled plasma mass spectrometry (ICP-MS),[14] electrothermal atomic-absorption spectrometry (ET-AAS),[15] atomic fluorescence spectrometry (AFS),[16] and hydride generation atomic fluorescence spectrometry (HG-AFS) [17] have been reported for the speciation analysis of Sb. The FAAS is a robust element-selective detection technique, which is easy to use, high precise, inexpensive and no need experienced-user with high sample throughput in many research laboratories. It is widely used for the determination of trace amounts of metal ions. However, because of the presence of Sb in real samples at low levels, antimony separation from other elements present and also the use of a preconcentration step is usually necessary before determination. A sample pre-treatment such as liquid-liquid extraction (LLE),[18] solid phase extraction (SPE) with and without flow injection, which are not time-consuming and do not need large volume of sample, especially when on-line systems are employed,[19-21] and slurry [22] are usually necessary. Unfortunately, all of the remaining off-line methods are time-consuming, need a large sample volume and because excess use of organic solvents are not eco-friendly. So, the development of a simple, eco-friendly, rapid, cheap, selective and sensitive analytical method for determination of antimony in real samples was significant.

Recently, Cloud Point Extraction (CPE) has attracted attention for limiting the use of toxic organic solvents. So, it is an eco-friendly analytical technique. The main advantages of CPE to other separation techniques are cheap, simplicity, lower toxicity to the environment, high recoveries and high concentration factors. Cloud point temperature is defined as the temperature that micelles are formed and became turbid of surfactants in aqueous solution.[23] Above the temperature, the micellar solution is separated into two phases that a surfactant-rich phase of a small volume and a dilute aqueous phase. When analyte reacts with a suitable ligand and form an aqueous low solubility complex, the analyte-containing complex can be extracted from the aqueous solution into a small-volume of surfactant-rich phase. We have recently reported several CPE methodologies developed for the determination of As,[24] Sn [25] and Sb [11] by FAAS in different foods and beverages.

In the existing study, a new CPE approach combined with FAAS has been developed for determination of Sb(III, V) and total Sb in beverage and milk samples in PET containers without needing chelating agent. The method is based on ion-pairing complexation of anionic borate complexes of Sb(III, V) with Pyronin B in presence of SDS using Triton X-114 as extracting agent at pH 2.0 Britton-Robinson (BR) buffer. The CPE approach was successfully used for the separation and preconcentration of inorganic Sb species from milk and beverage matrices with a measurable sensitivity difference. The method was also verified by analysis of the Sb contents of two CRMs after separation and preconcentration with CPE.

2. Experimental

2.1. Reagents and standard solutions

All chemicals and reagents used were of analytical-reagent grade or higher purity. Ultra-pure water (18.2 MΩ cm, Milli-Q, Gradient with Elix10, Millipore, USA) was used in all preparations and dilutions. The standard solutions, 1000 mg L^{-1} of Sb(III, V) ions were prepared by taking appropriate amounts of $SbCl_3$ and $SbCl_5$ for Sb(III) and Sb(IV), respectively. Then, the working solutions were prepared by stepwise dilution of the stock solutions with water. A 5.0 % (v/v) Triton X-114 and 1.0×10^{-3} mol L^{-1} sodium dodecyl sulfate (SDS) were prepared by dissolving 5.0 mL of Triton X-114 and a suitable amount of SDS in water in 100 mL volumetric flask, respectively. 3.0×10^{-4} mol L^{-1} of Pyronin B was prepared by dissolving an appropriate amount of reagent (Sigma, St. Loius, MO, USA) in water. A BR buffer solution of pH 2.0 was prepared by using a mixture of 0.04 mol L^{-1} boric acid (Merck, Darmstadt, Germany), 0.04 mol L^{-1} phosphoric acid (Merck) and 0.04 mol L^{-1} acetic acid (Merck) that has been titrated to the desired pH with 0.2 mol L^{-1} NaOH. All the glass and plastic vessels used for trace analysis were kept in 10 % (v/v) HNO_3 for at least 24 h and subsequently washed five times with water.

2.2. Instrumentation

An atomic absorption spectrometer (AAS-6300, Shimadzu, Kyoto, Japan) equipped with D_2-background correction, an antimony hollow cathode lamp and an air-acetylene flame atomizer, was used for all determinations. The wavelength, lamp current, spectral bandwidth, burner height, acetylene and air flow rates were 217.6 nm, 10 mA, 0.5 nm, 7 mm, 2.0 L min^{-1} and 10.0 L min^{-1}, respectively. A refrigerator was used to keep the beverage samples fresh and cool till the analysis. A thermostatic water bath (EPC 4420, Thermal, and Istanbul, Turkey) was used to maintain the temperature in CPE experiments. A centrifuge (Universal-320, Hettich Centrifuges, DJB Labcare Ltd., Newport Pagnell, and Buckinghamshire, England) was used to accelerate the phase separation process. The pH measurements were carried out with a pH meter (pH-2005, JP Selecta, Barcelona, Spain). Eppendorf vary-micropipettes (10–100 and 200–1000 μL) supplied from Sigma-Aldrich were used to deliver accurate volumes. An ultrasonic cleaner (UCS-10 model, Jeio Tech, Co., Ltd., Seoul, Korea) was used to degas and digest the milk and beverages with and without alcohol under the ultrasonic effect (150 watt, 40 Hz).

2.3. The general CPE procedure

A cloud point experiment has been carried out according to the following procedure. An aliquot of the sample or standard solution containing Sb (III, V) in the range of 5-400 and 15-500 μg L^{-1} respectively, 0.3 mL of 5.0 % (v/v) of Triton X-114, 1.75 mL of 1.0×10^{-3} mol L^{-1} of SDS, 2.5 mL of 1.0×10^{-3} mol L^{-1} of Pyronin B and 3.5 mL of pH 2.0 BR buffer solution were mixed into centrifuge tubes (50 mL in capacity). Then, the mixture was kept at 30 °C for 5 min in the thermostatic bath for equilibration. The separation for two phases was accelerated by centrifuging at 4000 rpm for 5 min. The mixtures were then cooled in an ice-bath for 10 min in order to increase the viscosity of the surfactant-rich phase and facilitate the removal of the aqueous phase. Then, aqueous phase of the mixture was easily separated from surfactant-rich phase by inverting the tube. The remaining surfactant-rich phase was dissolved in 0.75 mL of 0.1 mol L^{-1} HNO_3 in order to reduce its viscosity. Then, the resultant solution was directly introduced into nebulizer of FAAS for determination of Sb species. Finally, the Sb contents of selected samples were determined by using either direct calibration curves or standard additions approach based on detection by FAAS in order to suppress the matrix effect when necessary.

2.4. Digestion of CRM$_S$, beverage and milk samples by ultrasonic-assisted extraction

The beverages with and without alcohol, and milk samples in PET containers were purchased from local open-markets and in a Turkish store in Sivas, Turkey. Beverage and milk samples were subjected to ultrasonic assisted digestion prior to analysis. A known volume of each beverage and milk samples (5-15 mL) in PTFE flasks, in which the approximately 0.5 g and 20 mL of solid and liquid certified samples, SRM 1549 Nonfat milk powder and SRM 1643e Trace elements in water are analyzed respectively, was added to flasks of 50 mL. The flasks were placed in an ultrasonic water-bath, and were subjected to ultrasonic effect (150 watt, 40 Hz) for different time intervals of 3, 6, 9, 12, 15, 20 and 30 min. The temperature of the water bath initially was kept at 40 °C. After each time intervals the mixture solution of each flask were centrifuged at 3000 rpm for 10 min and the liquid phase was separated with a micropipette. After a sonication time of 12 min, a maximum signal was obtained for both Sb(III) and Sb(V) spiked at levels of 25 µg L^{-1}. Then, added 2 mL of 1.0 mol L^{-1} HNO_3 and 1 mL of 1.0 mol L^{-1} HCl (2:1, v/v) to each extracted solution and heated on ultrasonic bath for a time of 12 min at 40 °C according to matrix type and concentration level of analyte until a complete clear solution is obtained, then made volume 50 mL in a volumetric flask with water and kept at 4 °C until analysis. A mixture of KI and ascorbic acid in acidic media for measurement of total Sb levels of samples was used for reducing quantitatively Sb(V) to

Sb(III) before a reliable speciation analysis. An analyte blank including two CRMs for quality control was also submitted to the procedure in a similar way. After that, the digested samples were submitted to CPE procedure, and analyzed by FAAS according to the present method at pH 2.0 under the optimized reagent conditions. The three pointed calibration curve approach for spiked samples was preferably adopted in order to calculate recovery values and check the accuracy of results.

3. Results and Discussion

3.1. The general considerations related to method development

Based on enhancement of acidity of boric acid (pK_a: 9.24) in presence of *cis*-1,2-diols, *cis*-2-hydroxy acids, *cis*-1,2-dicarboxylic acids and polyhydric alcohols such as glycerol and mannitol and anhydrous sulfuric acid due to the formation of chelated structures, [26-28] it may be expected that acidity of aqueous solution of H_3BO_3 enhances in the presence of $Sb(OH)_3$ and $SbO(OH)_3$, in which it is available in form of neutral species in aqueous micellar media at pH 2.0 with pK_a values of 11.8 and 2.7, respectively. Based on changing acidity of boric acid as a component of BR buffer used to control pH of micellar media, without using a chelating agent for speciation of Sb species, it can be concluded that the anionic mononuclear (i) and binuclear (ii) mixed antimony-borate complexes form through condensation reactions based on coordinated-covalent bond formation and acid-base interaction, in which Sb–OH bonds interact with B-OH bonds with elimination of 1 mole water, and electron pair on Sb(III) especially transfers on the electron-deficient B(III) center as follows:

(i) Anionic mononuclear and binuclear Sb(III)-borate complexes, $HOSbO_2B(OH)O^-$ and $HOSbO_2B^-O_2SbOH$

(ii) Anionic mononuclear and binuclear Sb(V)-borate complexes, $(HO)O{=}SbO_2B(OH)O^-$ and $(HO)O{=}SbO_2B^-O_2Sb{=}O(OH)$

Also, the stabile complex formation between boric acid and neutral oxyacids of Sb at pH 2.0 may be supported by analysis of antimony and indium with ET-AAS in presence of boric acid used as matrix modifier. [29,30]

As a consequence, the determination of inorganic Sb species is based on competitive ternary complexation of the anionic Sb(III,V)-borate complexes formed in chelate structure with cationic xanthene dye, Pyronin B at pH 2.0, and then to be extracted into the micellar core of nonionic surfactant, Triton X-114 in presence of SDS as both a sensitivity improving agent and oppositely charged auxiliary ligand. Since Pyronin B has a conjugation in its planar structure, it is a highly resonance-stabilized ion-pairing reagent interacting with anionic Sb-borate complexes at pH 2.0. This stability is due to distribution of positive charge on diethyl amine groups and hetero-oxygen atom. Hence, the ternary complex formed in presence of SDS as micellar improving agent is easily and fastly transferred to surfactant-rich micellar phase of Triton X-114. Moreover, the anionic Sb-complexes, formed in micellar media at pH 2.0, are structurally compatible with resonance-stabilized planar Pyronin B molecule, with regard to the enhancement of acidity of boric acid in aqueous solution due to the formation of mononuclear and binuclear mixed Sb-borate complexes of the types $HOSbO_2B(OH)O^-$ /$HOSbO_2B^-O_2SbOH$ and $(HO)O{=}SbO_2B(OH)O^-$/$(HO)O{=}SbO_2B^-O_2Sb{=}O(OH)$ respectively, in which the electron deficient B(III) attains tetra-covalence through chelation. It can be concluded that the binuclear Sb(III, V)-borate complexes will be more stable as compared to the mononuclear Sb-borate complexes in terms of hydrophobicity. Therefore, for further applications, before detection with FAAS, the various analytical variables affecting CPE efficiency such as pH, buffer concentration, concentrations of Pyronin B, SDS and Triton X-114 including other operational variables were optimized in order to achieve the maximum sensitivity at fixed Sb(III) and Sb(V) concentrations of 100 µg L^{-1} in detail. Each point in optimization step was three times repeated, and the measurement results as average plus standard deviation were represented with error bars on figures.

3.2. Effect of pH and buffer concentration on CPE efficiency

The formation of a stable metal complex is the two important factors involved in the separation and preconcentration of metal ions by CPE. The pH is a critical factor for the formation of these conditions. Thus, the effects of different buffer solutions such as citrate, phthalate and BR buffers were examined for the formation of hydrophobic metal complex in the range of 1.0–6.0 in the presence of SDS as sensitivity improving agent and Triton X-114 as extracting agent. From the results in Figure 1(a), the best signal for Sb(III, V) has been obtained when BR buffer solution at pH 2.0 is used. It can be seen that chelating agent, Pyronin B approximately three times behaves more sensitive and selective to Sb(III) than Sb(V) in presence of SDS at pH 2.0. Also, the effect of buffer concentration on the analytical signal was examined in the range of (0.0–4.0)×10^{-3} mol L^{-1} concentration (Figure 1(b)), and the best analytical signal was obtained using a buffer solution of 2.0×10^{-3} mol L^{-1}. Thus, a buffer concentration of 2.0×10^{-3} mol L^{-1} at pH 2.0 was adopted as optimal value for further studies.

3.3. Effect of ion-pairing reagent's concentration on CPE efficiency

Pyronin B, which is a cationic xanthene dye at planar structure with the pK$_a$ values of 6.9 and 10.1,[31] forms a hydrophobic complex with both Sb(III) and Sb(V) with a significant sensitivity difference at pH 2.0, but Sb(III)–Pyronin B complex is more sensitive and suitable for measurements with FAAS owing to formation of more stable ternary complex in presence of SDS. The effect of the concentration of Pyronin B on analytical signal intensity of Sb(III) and Sb(V) were examined in the range of (0.00-0.10)×10^{-4} mol L^{-1}. The results were shown in in Figure 2(a). The signal intensities of Sb(III) and Sb(V) strongly depend on the amount of Pyronin B. From the results obtained, with increasing concentration of Pyronin B, the signal intensity of both Sb(III) and Sb(V) has increased in initial and the maximum signal intensity has achieved in 0.05×10^{-4} mol L^{-1}. Then, the analytical signal for both species gradually has decreased due to aggregation of Pyronin B, in which the concentration dependent monomer-dimer equilibrium is occurred in range of (0.05-0.10) $\times10^{-4}$ mol L^{-1}; $2InH^{+} \leftrightarrow (InH)_2^{2+}$. Thus, Pyronin B concentration of 0.05×10^{-4} mol L^{-1} has been selected as optimal value for further studies.

3.4. Effect of amount of ionic surfactant on CPE efficiency

The variations in analytical signal as a function of ionic surfactant concentration such as CPC, CTAB and SDS are examined. These ionic surfactants were chosen as auxiliary signal enhancing ligands. The dependence of CPE to concentration of ionic surfactants was studied in the range of $(0.00\text{-}0.06)\times10^{-3}$ mol L^{-1} in the presence of both Sb(III) and Sb(V) for each surfactant. The highest analytical signal has been obtained when SDS is used as ionic surfactant for both species. As can be seen from the in Figure 2(b), the CPE efficiency increases up to SDS concentration of 0.035×10^{-3} mol L^{-1}, and gradually decreases in higher concentrations. The reason of signal decrease may be increase in blank signal in absence of analyte due to ion-pairing complex formation between cationic Pyronin B and anionic SDS. Thus, SDS as ionic surfactant at optimal concentration of 0.035×10^{-3} mol L^{-1} was selected for further studies.

3.5. Effect of amount of nonionic surfactant on CPE efficiency

To the present time, non-ionic surfactants (mainly polyoxyethylenated alkylphenols) are those most widely employed for metal analysis with CPE. The surfactants are commercial availability, high purity grade, stable, non-volatile, low toxicity and eco-friendly. A successful

CPE should maximize the preconcentration factor by minimizing volume of surfactant-rich phase. Therefore, the effects of different nonionic surfactants such as Ponpe 7.5, Triton X-114 and Triton X-100 on the analytical signals of 100 μg L^{-1} of Sb(III, V) were studied in range of 0.01–0.10 % (v/v). The signals obtained from Triton X-114 are bigger and higher than those of Ponpe 7.5 and Triton X-100 with the maximum signals value at concentration of 0.03 % (v/v) of Triton X-114 for both Sb species. Antimony signal reaches up to the largest value when the surfactant concentration increases up to a value of 0.03 % (v/v). In higher concentrations of Triton X-114 (Figure 2(c)), the analytical signal starts gradually to decrease. This can be attributed to an increase in volume and viscosity of the surfactant phase. Thus, Triton X-114 concentration of 0.03 % (v/v) was selected as optimal value for further studies.

3.6. The effect of other experimental parameters

As a result of experimental studies performed, the results for both Sb(III) and Sb(V) show that an equilibration temperature of 30 °C and a time of 5 min were sufficient to achieve quantitative extraction. Moreover, from the studied centrifuge time (in range of 0-30 min) and rates (in range of 1000-5000 rpm), it was observed that centrifugation for 5 min at 4000 rpm leads to the maximum recovery and sensitivity for both Sb(III) and Sb(V).

4. The performance properties of the present method

Analytical parameters of the method proposed for determination of Sb species by the CPA/FAAS were determined. These parameters are summarized in Table 1. Under the optimized conditions, the calibration curves were obtained by preconcentration of 50 mL of sample. After preconcentration, the linear working ranges were 5-400 and 15-500 μg L^{-1} with changing calibration sensitivity for Sb(III, V), respectively. The method detection limits (DLs) are 1.68 and 4.28 μg L^{-1} for Sb(III) and Sb(V) along with preconcentration factor of 62.5 and sensitivity enhancement factors of 117.6 and 83.4, respectively. Sensitivity enhancement factor (EF) is calculated as the ratio of slopes of calibration curves obtained with and without preconcentration. Preconcentration factor (PF) is defined as the ratio of the initial solution volume to the volume of surfactant rich phase. The obtained results are consistent with each other. The relative standard deviations (RSDs) after preconcentration with CPE have been in the range of 3.42-2.15 and 3.75-2.54 % for six replicate measurements of 50 and 250 µg L^{-1} of Sb(III) and Sb(V), respectively while it is in the range of 2.65 and 2.50 % for six replicate measurements of 500 µg L^{-1} Sb(III) and Sb(V) in the conditions without preconcentration.

5. Matrix effect

The matrix effects of the common cations and anions in selected samples on the extraction of 100 µg L^{-1} Sb(III) due to give more sensitive responses than those of Sb(V) were examined. The tolerated amounts of an ion were the concentration values tested that caused error more than 5.0 % the recovery alteration. According to the results obtained, the recoveries are quantitative and satisfactory in the presence of a large amount of foreign common cations (Na^+, K^+, NH_4^+,Ca^{2+}, Mg^{2+}, Zn^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Al^{3+}, Pb^{2+}, Cd^{2+}, Hg^{2+}) and anions (Cl^-, Br^-, I^-, NO_3^-, NO_2^-, SO_4^{2-}, $H_2PO_4^-$, SeO_3^{2-}) in the selected matrices do not cause interference on CPE of Sb(III, V) ions up to a tolerance ratio of maximum 1500. The most serious interference with tolerance ratio ranging from 1 to 25 has been observed from oxidizing species such as CrO_4^{2-}, MoO_4^{2-}, Fe^{3+} and Cu^{2+} in acidic media including F^- and As^{3+} ions. The interference effect may be due to fact that the oxidant ions oxidize ion-pairing reagent, Pyronin B at pH 2.0, and this would make the ternary complex formation between Sb(III, V) and Pyronin B less efficient. The interference effects of F^-, which is available in forms either $SbOF_2^-$ or BF_4^- and As(III), which is available in forms of AsO(OH) or $As(OH)_3$ at pH 2.0 BR buffer, may result from the fact that they give stable complexes, which can transfer into the surfactant-rich phase. To solve these problems, their interfering effects were successfully eliminated up to a tolerance ratio changing in range of 100-150 by using suitable masking agents like thiosulfate, pyrophosphate and citric acid after reduction of oxidant ions with ascorbic acid at pH 2.0. Moreover, at initial, the interferences arising from organic matrix components such as lactose, fat, proteins, vitamins and enzymes were completely removed by pretreatment with acid mixture (2:1, v/v, HNO_3/HCl) at 40 ^oC under ultrasonic power.

6. Optimization of oxidation of Sb(III) to Sb(V) and determination of total Sb

After the CPE procedure under the optimized reagent conditions at pH 2.0, 1.5 mL the resultant solution was directly introduced into the conventional nebulizer of FAAS for determination of Sb(III) and Sb(V) with a significant sensitivity difference in presence of each other. For accurate and reliable determination of total Sb, initially reduction conditions of Sb(V) to Sb(III) was also tested. For this purpose, different types of reducing agents such as ascorbic acid, KI, L-cysteine, sulfite, thiourea and thiosulfate have been used for reduction of Sb(V) to Sb(III) so far. These reducing agents are different advantages and disadvantages. In present study, a mixture of ascorbic acid and KI in acidic media was chosen as it is stable, readily available and easy to use. Effect of reducing agent volume was studied in the range of (0–20) mL of mixture of ascorbic acid (1.0×10^{-2} mol L^{-1}) and KI (1.0×10^{-3} mol L^{-1}) for reduction 100 µg L^{-1} of Sb(V) to Sb(III) with sonication time 15 min at 40 ^oC. A volume of reducing mixture of 8.0 mL was enough in order to completely reduce Sb(V) to Sb(III) in the presence of 0.5 mol L^{-1} HCl. Total Sb was detected and determined as equivalent analytical signal to Sb(III) by FAAS after pre-reduction of Sb(V) to Sb(III) in binary mixtures. For direct analysis of Sb(V), the binary mixtures at Sb(III)/Sb(V) ratios ranging from 1:9 to 9:1 were submitted to CPE procedure prior to detection of the total Sb by FAAS. The analysis results of Sb(III), Sb(V) and total Sb are shown in Table 2. From the results, it has been seen that the direct simultaneous analysis can quantitatively be conducted in binary mixtures with a recovery ranging from 95.0 % to 99.1 % and a RSD ranging from 1.9 % to 5.3 % as a measure of accuracy and precision. Also, the accuracy of speciation Sb(V) analysis without any interference effect can be seen from the difference between Sb(III) and total Sb contents.

6.1. Accuracy and determination of Sb species in real samples

In order to control the accuracy of the method, it was applied into analysis of two CRMs (SRM-1643e Trace elements in water and SRM 1549 Nonfat milk powder, which are supplied from NIST (Gaitersburg, MD, USA). In addition to, the recovery experiments were performed for five replicate measurements after additions of standard Sb solutions into the CRMs at known concentrations. It has been found that the recoveries are quantitative in range of 96.0–99.6 % with a RSD in range of 2.5-4.2 % for both Sb(III) and Sb(V). The accuracy parameters are summarized in Table 3. A good agreement was found on both CRM samples with the certified values and the total Sb concentration determined by the proposed CPE method, respectively. In order to control the possible matrix effect, the total Sb content of SRM 1549 Nonfat milk powder with a reasonable RSD of 7.7 % was determined by means of standard addition method after pre-reduction with a mixture of KI and ascorbic acid in HCl media. The Sb(III) and Sb(V) contents of the certified milk sample were directly not detected by using the proposed method due to be under method detection limit. The accuracy of the method was statistically verified by using the one-paired t-test. It can be seen that the experimental t-values are statistically lower than the theoretical t-values at 95 % confidence level. We have investigated the applicability of the CPE methodology using preconcentration with Pyronin B in mixed surfactant media for the determination of amounts of Sb(III), Sb(V) and total Sb in milk and beverage samples with and without alcohol. After ultrasonic-assisted digestion, the digested samples were analyzed for the presence of trace Sb species, Sb(III) and Sb(V), and total Sb without and with reduction with a mixture of KI and ascorbic acid with the present CPE/FAAS method. The results are summarized in Table 4. The results indicate the successful applicability of the method for determination of Sb species in the selected samples. The recoveries for the spiked Sb(III) and Sb(V) at different concentration levels into the samples were evaluated, and were found to change in the range of 96.5–99.0 %. Also, the precision of the method as RSD % was in range of 2.7-6.1 %.

When it is compared with the other preconcentration techniques previously published in literature,[11, 32, 34, 36 and 44] it is clear in Table 5 that the present method has advantages such as very good sensitivity, selectivity, accuracy, precision, quantitative recovery, wider working range of 80-fold, lower and comparable detection limits and high sensitivity enhancement factors of 117.6 and 83.4 for Sb(III) and Sb(V), respectively. The LDs obtained in a similar speciation study conducted by CPE-FAAS [32] are of the same order as those in our present work. However, when it is considered other advanced preconcentration techniques such as DDLM, SDME, LSME, HF-LPME and USAE-ME, [12, 21, 33, 35-42] in which more sensitive but cost-effective detectors like Total XRF, ET-AAS, ICP-OES and ICP-MS for speciation analysis are used, the advantage of the presented method can be reliably applicability to more complex samples such as milk and beverages with a good accuracy and precision at especially low concentrations. In order to obtain a more accurate and reliable results, the proposed CPE/FAAS method does not additionally need to use chemometric tools such as PLS model and Box-Behnken design for optimization of analytical variables. Moreover, due to stabilize internal conversion of Sb(III, V) species of boric acid as a component of pH buffer used (via complexation at different stabilities), there is the advantages of avoiding the use of chelating agent over the existing methods in literature, that needs to bind only one of Sb(III, V) species with a significant sensitivity difference. Also, the present method is reasonably cheaper, more simple and convenient for analytical purposes when compared with other methods.

7. Conclusions

In the present study, a new CPE method based on mixed surfactants was efficiently used as a separation and preconcentration tool to detect the inorganic Sb species in milk and beverage samples in PET containers. Advantages of the cloud point methodology such as easy, safe, rapid, inexpensive and the Pyronin B as a new selective and sensitive ion-pairing reagent for dissolved Sb species, Sb(III, V) were successfully utilized for their determination in selected samples. In contrast to other preconcentration techniques, no organic solvent is used; thus, the environmental pollution is limited to a little amount of surfactant, Triton X-114, which is commercially available at low cost. The present method is inexpensive, because it consists of much low equipment and running costs. The combination of CPE with FAAS can be considered as an alternative tool to costly, tedious and requiring an experienced-user detection techniques such as ICP-MS, ETV-ICP-OES, and GF-AAS. Finally, it may be concluded that the present method will be successfully applied to preconcentration and determination of inorganic Sb species in different matrices for a local laboratory.

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Conflict of interest

The authors declare that it is not any conflict of interest.

Compliance with Ethics Requirements

This article does not contain any studies with human or animal subjects.

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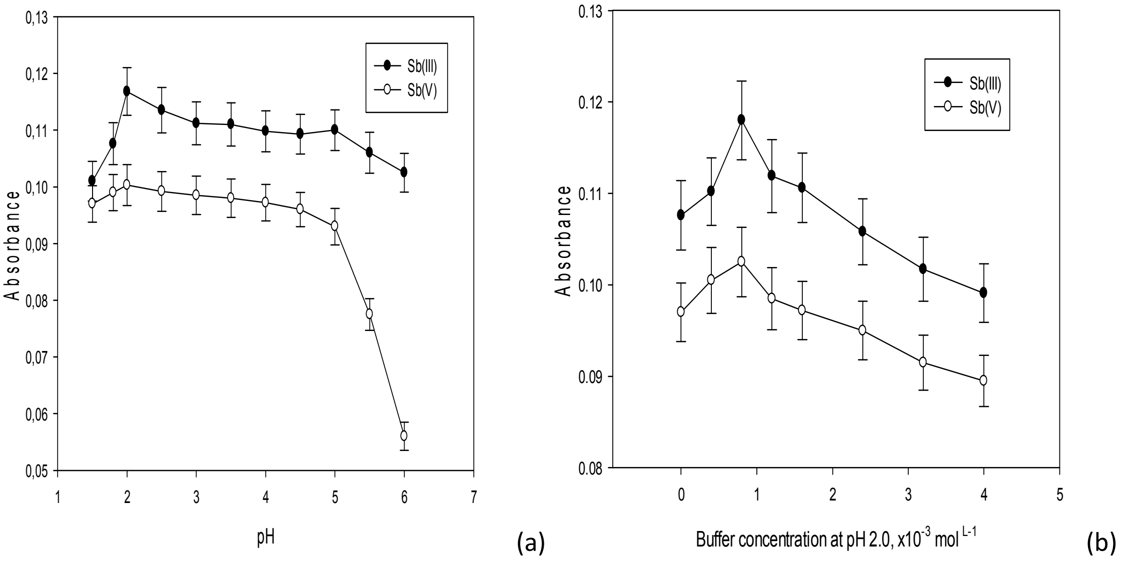

(a) (b)

Figure 1 The effect of (a) pH and (b) buffer concentration on CPE efficiency

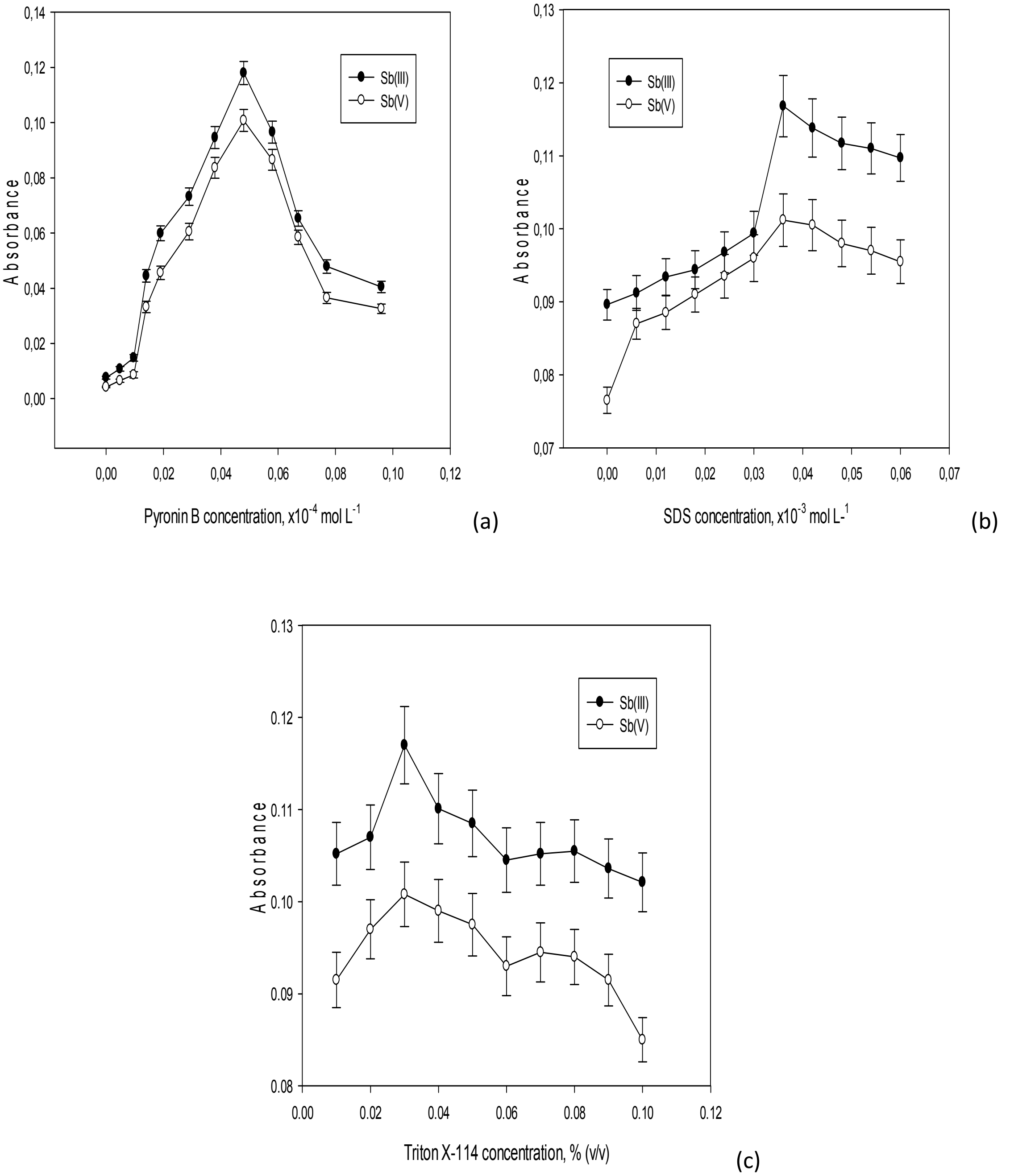

Figure 2 The effect of (a) ion-pairing reagent concentration, (b) anionic surfactant, SDS concentration and (c) nonionic surfactant, Triton X-114 concentration on CPE efficiency

Table 1 Analytical features of the proposed CPE/FAAS method

Parameter	Analytical features			
	After preconcentration		Before preconcentration	
Analytical species	Sb(III)	Sb(V)	Sb(III)	Sb(V)
Linear range, μg L^{-1}	5-400	15-500	250-3500	350-4500
Slope (m)	0.5055	0.1751	0.0043	0.0021
Intercept (b)	0.0661	0.0831	0.0102	0.0156
Correlation coefficient, r^2	0.9960	0.9844	0.9948	0.9924
RSDs (%) (n: 6, 50, 250 and 500 μg L^{-1})	3.42-2.15	3.75-2.54	2.65	2.50
Detection limit, DL (n: 12, $3\sigma_b/m$), μg L^{-1}	1.68	4.28	68.3	93.2
Quantification limit, QL (n: 12, $10\sigma_b/m$), μg L^{-1}	5.60	14.3	227.7	310.7
[a]Enhancement factor, EF	117.6	83.4	-	-
[b]Preconcentration factor, PF	62.5	62.5	-	-

[a] Enhancement factor is calculated as the ratio of slopes of calibration curves obtained with and without preconcentration

[b] Preconcentration factor is defined as the ratio of the initial solution volume to the volume of surfactant rich phase

Table 2 The results of speciation analysis for artificially prepared binary mixtures containing Sb(III) and Sb(V)

Sb(III)/Sb(V) ratio	Added Sb(III) (μg L^{-1})	Added Sb(V) (μg L^{-1})	*Found Sb(III) (μg L^{-1})	RSD %	Recovery %	**Total Sb (Sb(III)+Sb(V), μg L^{-1})	RSD %	Recovery %	*Found Sb(V) (μg L^{-1})	RSD %	Recovery %
4:1	80	20	79.5±1.7	2.1	99	98.5±2.1	2.1	98	19.2±0.6	3.1	96.0
3:2	60	40	59.6±1.6	2.7	99	97.8±2.1	2.1	98	39.4±1.3	3.3	98.5
1:1	50	50	49.4±1.4	2.8	99	97.5±2.2	2.3	98	49.2±1.4	2.8	98.4
2:3	40	60	39.7±1.3	3.3	99	96.8±2.2	2.3	97	59.4±1.6	2.7	99.0
1:4	20	80	19.8±0.7	3.5	99	96.7±2.2	2.3	97	79.3±1.7	2.1	99.1
1:9	10	90	9.7±0.4	4.1	97	96.3±2.4	2.5	96	88.3±2.1	2.4	98.1
9:1	90	10	89.3±2.1	2.4	99	99.2±2.2	2.2	99	9.5±0.4	4.2	95.0

* The average values plus their standard deviations for direct five replicate measurements of Sb(III) and Sb(V)

**The total Sb values found as the equivalent to Sb(III) from the calibration curve after reduction with a mixture of KI/Ascorbic acid in acidic medium

Table 3 The Sb(III), Sb(V) and total Sb levels for five replicate measurements of Sb contents of CRMs after application of the CPE/FAAS method

CRMS	Certified value, µg kg^{-1} or µg L^{-1}	Sb(III)				Sb(V)				Total Sb	***The experimental t-value
		Added,	*Found,	RSDs %	Recovery %	Added,	*Found,	RSDs %	Recovery %	**Found,	
SRM-1549 Nonfat milk powder	^{a} 0.27	-	under MDL[b]	-	-	-	under MDL[b]	-	-	0.26±0.02	1.12
		10	9.7±0.4	4.1	97.0	10	9.6±0.4	4.2	96.0	19.2±0.7	-
		25	24.5±0.7	2.8	98.0	25	24.5±0.8	3.3	98.0	48.5±1.5	-
SRM-1643e Trace elements in water	56.88±0.60	-	7.6±0.3	3.9	-	-	49.4±1.8	3.6	-	57.0±2.0	0.37
		10	17.4±0.6	3.4	98.0	15	64.1±2.0	3.1	98.0	82.3±2.5	-
		25	32.5±1.0	3.1	99.6	75	124.1±3.1	2.5	99.6	156.2±3.5	-

*The average values plus their standard deviations of five replicate measurements

**Samples were initially digested under ultrasonic power (150 watt, 40 Hz) using 2 mL of 1.0 mol L^{-1} HNO_3 and 1 mL of 1.0 mol L^{-1} HCl (2:1, v/v). After pre-reduction of Sb(V) to Sb(III) with a mixture of KI and ascorbic acid in acidic media, the average value plus standard deviation found for total antimony by using three pointed standard addition curve method under the optimized conditions

*** The tabulated Student's t- is 2.78 for 95 % confidence level and four degrees of freedom

[a] The certified value (or informed value) determined with ICP-MS for m/z: 123 in which the major and minor/trace species in sample matrix are Na, K, Mg, Ca, P, S, Zn, Al, Fe, Mn, Cu, Mo, Se, Pb, Co, Cr, As, Sb, Cd and Hg

[b] MDL, method detection limit

Table 4 The Sb(III), Sb(V) and total Sb contents of liquid samples after preconcentration by FAAS/CPE (sample volumes ranging from 5 to 15 mL, n: 5)

Samples	Added Sb(III), µg L^{-1}	[a]Found Sb(III), µg L^{-1}	Recovery %	RSD %	Added Sb(V), µg L^{-1}	[b]Found Sb(V), µg L^{-1}	Recovery %	RSD %	[c]Total Sb, µg L^{-1}
Milk and fruit-flavoured milk									
Cow milk	-	2.20±0.12	-	5.4	-	4.10±0.2	-	4.9	6.35±0.3
	15	16.90±0.6	98.0	3.6	10	13.90±0.5	98.0	3.6	31.10±0.9
Goat milk	-	3.80±0.2	-	5.3	-	8.50±0.4	-	4.7	12.50±0.6
	15	18.65±0.6	99.0	3.2	10	18.30±0.6	98.0	3.3	37.30±1.2
Sheep milk	-	2.55±0.13	-	5.1	-	5.80±0.3	-	5.2	8.50±0.4
	15	17.35±0.6	98.7	3.5	10	15.60±0.6	98.4	3.8	33.40±1.0
Banana-flavoured milk	-	2.65±0.13	-	4.9	-	6.10±0.3	-	4.9	8.60±0.4
	15	17.45±0.6	98.7	3.4	10	15.90±0.6	98.0	3.8	33.40±1.0
Strawberry-flavoured milk	-	3.50±0.2	-	5.7	-	8.30±0.4	-	4.8	11.60±0.5
	15	18.30±0.6	98.7	3.3	10	23.10±0.7	98.7	3.0	36.30±1.2
Peach-flavoured milk	-	2.50±0.13	-	5.2	-	5.80±0.3	-	5.2	6.10±0.3
	15	17.30±0.6	98.7	3.5	10	15.50±0.6	97.0	3.9	30.80±0.9
Beverages with alcohol									
Red wine, Doluca	-	3.40±0.2	-	5.9	-	4.90±0.3	-	6.1	8.50±0.4
	15	18.15±0.7	98.3	3.9	10	14.60±0.7	97.0	3.6	33.30±1.2

White wine, Doluca	-	3.35±0.2	-	6.0	-	6.85±0.4	-	5.8	10.40±0.5
	15	18.20±0.7	99.0	3.8	10	16.65±0.6	98.0	3.6	35.15±1.2
Beer, Tuborg	-	2.10±0.12	-	5.7	-	3.00±0.15	-	5.0	5.30±0.3
	15	16.90±0.7	98.7	4.1	10	12.80±0.6	98.0	4.7	30.15±1.0
Beer, Carlsberg	-	3.40±0.2	-	5.9	-	3.70±0.2	-	5.4	7.20±0.4
	15	18.15±0.7	98.3	3.8	10	13.50±0.6	98.0	4.4	31.80±1.0
Beer, Marmara gold	-	2.65±0.15	-	5.7	-	3.55±0.2	-	5.6	6.40±0.3
	15	17.50±0.7	99.0	4.0	10	13.40±0.6	98.5	4.5	31.15±1.0
Beverages without alcohol									
Apple Cider Vinegar	-	4.10±0.2	-	4.9	-	5.35±0.2	-	3.7	9.38±0.3
	10	13.90±0.4	98.0	2.9	10	15.05±0.4	97.0	2.7	29.20±0.7
Grape Vinegar	-	3.45±0.15	-	4.3	-	4.35±0.2	-	4.6	7.74±0.3
	10	13.25±0.4	98.0	3.0	10	14.20±0.4	98.5	2.8	27.60±0.7
Cola (Le cola)	-	5.15±0.2	-	3.9	-	8.50±0.3	-	3.5	13.72±0.2
	10	14.90±0.4	97.5	2.7	10	18.15±0.5	96.5	2.8	32.80±0.8
Orange juice (Le Fanta)	-	-	-	-	-	-	-	-	0.90±0.05*
	10	9.70±0.3	97.0	3.1	10	9.85±0.3	98.5	3.0	21.10±0.6
Lemonade	-	5.35±0.2	-	3.7	-	12.75±0.5	-	3.9	18.22±0.3
	10	15.25±0.5	99.0	3.3	10	22.60±0.7	98.5	3.23	37.65±0.9

[a] The average value plus standard deviation found for Sb(III) by using the calibration curve method under the optimized conditions

[b] The average value plus standard deviation found for Sb(V) by using the calibration curve method under the optimized conditions

[c] Samples were initially digested under ultrasonic power (150 watt, 40 Hz) using 2 mL of 1.0 mol L^{-1} HNO_3 and 1 mL of 1.0 mol L^{-1} HCl (2:1, v/v). After pre-reduction of Sb(V) to Sb(III) with a mixture of KI and ascorbic acid in acidic media, the average value plus standard deviation found for total antimony by using standard addition curve method under the optimized conditions

* After pre-reduction the average value plus standard deviation found by using standard addition curve method under the optimized conditions

Table 5 Comparison of analytical performance of the proposed CPE/FAAS method with those of other preconcentration methods in literature

Sample matrix	Analytical species	Detection tool	Chelating agent	Preconcentration procedure	Detection limit	Precision, RSD %	References
Beverages and biological samples	Sb(III,V)	FAAS	VBO^+	CPE	0.25, 5.15 µg L^{-1}	0.24-2.35 % (10 and 100 µg L^{-1}, *n*: 5)	11
Water samples and urine samples	Sb(III, V)	ETV-ICP-OES	APDC	CPE	0.09 µg L^{-1}	4.3 %	12
Water and food samples	Sb(III,V)	STAT-FAAS	DDTC	FI-on line SPE	6, 8 ng L^{-1}	2.8 and 3.5 % (intra-day precision, 7.1 and 8.4 % (inter-day precision)	21
Seawater, antileishmania drug and human serum	Sb(III)	Spectrophotometry, 330 nm	Iodide	CPE	0.23 µg L^{-1}	3.32 and 1.85 % (10 and 70 µg L^{-1}, n: 8)	23
Artificial seawater and wastewater	Sb(III), total Sb	FAAS	BPHA	CPE	1.82, 2.08 µg L^{-1}	2.6 and 2.2 %	32
Well and lake Water samples	Sb(III)	ET-AAS	APDC	CPE	0.03 µg L^{-1}	6.5 % (0.4 µg L^{-1}, n: 10)	33

Commercial glucantime sample	Sb(III,V)	Spectrophotometry at 220-290, 220-320 nm	Pyrogallol	Speciation possibility with PLS calibration model at pH 2.0	(3.96-3.98)×10^{-5} mol L^{-1}	The relative error of 4.8 and 0.5 % in calibration of Sb(V) and Sb(III)	34
Water and wine samples	Sb(III), total Sb	HG-ICP-OES	DDTP	CPE	0.08 μg L	-	35
Natural waters	Sb(III,V)	Total reflection XRF	DDTC/1-octanol or APDC/CCl_4,	HF-LPME or DDLM	1.1 or 0.09 μg L^{-1}	-	36
Water samples	Sb(III,V)	ET-AAS	DDTC	LSME	2 ng L^{-1}	≤ 9.0 %	37
Natural water samples	Sb(IIl), total Sb	ET-AAS	BPHA/$CHCl_3$	SDME	8.0, 9.2 ng L^{-1}	6.6 and 7.1 % (0.2 μg L^{-1}, n: 7)	38
Natural water samples	Sb(IIl), total Sb	ET-AAS	BPHA/$CHCl_3$	DLLM	5, 6 ng L^{-1}	3.8 and 4.1 % (0.4 μg L^{-1}, n: 8)	39
Water samples	Sb(III)	FAAS	Bromopyrogallol	USAE-ME	0.62 μg L^{-1}	3.6 % (100 μg L^{-1}, n:	40

			red/undecanol			10)	
Environmental and biological samples	Sb(III,V)	TS-FF-AAS	DDTC/1-octanol	HF-SLME	0.8 µg L^{-1}	6.2 % (50 µg L^{-1}, n: 5)	41
Water and blood serum	Sb(III, V)	ET-AAS	DDTP	CPE	0.08 µg L^{-1}	4.0 %, 1.5 %	42
Food packaging materials	Sb(III), Total Sb	ET-AAS	APDC	CPE	0.02 µg L^{-1}	7.8 %	43
Seawater, antileishmania drug and human serum	Sb(III)	FI-Spectrophotometry, 345 nm	Iodide	CPE/optimization of the variables with Box–Behnken design	1.8 µg L^{-1}	1.6 % as CV (15 µg L^{-1}, n: 5)	44
Water samples	Sb(III), Se(IV)	Batch-ETV-ICP-MS	DDTC	CPE	0.03 µg L^{-1}	4.2 % as CV (1.00 µg L^{-1}, n: 5)	45
Different water samples	Sb(V)	Spectrophotometry	Rhodamine B	USAE-ME	0.6 µg L^{-1}	2.4 % (100 µg L^{-1}, n: 5)	46
Milk and beverage samples	Sb(III,V),	FAAS	Pyronin	CPE	1.68, 4.28	2.15-3.75 % (n: 6, 50,	The

Total Sb	B/SDS	μg L^{-1}	250 and 500 μg L^{-1})	proposed method

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DDTC, Diethyldithiocarbamate; APDC, ammonium pyrrolidine dithiocarbamate; DDTP, ammonium o,o-diethyl dithiophosphate; BPHA, N-benzoyl-N-phenyhydroxylamine; VBO^{+}, Victoria Pure Blue; Pyronin B, 3,6-Bis(diethylamino)xanthylium chloride

Experimental steps of the CPE procedure for detection of Sb(III), Sb(V) and total Sb in real samples.