

Analytical Methods

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3 **Ultrasound assisted-cloud point extraction combined with flame atomic absorption**
4 **spectrometry for selective preconcentration and determination of As(V) in selected**
5 **water and beverage samples**
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18 **Abstract**

19 In present study, to evaluate the inorganic arsenic species at $\mu\text{g L}^{-1}$ levels, a simple, low-cost
20 analytical method has been developed using flame atomic absorption spectrometry (FAAS).
21 The ultrasound-assisted cloud point extraction (UA-CPE) was efficiently used to separate and
22 preconcentrate As(V) in presence of excess As(III) in different samples of water and
23 beverages. The selective ion-association complex and/or π -stacking complex of As(V) with 3-
24 amino-7-dimethylamino-2-methylphenazine (Neutral red, NRH^+ or NR) in presence of
25 Pyrogallol at pH 8.0 were extracted into the surfactant-rich phase of polyethyleneglycolmono-
26 *p*-nonyphenylether (PONPE 7.5) from the sample matrix. After optimization of the UA-CPE
27 conditions, the limits of detection and quantification (LOD and LOQ) obtained for As(V) with
28 sensitivity enhancement factor of 47.3 at the linear range of 1.5-170 $\mu\text{g L}^{-1}$ were 0.45 and 1.5
29 $\mu\text{g L}^{-1}$, respectively. The relative standard deviation (RSD) as a measure of precision was
30 found in range of 2.7-4.2 %. The proposed method was successfully applied to the
31 determination of As(V) and total As in selected water and beverage samples after rapid and
32 efficient ultrasonic- and microwave-assisted extraction approaches before and after pre-
33 oxidation with KMnO_4 in acidic media. The concentration of As(III) was calculated from
34 difference between total As and As(V) concentrations. The accuracy was validated by
35 analysis of two certified standard reference materials (SRMs) including recovery rates of
36 spiked samples.
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50 **Keywords:** Inorganic Arsenic Species, Neutral Red, Ultrasound-Assisted Cloud Point
51 Extraction, Waters, Beverages, Flame Atomic Absorption Spectrometry
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Highlights

- A new UA-CPE method was efficiently combined with FAAS for selective detection of As(V).
- The method is based on the ion-association of As(V) with NRH^+ in presence of Pyrogallol.
- A detection limit of $0.45 \mu\text{g L}^{-1}$ for As(V) at the linear range of $1.5\text{--}170 \mu\text{g L}^{-1}$ was achieved.
- It is a selective and sensitive method for monitoring of trace As(V).

1. Introduction

Arsenic (As) is one of the most toxic elements that occurs in both inorganic and organic forms. Both forms of As can be found in the environment from natural sources^{1,2} and anthropogenic activities.^{3,4} Arsenic has received increased attention in recent years because of its carcinogenic and other toxic properties such as dermal changes, pulmonary, cardiovascular, gastrointestinal, hematological, renal, neurological, reproductive, immunologic and genotoxic, mutagenic.⁵⁻⁷ Speciation of inorganic arsenic in terms of As(III) and As(V) is often as important as total As quantification because of its varying degrees of toxicity.^{8,9} Arsenic in food and beverages has recently drawn the attention of analytical chemistry. Conventional atomic and molecular spectrometric methods typically measure total arsenic and are unable to differentiate the different arsenic species. Because different arsenic species have greatly different toxicities, inorganic arsenic is more toxic as compared to organic arsenic. Thus, new analytical methods are simultaneously needed to separate and quantify the inorganic arsenic species, As(III), As(V) and total As in order to assess the risks on human health related to the presence of arsenic in beverage and foods.

In general, the widely used analytical techniques for the direct detection of the arsenic species in water and beverage samples are high performance liquid chromatography coupled to inductively coupled plasma mass spectrometry (HPLC-ICP-MS),¹⁰ inductively coupled plasma optical emission spectrometry (ICP-OES),¹¹ hydride generation-atomic fluorescence spectroscopy (HG-AFS),¹² hydride generation atomic absorption spectrometry (HG-AAS),¹³ flame atomic absorption spectrometry (FAAS),¹⁴ inductively coupled plasma mass spectrometry (ICP-MS),¹⁵ graphite furnace atomic absorption spectrometry (GF-AAS),¹⁶ and electrothermal atomic-absorption spectrometry (ET-AAS)¹⁷ until now. Among these techniques, FAAS is still widely used in analytical chemistry. Moreover, the device has advantages such as convenience, selectivity, speed, precision and accuracy than others. Because of matrix effect and being at trace levels of arsenic in water and beverage samples, separation and preconcentration steps are still necessary for especially FAAS method with low sensitivity.¹⁸ Thus, preliminary separation and preconcentration are required prior to FAAS determination. For the determination of As(III) and As(V), the separation and preconcentration methods reported in the literature are usually based on atomic spectroscopic

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3 techniques after solid phase extraction (SPE),¹⁹ dispersive liquid–liquid microextraction
4 (DLLME),²⁰ single-drop microextraction (SDME),²¹ liquid–liquid extraction (LLE),²²
5 coprecipitation²³ and ultrasound assisted emulsification of solidified floating organic drop
6 microextraction (USAESFODME).²⁴ But these procedure have drawbacks such as time-
7 consuming, unsatisfactory enrichment factors, using toxic organic solvents and forming
8 secondary wastes. Unlike them, Cloud point extraction (CPE)¹⁴ is a versatile method for the
9 separation and preconcentration of metal ions having considerable properties such as
10 simplicity, inexpensive compared to organic solvents, fast, selectivity and sensitivity.

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12 The main aim of the present work was to develop a rapid, sensitive, selective, accurate
13 and reliable method for separation and preconcentration of only one specie, As(V) or As(III),
14 of inorganic As species in water and beverage samples using ultrasonic assisted (UA)-CPE
15 technique prior to FAAS. The method is based on the selective ion-association of As(V) with
16 3-amino-7-dimethylamino-2-methylphenazine (Neutral red, NRH⁺ or NR) in presence of
17 Pyrogallol at pH 8.0, and then extraction of ternary complex to micellar phase of
18 polyethyleneglycolmono-*p*-nonyphenylether (PONPE 7.5) as extracting agent. The factors
19 influencing the efficiency of UA-CPE for accelerating ion-association reaction and facilitating
20 phase separation were systematically investigated. The method was applied successfully to
21 the determination of As(III), As(V) and total As after selective separation and
22 preconcentration of trace As(V) in water and beverage samples with UA-CPE.
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36 2. Experimental

37 2.1. Instrumentation

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40 An atomic absorption spectrometer (AAS-6300, Shimadzu, Kyoto, Japan), equipped
41 with D₂-background correction, arsenic hollow cathode lamp and an air-acetylene flame
42 atomizer, was used for arsenic determinations. The wavelength, lamp current and spectral
43 bandwidth were 197.2 nm, 10 mA, 0.2 nm, respectively. A centrifuge (Universal-320, Hettich
44 Centrifuges, and England) was used to accelerate the phase separation process. The pH
45 measurements were carried out with a pH meter (pH-2005, JP Selecta, Spain). Eppendorf
46 vary-pipettes (10–100 and 200–1000 µL) were used to deliver accurate volumes. An
47 ultrasonic power (UCS-10 model, Jeio Tech, Co., Ltd., Seoul, Korea) was used to degas and
48 extract the water and beverage samples, also used to maintain the temperature in UA-CPE
49 experiments. A refrigerator at 4 °C was used to keep the samples fresh, and cool till the
50 analysis.
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2.2. Chemicals and Reagents

All chemicals and reagents used were of analytical-reagent grade or higher purity. Ultra-pure water with a resistivity of 18.2 M Ω cm was prepared using a Labconco (USA) water purification system. All solutions were prepared with this ultra-pure water. Stock solutions of As(III) and As(V) (1000 $\mu\text{g mL}^{-1}$) were prepared by dissolving appropriate amounts of As₂O₃ and Na₂HAsO₄ (Merck, Darmstadt, Germany) in 1 mol L⁻¹NaOH and 3 mol L⁻¹HCl solution, and then adjusting the pH to 7.0 with the water, respectively. The standard solutions used for construction of calibration curves were prepared by dilution of the stock solution with the water just before use. Stock solutions of 1.0 $\times 10^{-3}$ mol L⁻¹ NRH⁺ (Sigma, St. Louis, MO, USA) were prepared fresh daily by dissolving the reagents in ethanol (Merck) and diluting with the water. As it is not possible to obtain a real aqueous solution of the nonionic surfactant, PONPE 7.5 due to low cloud point, it was practically convenient to prepare a stock solution as follows: 2.5 mL of PONPE 7.5 (Sigma) and 5 mL of ethanol were mixed and made up to 100 mL with water. For the preparation of 100 ml pH 8.0 borate buffer solution, 50 mL of 0.05 mol L⁻¹ trisodiumtetraborate (Merck) and 44 mL of 0.1 mol L⁻¹HCl (Merck) solutions were mixed. The Pyrogallol solution, 1.0 $\times 10^{-3}$ mol L⁻¹ were prepared by dissolving an appropriate amount of chemicals (Sigma) in the water. The vessels and pipettes used for trace analysis were kept in 10% (w/v) HNO₃ for at least 24 h and subsequently washed five times with the water.

2.3. Preparation of water and beverage samples to analysis

All of the water and beverage samples selected for analysis were supplied from local supermarket in Sivas, Turkey. For the present study, four water samples, two non-alcoholic and three alcoholic beverages of different brands were randomly selected. Before preconcentration procedure, all water samples were pre-filtered using a 0.45 μm membrane filter. Initially, they were passed through a micro-column filled with a cation-exchange resin (Chelex 100 chelating resin, which is a styrene divinylbenzene copolymer that contains paired imino-diacetate, functional groups) around pH 6.0 in order to remove interfering ions like Cu(II), Fe(III), Mn(II), Ca(II) and Mg(II), which can be available at trace, minor and/or major levels in water and beverages. Thus, the effects of the possible interfering ions were greatly reduced in determination of inorganic As species. Then, the water samples were stored at 4 °C until analyzed. 50 mL of the water samples were preconcentrated by evaporation approximately to final volume of 10 mL. After that, the preconcentrated water samples for As(V) and total As were submitted to UA-CPE procedure, and analyzed by standard addition

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3 method before and after pre-oxidation for speciation analysis in order to control the matrix
4 effect. The As(III) contents were calculated from differences between the contents of As(V)
5 and total As.
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9 All beverage samples were subjected to total matrix digestion prior to analysis. Two
10 different extraction methods are applied for the all samples. In the first extraction procedure
11 for beverage samples, a mixture of 2.0 mol L⁻¹ HNO₃ and 1.5 mol L⁻¹ H₂O₂ (v/v, 5:1) were
12 added to 25 mL of beverage samples into beaker of 150 mL. Then, the samples were
13 submitted to the microwave oven programs of 5 min at 250 W, 5 min at 450 W and finally
14 5 min at 650 W. After extraction under microwave power, samples were cooled, transferred
15 and diluted to calibrated flasks of 50 mL with water after filtration with a membrane filter of
16 0.45 μm for subsequent analysis. It is worth underlining that beverages should be diluted as
17 little as possible to avoid diluting the low arsenic content. For oxidation of As(III) to As(V) in
18 the extracted samples, 1.5×10⁻⁴ mol L⁻¹ of KMnO₄ solution containing 0.25 mol L⁻¹HCl was
19 added to each, and in a similar way, the samples were submitted to the microwave oven
20 programs of 2 min at 250 W, 5 min at 450 W, 2 min at 650 W and finally 3 min at 900 W.
21 After oxidation with KMnO₄ in acidic media, total As analysis was also made by using FAAS
22 after preconcentration with UA-CPE under the optimized conditions.
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33 The steps of the second extraction (ultrasonic power) process are as follows; (1) 25
34 mL of the beverage samples was transferred into beaker of 100 mL. (2) Then, the samples
35 were added 10 mL of diluted HNO₃ (2.0 mol L⁻¹) and 5.0 mL of diluted HClO₄ (1.5 mol L⁻¹).
36 (3) The final volume of the mixture was completed to 100 mL with ultra-pure water. (4) The
37 mixture was initially heated in an ultrasonic bath at 65 °C for 15 min (300 watt, 60 Hz). (5)
38 The pH of the digested samples was adjusted to 7.0 by using diluted NaOH (2 mol L⁻¹). (6)
39 After centrifugation at 4000 rpm for 5 min, the extracted samples were filtered using the
40 membrane filter into a volumetric flask before analysis. Similarly, the preconcentrated
41 beverages for As(V) and total As were submitted to UA-CPE procedure, and analyzed by
42 standard addition method before and after pre-oxidation for speciation analysis in order to
43 control the possible matrix effect. The As(III) contents were calculated from differences
44 between the contents of As(V) and total As. Also, two different SRMs were studied in order
45 to verify the accuracy and precision of the method. The selected certified samples are SRM
46 1575a pine needles and SRM-1643e trace elements in water. The certified reference values
47 are available for arsenic for assessment of the method accuracy. The solid reference material
48 (0.5 g) was initially submitted to the extraction process under ultrasonic and microwave
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3 powers. Due to contain its interfering cationic ions at high levels, in a similar way to those of
4 water samples, it was separated from them by passing through a chelating cation-exchange
5 resin prior to UA-CPE. The liquid reference material was not submitted to any digestion
6 process. It was directly analyzed with the proposed UA-CPE method.
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10 11 12 13 **2.4. The general UA-CPE procedure**

14 In a set of 50 mL volumetric tubes containing 2.2 mL of pH 8.0 borate buffer, 1.75 mL
15 of 1.0×10^{-3} mol L⁻¹ Pyrogallol, 1.25 mL of 1.0×10^{-3} mol L⁻¹ NRH⁺, 0.75 mL of 0.01 mol L⁻¹
16 Na₂SO₄ and 0.8 mL of 2.5% (v/v) PONPE 7.5 in the range of 1.5–170 µg L⁻¹ As (V) were
17 mixed and kept in a ultrasonic bath (300 watt, 40 Hz). Ultrasound was applied to assist and
18 accelerate CPE under the equilibrium temperature of 35 °C for 5 min to start the process of
19 extraction and preconcentration of As(V) in the surfactant-rich phase, which accomplished in
20 an ultrasonic cleaner. The phase separation was accelerated by centrifuging at 4000 rpm for 5
21 min. Then, the resulting mixtures were cooled in an ice-bath for 5 min to increase the
22 viscosity of the surfactant-rich phase and make easy the extracted of the aqueous phase. Then,
23 the aqueous phase was easily separated from surfactant-rich phase by inverting the tube. The
24 surfactant-rich phase was dissolved and diluted to 1.0 mL of methanol containing 1.0 mol L⁻¹
25 HNO₃, and then the resultant solution was directly introduced into FAAS for determination of
26 As(V) as analyte. Finally, the inorganic As contents of water, beverage and certified samples
27 were determined by using standard addition method in order to control the possible matrix
28 effect around the detection limit.
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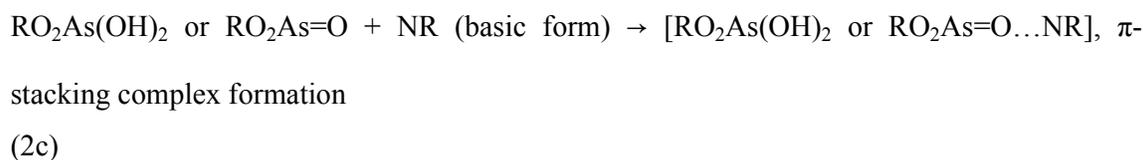
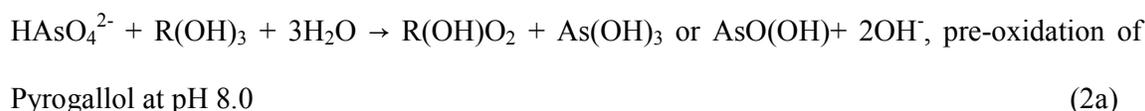
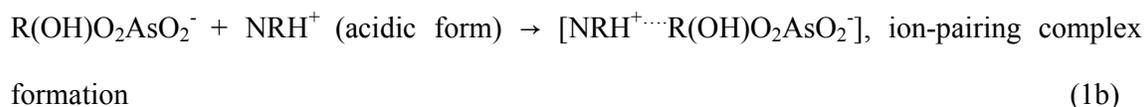
40 41 **2.5. Speciation study**

42 In the sense of method development, Neutral red as ion-pairing reagent is a cationic phenazine
43 group dye with a pK_a value of 6.8. At lower acidic pHs than 6.8, it is in form of NRH⁺ while
44 it is in form of NR at basic pHs. At pH 8.0, As(III) is predominantly in neutral form of
45 As(OH)₃/AsO(OH) with a pK_a value of 9.3 whereas As(V) is predominantly in ionic form of
46 AsO₃(OH)²⁻ or AsO₂(OH)⁻ with an acid ionization constants of pK_{a1}: 2.24, pK_{a2}: 6.96 and
47 pK_{a3}: 11.50. From prior studies conducted in pH range 3-10, it was observed that As(V)
48 according to As(III) at levels of 10 µg L⁻¹ gave selectively a more stable complex with a
49 significant sensitivity difference at pH 8.0. From the literature information's²⁵⁻²⁸ and these
50 findings based on selection of As(V) as analyte for further studies, it can be concluded that the
51 formed hydrophobic ternary complex is easily and rapidly extracted into the surfactant-rich
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phase of PONPE 7.5 under ultrasonic power. The possible complex formation mechanisms based on ion-association between anionic As(V)-Pyrogallol complex and NRH^+ or acid-base and π - π stacking interactions between As(III)-Pyrogallol condensation adduct and basic dye, NR after reduction of As(V) to As(III) at pH 8.0 may be postulated as follows:



(1a)



In speciation study as a consequence of the proposed method to be a selective and sensitive to only As(V), different types of oxidizing agents such as H_2O_2 , $\text{K}_2\text{Cr}_2\text{O}_7$ and KMnO_4 were studied for oxidation of As(III) to As(V) with different advantages and disadvantages. In this study, $1.5 \times 10^{-4} \text{ mol L}^{-1}$ of KMnO_4 solution containing 0.25 mol L^{-1} HCl was used as an oxidizing agent which allowed rapid and complete oxidation of As(III) to As(V) at room temperature. After the quantitative oxidation of As(III) to As(V), pH of the solutions was adjusted to 7.0 with diluted NaOH solution (1.0 mol L^{-1}). Then, the proposed method was applied for the determination of the total As by means of FAAS. Also, the concentration of As(III) is calculated from difference between total As and As(V) concentrations.

3. Results and Discussions

The effects of concentrations of reagents and nonionic surfactant, pH, temperature and time of equilibration, centrifugation rate and time on the analytical signal were investigated and optimized in order to reach the best analytical performance for the UA-CPE procedure.

3.1. Effect of pH and buffer volume on UA-CPE

The separation of As(V) or As(III) by UA-CPE method involves previous formation of a stable complex, which needs to present sufficient hydrophobicity to be extracted into the small volume of the surfactant-rich phase. Thus, the pH is a critical factor affecting both the reaction between ion-pairing reagent and As(III) or As(V)-Pyrogallol adducts, and the extractability of complex into the surfactant-rich phase for UA-CPE. Thus, in this part of experiment, the effects of different buffers were extensively studied for the extraction and determination of arsenic in the surfactant-rich phase in the range pH 5.5-11.0 (Figure 1). The maximum absorbance was obtained with borate buffer system with a significant sensitivity difference for As(V) at pH 8.0. Below the pH 8.0, extraction efficient is very low because of complex formation is inadequate as a measure of protonation of ion-pairing reagent, NRH^+ and dimerization equilibrium depending on its concentration and pH, $2\text{NRH}^+ \leftrightarrow (\text{NRH})_2^{2+}$ or $2\text{NRH}_2^{2+} \leftrightarrow (\text{NRH}_2)_2^{4+}$. It is implied in literature^{29,30} that the dye in low concentrations of $3.94 \times 10^{-5} \text{ mol L}^{-1}$ at $\text{pH} \leq 7.0$ is aggregated with a dimerization constant of K_D : 5.31. Also, above pH 8.0, the reason of decrease in extraction efficient can be deprotonation of Pyrogallol to phenolate anion due to slow and incomplete of redox reaction before hydrophobic complex. Hence, an optimal value was selected as a pH of 8.0 in order to give the highest sensitivity. Furthermore, the concentration of borate buffer solution as a function of volume of buffer solution at a fixed concentration on the analytical signal was also studied in range of 0.5–4.0 mL, and the best analytical signal was obtained by using 2.0 mL buffer solution in final volume of 50 mL.

3.2. Effect of complexing agents volume on UA-CPE

The UA-CPE efficiency depends on the hydrophobicity of the ligand and the complex formation. Hence, the effect of the volume of the NRH^+ at fixed concentration of $1.0 \times 10^{-3} \text{ mol L}^{-1}$ on the analytical signal was examined in range of (0.0–4.0) mL and the results were illustrated in Figs. 2. It could be seen that the signal intensity of As(V) strongly depended on the amount of NRH^+ . With the volume of NRH^+ increased from 0.0 to 1.25 mL, the signal intensity initially increased, and maximum signal intensity was achieved after the amount of the NRH^+ approached to 1.25 mL at fixed concentration of $1.0 \times 10^{-3} \text{ mol L}^{-1}$ and then

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3 gradually decreased due to degradation of ligand. Thus, 1.25 mL of 1.0×10^{-3} mol L⁻¹NRH⁺
4 was selected as optimal value for further studies.
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7 The variation of the analytical signal as a function of the volume of Pyrogallol at fixed
8 concentration of 1.0×10^{-3} mol L⁻¹ in the presence of 10 µg L⁻¹As(V) was studied in range of
9 (0.0–4.0) mL, and the results in Figs. 2 indicated that the signal intensity of the analyte
10 linearly increases with Pyrogallol volume up to 1.75 mL. The maximum signal intensity
11 linearly decreased with increasing slope at the higher volumes. The cause of this decrease in
12 signal may be complexation of Pyrogallol based on acid-base and π -stacking interactions with
13 NRH⁺ in absence of As(V) due to increase in blank signal. So, a Pyrogallol volume of 1.75
14 mL at fixed concentration of 1.0×10^{-3} mol L⁻¹ was selected as optimal value for further
15 studies.
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23 **3.3. Effect of amount of nonionic surfactant on UA-CPE**

24 In UA-CPE choosing an appropriate surfactant is important, since the temperature
25 corresponding to cloud point is correlated with the hydrophilic property of a surfactant. A
26 successful CPE should maximize the extraction efficiency by minimizing the phase volume,
27 thus increasing its concentrating capability. To the present time, non-ionic surfactants (mainly
28 polyoxy ethylenated alkylphenols) such as Ponpe 7.5, Tween series and Triton series are those
29 most widely employed for metal analysis with UA-CPE. The surfactants are commercial
30 availability, high purity grade, stable, non-volatile, relatively non-toxic and eco-friendly
31 reagents. In the preliminary experiments, it is observed that the addition of the nonionic
32 surfactants such as Triton X-45, Triton X-114 and PONPE 7.5 to ternary complex system of
33 As(III) or As(V) and heating the solution provides a successful extraction in Figure 3.
34 Therefore, the effect of volume of the surfactants at fixed concentration 5.0 % (v/v) on the
35 analytical signal of 10 µg L⁻¹ was examined in range of (0.0–3.0) mL. The best quantitative
36 extraction was observed with 0.6 mL of 2.5 % (v/v) PONPE 7.5. This value corresponds to a
37 maximum concentration of 0.0306 % (w/w) PONPE 7.5 above its critical micelle
38 concentration of 0.009 % (w/w). Therefore, 0.6 mL of 2.5 % (v/v) PONPE 7.5 was selected as
39 optimal value for further studies.
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52 **3.4. Effect of salting out agent concentration on UA-CPE**

53 Studies on the effects of some additives, such as anionic, non-ionic surfactants and inorganic
54 electrolytes such as NaCl, KCl, Na₂SO₄ and NH₄Cl, on the cloud point behavior of non-ionic
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3 surfactants have been reported.³¹⁻³³ It was observed that the presence of electrolytes decreases
4 the cloud point (salting-out effect), resulting in a more efficient extraction. The lower cloud
5 point is attributed to electrolytes promoting dehydration of the poly (oxyethylene) chains.
6 According to Komaromy-Hiller, the salting-out phenomenon is directly related to desorption
7 of ions to the hydrophilic parts of the micelles, increasing interaction between micelles and
8 consequently leading to the precipitation of surfactant molecules. Based on this discussion,
9 the influence of ionic salts strength such as NaCl, KCl, Na₂SO₄ and NH₄Cl on extraction
10 efficiency was studied in the range of (0.0–2.0) mL at a fixed inorganic salts concentration of
11 0.01 mol L⁻¹ under the optimized reagent conditions. The maximum absorbance was obtained
12 at 0.75 mL of 0.01 mol L⁻¹Na₂SO₄ as sensitivity enhancement agent. The absorbance
13 considerably decreased for increasing Na₂SO₄ volumes in range of 0.75–2.0 mL. This effect
14 might be explained by the additional surface charge when the Na₂SO₄ concentration is very
15 high, thus changing the molecular architecture of the surfactant and consequently the micelle
16 formation process. It is necessary to emphasize that different blank solutions were also
17 evaluated and no significant signal was obtained. Therefore, 0.75 mL of 0.01 mol L⁻¹ Na₂SO₄
18 was selected as optimal value for further studies.
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30 **3.5. Effects of equilibrium temperature and sonication time**

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33 Equilibrium temperature and time are important parameters to complete quantitatively
34 the complex formation and achieve an easy phase separation and preconcentration on UA-
35 CPE. Due to ultrasonic cavitation, ultrasound can accelerate the interactive rate between the
36 surfactant and aqueous phase, so that the target analyte could be well extracted into the
37 surfactant-rich phase. Hence, the effect of equilibrium temperature was investigated in range
38 of 30–60 °C under ultrasonic power (300 watt, 40 Hz). As a result of experimental studies, the
39 solutions became turbid as soon as the solutions were put into the ultrasonic bath with
40 temperature higher than 35 °C. The temperature had no considerable effect upon the
41 extraction efficiency and the analytical signal kept constant at temperature range of 30–60 °C.
42 Keeping the equilibrium temperature of 35 °C, the influence of sonication time on UA-CPE
43 was investigated in range of 2–30 min. It was seen that, 5 min was sufficient to achieve a
44 quantitative extraction of As(V). Thus, 35 °C and 5 min at fixed ultrasonic power (300 watt,
45 40 Hz) were chosen as the equilibrium temperature and sonication time for the propose UA-
46 CPE method respectively.
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56 **3.6. Effects of centrifugation rate and time**

Centrifuge time and rate are very necessary to preconcentrate trace amounts of As(V) with high efficiency in a short time. Thus, under optimal conditions obtained, the effect of the centrifuge time and rate were studied in range of 2-20 min and 500-4000 rpm, respectively. The results showed that centrifugation for 5 min at 4000 rpm and cooling for 10 min in an ice-bath leads to the maximum recovery and sensitivity for As(V).

3.7. Selection of diluting agent

The volume of the surfactant-rich phase acquired after UA-CPE is very viscous and small for detection by FAAS. Thus, before detection by FAAS the volume of the surfactant-rich phase can be decreased using diluting agents. It is very important to choose the suitable solvent for maximum extraction efficiency. The effect of various solvents such as methanol, acetone, acetonitrile, acidic methanol, acidic ethanol, ethanol and THF in range of 0.5-2.0 mL was studied to obtain the maximum analytical sensitivity and the best regression coefficient, r^2 after UA-CPE. The best absorbance as a measure of analytical sensitivity (m/s) and regression coefficient (r^2) was obtained in the presence of surfactant-rich phase diluted to 1.0 mL with methanol containing $1.0 \text{ mol L}^{-1} \text{ HNO}_3$ from calibration curves obtained for fixed As(V) concentrations of 5, 15 and $25 \text{ } \mu\text{g L}^{-1}$.

4. Optimization of oxidation of As(III) to As(V) and determination of total As

Different types of oxidizing agents such as H_2O_2 , $\text{K}_2\text{Cr}_2\text{O}_7$ and KMnO_4 have been studied for oxidation of As(III) to As(V) with different advantages and disadvantages. In this study, $1.5 \times 10^{-4} \text{ mol L}^{-1}$ of KMnO_4 solution containing $0.25 \text{ mol L}^{-1} \text{ HCl}$ was used as an oxidizing agent which allowed rapid and complete oxidation of As(III) to As(V) at room temperature out of any interfering of excess amount of KMnO_4 in total As determination step. Effect of KMnO_4 concentration at fixed concentration of $0.25 \text{ mol L}^{-1} \text{ HCl}$ was examined in the range of $(0.1-3.0) \times 10^{-4} \text{ mol L}^{-1}$ for oxidation of $25 \text{ } \mu\text{g L}^{-1} \text{ As(III)}$ with oxidation time of 5 min. It was found that KMnO_4 concentration is enough $1.5 \times 10^{-4} \text{ mol L}^{-1}$ in order to completely oxidize As(III) to As(V). After pre-oxidation, the mixtures were analyzed by proposed method to determine the total As levels.

The proposed UA-CPE/FAAS method was studied for speciation of the inorganic As species after oxidation of As(III) to As(V) using a KMnO_4 solution. For this step, the changing concentrations of As (III) in the range of $5-100 \text{ } \mu\text{g L}^{-1}$ in the presence of the fixed As(V) concentration of $5 \text{ } \mu\text{g L}^{-1}$ were spiked into the solution media. After oxidation with

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KMnO₄, total speciation analysis was also made by using FAAS based on preconcentration with UA-CPE under optimum conditions. The amount of As(III) ions were calculated from the difference between the amounts of As(V) and total As before and after oxidation due to give more reliable results. The same procedure was applied to determine the inorganic As species in real samples. The speciation results are given in Table 1. As can be seen from Table 1, the recoveries were reasonable for trace analysis, ranging from 98.2 % to 101 %. The REs and RSDs for five replicate measurements in range of 5-100 µg L⁻¹ As(III) in the presence of 5 µg L⁻¹ As(V) were between -(1.3-4.0) % and 2.2-4.1 %, respectively.

5. Interference study

Interfering ions may react with NRH⁺ or Pyrogallol and may decrease the formation of hydrophobic complexes with As(V) and NRH⁺. Thus, the effects of foreign ions on the preconcentration and determination of 20 µg L⁻¹ of As(V) under the optimized conditions by the proposed method were investigated. The tolerance limit was identified as the concentration of added ion that caused greater than ± 5.0 % relative error. As can be seen from Table 2, the results demonstrated that large excess most of the cations and anions did not interfere on the determination of 20 µg L⁻¹ of As(V). At initial, in order to suppress the effect of possible interfering cations, which can be available in sample matrix including solid SRM, samples were passed through a chelating cation-exchange resin acting as an anion-exchange resin ≤ pH 4.0 and a cation-exchange resin between pH 4.0-7.0. Thus, their interfering effects greatly were reduced for accurate and reliable determination of As(V) in real samples. Additionally, the interfering effects of interfering anionic and cationic species such as Cr³⁺, Fe²⁺, Fe³⁺ and C₂O₄²⁻ can be efficiently removed by the addition of suitable masking agents to the solution before preconcentration with UA-CPE. The results clearly point out the analytical performance of the propose method for real sample applications.

6. Analytical figures of merits

In order to evaluate the developed method, a series of experiments have been carried out under the optimized conditions. The calibration curve was constructed after preconcentration with UA-CPE. A good linear response was obtained in range of 1.5-170 µg L⁻¹ with regression coefficient of 0.9967 for As(V). After optimization studies by FAAS based on improvement of selectivity with a suitable reagent and pH for As(V) instead of selective detection tool such as HG-AAS, the limits of detection (LOD) and quantification (LOQ), which are defined as 3σ_{blank}/m and 10σ_{blank}/m (n: 10) (where σ_{blank} is the standard deviation of

twelve replicate measurements of the blank and m is the slope of the calibration curve) were 0.45 and $1.5 \mu\text{g L}^{-1}$, respectively. Also, the RSDs as a measure of precision for 5 replicate determinations of 5, 20 and $50 \mu\text{g L}^{-1}$ of As(V) were in the range of 2.2-4.7 %. The preconcentration factor (PF), which is calculated as the concentration ratio of analyte in the final diluted surfactant rich extract ready for FAAS determination and the initial solution was averagely 50 for As(V). The sensitivity enhancement factor (EF) was 47.3, which was calculated by using the ratio between the slopes of calibration curves obtained with and without the preconcentration with UA-CPE.

6.1. Accuracy of the UA-CPE method and analytical applications

The accuracy of the method was analyzed two SRMs: SRM 1575a (pine needles) and SRM-1643e (trace elements in water) after dilution of 15-fold and the results seen in Table 4(a). As can be seen from Table 4(a), the observed values (39.4 ± 0.8 and $58.3 \pm 1.0 \mu\text{g kg}^{-1}$ or $\mu\text{g L}^{-1}$ for five replicate measurements) found by using UA-CPE-FAAS for SRM 1575a pine needles and SRM-1643e trace elements in water were statistically in good agreement with the certified values of 39.0 ± 2.0 and $59.0 \pm 0.7 \mu\text{g L}^{-1}$. As the certified values were within the 95 % confidence interval about the mean of the experimentally determined values, there is no significant difference between the values. It can be concluded that the proposed method is accurate and free from systematic errors. Also, in order to confirm the accuracy of the proposed method, spiking was performed in five replicate at three concentration levels (5, 20 and $50 \mu\text{g L}^{-1}$) of As(V) for both SRMs. As a result, it has been found that the recoveries are highly quantitative in range of 98.6–101 % with a RSD ranging from 0.8 to 1.7 % for total As. The applicability of the method was successfully investigated by determining of inorganic As species in selected water and beverages. The beverages were pretreated with extraction under microwave and ultrasonic powers, according to procedure explained in preparation of water and beverage samples to analysis. 5.0 mL of the prepared solution samples were individually transferred into volumetric tubes of 50 mL. Then, the proposed method at levels of 5, 10 and $50 \mu\text{g L}^{-1}$ As(V) was applied to determine the amounts of As(V) by using the standard addition method. After oxidation of As(III) to As(V), the method was applied to determine the amount of total As. The amount of As(III) was determined by calculating the difference between total As and As(V). The results for the beverages analyzed using the evaluated UA-CPE-FAAS method are shown in Table 4(b). In any event, the calibration was attained using the aqueous standard calibration curves. The recoveries from spiked solutions were varied in the range of 98–104 % for microwave-assisted extraction and 98–103 % for ultrasonic-

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3 assisted extraction. Also, the method has directly been applied for the determination of As(V),
4 As(III) and total As in both hot- and cold-spring water including drinking water samples.
5 Because the inorganic As contents of samples are below the method quantification limit,
6 instead of direct calibration curve approach, the As(V) and total As contents of samples were
7 established by using standard addition calibration curves of spiked samples at levels of 5, 10
8 and 15 $\mu\text{g L}^{-1}$ As(V) before and after pre-oxidation with KMnO_4 in acidic media.
9 Additionally, the recovery experiments of spiked As(V) at levels of 5, 10 and 50 $\mu\text{g L}^{-1}$ were
10 carried out by using calibration curve method, and the results are shown in Table 4 (c). The
11 results indicated that the recovery rates are quantitatively at reasonable levels for trace As
12 analysis, ranging from 96.7 to 103 %.

20 6.2. The comparison of proposed method with the methods published in literature

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22 In the light of all these results, the proposed UA-CPE method gives low LOD ($0.45 \mu\text{g L}^{-1}$),
23 good RSDs (2.7-4.2 %), linear working range (from 1.5 to 170 $\mu\text{g L}^{-1}$), good preconcentration
24 (50) and sensitivity enhancement factor (43.2) according to SPE,¹⁹ SPME,²¹ LLME²² and
25 coprecipitation²³ methods reported in literature. Moreover, from literature information's, a
26 sensitivity improvement has been achieved by the method when compared to previously
27 reported using works UV-Vis including FAAS, HG-AAS, HG-AFS, ET-AAS, ICP-OES and
28 ICP-MS.^{13, 34-39} Moreover, the instruments such as ET-AAS, HG-AAS, ICP-OES and ICP-
29 MS are expensive, time-consuming and need expert user in her/his area. Unlike these
30 techniques, FAAS is a simple, cheap, easy operated, rapid response time, available element-
31 selective instrument in many laboratories. Also, the detection limit of the method is lower
32 than those of the reported other methods. As a result, the proposed method is versatile for
33 analysis of trace inorganic As contents of selected water and beverage samples. Moreover, the
34 presented study can also be extended to the other complex matrices and possible sources of
35 contamination.

46 7. Conclusions

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48 Because there are at lower amounts of inorganic As species in water and beverage samples, it
49 usually needs a preconcentration step or more sensitive analytical instrumentation such as
50 ETV-ICP-OES, ICP-MS, HG-ICP-OES, which are very expensive. Thus, in the present study,
51 a new UA-CPE method for the determination of inorganic As species in the samples is
52 described and evaluated by a conventional FAAS. The method allows arsenic detection at
53 0.15 $\mu\text{g L}^{-1}$ levels in a wide linear range of 113-fold, thus represents a promising approach in
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3 the monitoring of toxic inorganic As in the real samples. Advantages of the UA-CPE
4 methodology are easy, safe, rapid and inexpensive. Also, FAAS is a comparatively simple,
5 economical and a versatile element-selective detection tool, which can be available in nearly
6 every research laboratory. So, it can be considered an alternative to expensive and time-
7 consuming analytical techniques such as ICP-MS, ETV-ICP OES, ICP-AES, HG-ICP-OES
8 and ET-AAS. Moreover, NRH⁺ forming a stable ion-pairing complex and/or π -stacking
9 complex with As(V) or As(III) in the presence of Pyrogallol at pH 8.0 was for the first time
10 studied for selective determination of As(V) in selected water and beverages. The results
11 clearly show the potential and versatility of the method, which could be applied to the
12 selective detection of As(V) in presence of excess As(III) in the different complex matrices.
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20 **Acknowledgments**

21 Financial assistance from the Cumhuriyet University Scientific Research Projects
22 Commission (CUBAP), Sivas in Turkey, is sincerely thanked.
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25 **Conflict of Interests**

26 The author declares that they have no conflict of interest.
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30 **Compliance with Ethics Requirements**

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



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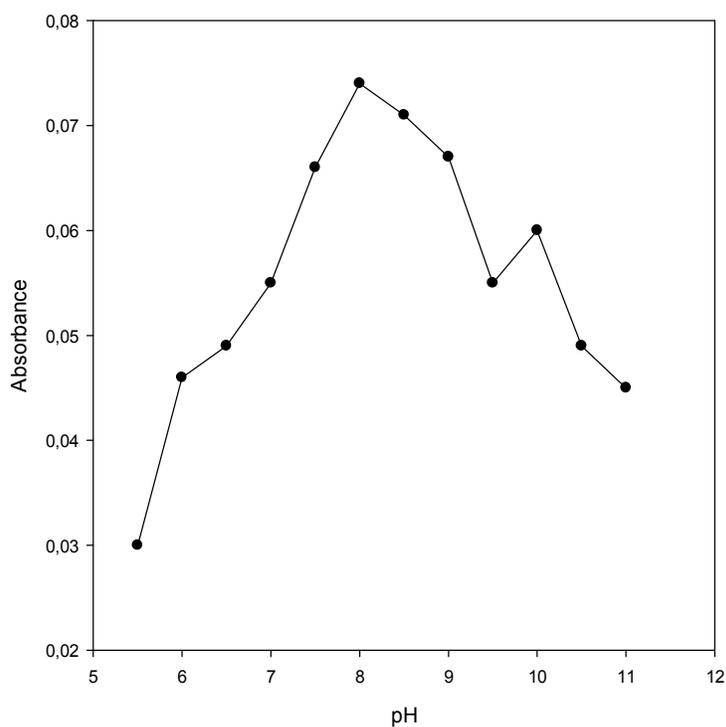


Figure 1 Effect of pH on UA-CPE efficiency. Optimal conditions: $10 \mu\text{g L}^{-1}$ As(V), 1.75 mL of $1.0 \times 10^{-3} \text{ mol L}^{-1}$ Pyrogallol, 1.25 mL of $1.0 \times 10^{-3} \text{ mol L}^{-1}$ NRH⁺, 0.75 mL of 0.01 mol L^{-1} Na₂SO₄ and 0.8 mL of 2.5 % (v/v) PONPE 7.5 under ultrasonic power (300 watt, 40 Hz) at 35 °C for 5 min and centrifugation time of 5 min at 4000 rpm

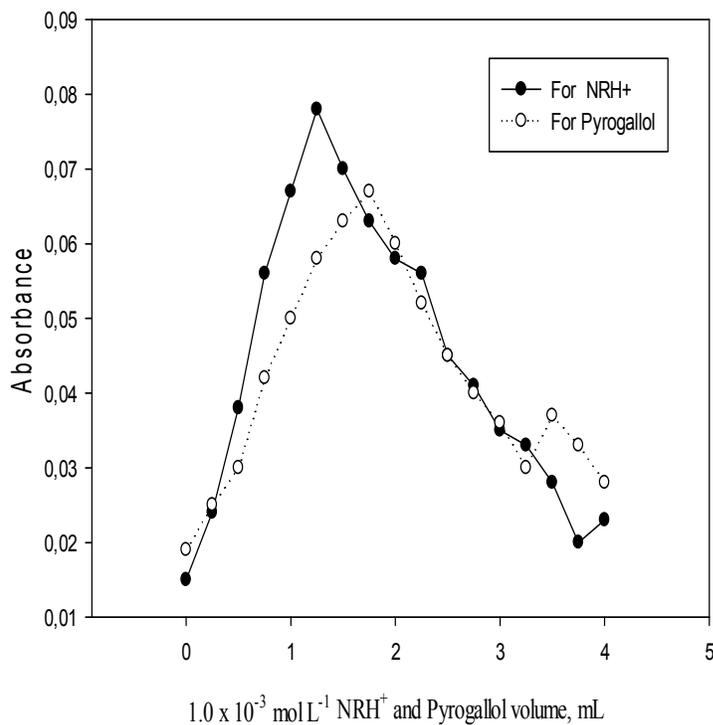


Figure 2 Effect of NRH⁺ and Pyrogallol volume on UA-CPE efficiency. Optimal conditions: $10 \mu\text{g L}^{-1}$ As(V), 2.2 mL of pH 8.0 borate buffer, 0.75 mL of 0.01 mol L^{-1} Na_2SO_4 and 0.8 mL of 2.5 % (v/v) PONPE 7.5 under ultrasonic power (300 watt, 40 Hz) at $35 \text{ }^\circ\text{C}$ for 5 min and centrifugation time of 5 min at 4000 rpm

Table 1 The determination of inorganic arsenic species (As(III), As(V) and total As) in artificially prepared binary mixtures

Sample, $\mu\text{g L}^{-1}$	Ratio	Added As(III), $\mu\text{g L}^{-1}$	Total As(V) +As (III), $\mu\text{g L}^{-1}$	Found As(III) $\mu\text{g L}^{-1}$	^a RSD (%)	^b RE (%)	Recovery (%)
-	-	5	4.90±0.2	4.9	4.1	-2.0	98.0
	1:1	5	9.8±0.3	4.8	3.1	-4.0	96.0
5	1:5	25	29.2±0.7	24.2	2.4	-3.2	96.8
	1:10	50	54.3±1.2	49.3	2.2	-1.4	98.6
	1:20	100	103.7±2.6	98.7	2.5	-1.3	98.7

^aThe relative standard deviation (RSD) of three replicate measurements after pre-oxidation with KMnO_4 in acidic media

^bThe relative error (RE) of three replicate measurements after pre-oxidation with KMnO_4 in acidic media

Table 2 Tolerance limits and recovery of interfering matrix ions for determination of 20 μgL^{-1} As(V) under the optimized conditions

Interfering species	*Tolerance limits, [Interferent]/[As(V)]	Recovery %
Na ⁺ , I ⁻ , NH ₄ ⁺ , Cl ⁻ and Mg ²⁺ ,	1500-1000	97-103
Br ⁻ , Sr ²⁺ , Zn ²⁺ , F ⁻ and Ca ²⁺	1000-750	98-103
SCN ⁻ , SO ₄ ²⁻ , Ag ⁺ and Sb ⁵⁺	750-500	96-103
Mo ⁶⁺ , K ⁺ , V ⁴⁺ , Sb ³⁺	500-300	97-103
Bi ³⁺ , Cu ²⁺ and HPO ₄ ²⁻	300-200	97-102
Sn ⁴⁺ , Hg ²⁺ and Mn ³⁺	200-150	98-104
Pb ²⁺ , Co ²⁺ , V ⁵⁺ , Mn ²⁺ and Al ³⁺	150-100	97-103
Ni ²⁺ , C ₂ O ₄ ²⁻ and Se ⁴⁺	100-50	96-103
Cr ³⁺ , Fe ²⁺ and Fe ³⁺	50-10	96-104

* The tolerance limit was identified as the concentration of added interfering ion that caused greater than $\pm 5.0\%$ relative error

Table 3 Analytical characteristics of the UA-CPE method

Analytical Parameters	After preconcentration with UA-CPE	Before preconcentration with UA-CPE
Linear calibration range, $\mu\text{g L}^{-1}$	1.5-170	25-240
Regression equation	$A=7.90 \times 10^{-3} C_{\text{As(V)}, \mu\text{g L}^{-1}} + 0.0054$	$A=1.67 \times 10^{-4} C_{\text{As(V)}, \mu\text{g L}^{-1}} + 0.094$
Correlation coefficient, r^2	0.997	0.991
RSD (%) (5, 20 and 50 $\mu\text{g L}^{-1}$, n: 5)	2.7-4.2	3.5-5.9
Detection limit (LOD) (n: 12, $3\sigma_b/m$), $\mu\text{g L}^{-1}$	0.45	17.8
Quantification limit LOQ (n: 12, $10\sigma_b/m$), $\mu\text{g L}^{-1}$	1.50	42.9
^a Preconcentration factor	50	-
^b Sensitivity enhancement factor	43.2	-

^aPreconcentration factor is defined as the ratio of the initial solution volume to the volume of surfactant rich phase

^bSensitivity enhancement factor is calculated as the ratio of slope of preconcentrated samples to that obtained without preconcentration

Table 4 (a) The total As contents of SRMs obtained by using the proposed UA-CPE-FAAS method

SRMs	Certified value (ng g ⁻¹ or µg L ⁻¹)	Added (µg L ⁻¹)	*Found (µg L ⁻¹)	Recovery %	RSD %	**Experimental t- value
		-	39.4±0.8	101	1.7	0.36
SRM 1575a Pine Needles	39.0±2.0	5	43.8±1.0	99.5	1.4	-
		20	58.2±0.7	98.6	1.2	-
		50	89.5±0.5	100	1.0	-
SRM-1643e Trace elements in water	59.0± 0.7	-	58.3±1.0	98.8	1.3	0.67
		5	63.6±0.8	99.4	1.1	-
		20	79.4±0.5	100	0.8	-
		50	109±0.4	100	0.8	-

*The average and its standard deviation of five replicate measurements at confidence interval of 95 %

** The experimental t-values calculated by using $\mu = x_{\text{average}} \pm tSD/N^{1/2}$ for five replicate measurements at confidence interval of 95 % in which the critical t-value is 2.78 for 4 degrees of freedom at confidence interval of 95 %

Table 4(b) Determination of inorganic arsenic levels of beverages and percent recoveries of spiked samples (5 mL of the sample, n: 5)

Samples	By microwave-assisted extraction (n: 5)				By ultrasonic-assisted extraction (n: 5)				*The variance ratio, F- test and Student' t- test		
	Added, $\mu\text{g L}^{-1}\text{As(V)}$	Found, $\mu\text{g L}^{-1}\text{As(V)}$	Recovery %	Total As(V) +As(III), $\mu\text{g L}^{-1}$	Found, $\mu\text{g L}^{-1}\text{As(III)}$	Added, $\mu\text{g L}^{-1}\text{As(V)}$	Found, $\mu\text{g L}^{-1}\text{As(V)}$	Recovery %		Total As(V) +As(III), $\mu\text{g L}^{-1}$	
	-	5.3±0.2	-	9.7±0.3	4.4	-	5.6±0.1	-	10.8±0.2	5.2	0.95 (0.77)
White wine	5	10.1±0.3	98.1	14.3±0.2	4.2	5	10.9±0.2	103	15.9±0.2	5.0	-
	10	15.6±0.3	102	20.0±0.4	4.4	10	16.0±0.2	102	21.4±0.3	5.4	-
	50	54.9±0.4	99.2	59.5±0.2	4.6	50	55.9±0.4	100	60.8±0.2	4.9	-
	-	7.6±0.5	-	13.6±0.4	6.0	-	8.2±0.3	-	15.0±0.1	6.8	1.36 (0.53)
Red wine	5	12.3±0.7	97.6	18.5±0.3	6.2	5	12.9±0.3	97.8	19.6±0.1	6.7	-
	10	17.2±0.6	97.7	23.3±0.3	6.1	10	18.5±0.4	102	25.2±0.3	6.7	-
	50	58.0±0.6	101	63.8±0.5	5.8	50	58.3±0.3	10	65.1±0.4	6.8	-
	-	4.6±0.2	-	7.9±0.1	3.3	-	5.2±0.1	-	9.3±0.1	4.1	1.17 (0.61)
Beer	5	9.4±0.4	97.9	12.9±0.2	3.5	5	10.0±0.2	98.0	14.3±0.1	4.3	-
	10	14.8±0.6	101	17.9±0.4	3.1	10	15.4±0.1	101	19.4±0.2	4.0	-
	50	55.1±0.5	101	58.2±0.5	3.1	50	55.3±0.2	100	59.5±0.3	4.2	-
	-	3.2±0.1	-	5.3±0.2	2.1	-	4.0±0.1	-	6.9±0.2	2.9	1.30 (0.28)
Apple Juice	5	8.5±0.3	104	10.4±0.2	1.9	5	9.2±0.2	102	12.2±0.1	3.0	-
	10	13.5±0.2	102	15.7±0.4	2.2	10	13.8±0.2	98.6	17.0±0.3	3.2	-

1												
2												
3												
4												
5		50	53.0±0.3	99.6	55.4±0.5	2.3	50	54.1±0.2	101	57.0±0.3	2.9	-
6												
7		-	4.8±0.4	-	7.5±0.1	2.7	-	5.5±0.1	-	9.3±0.09	3.8	1.08(0.69)
8												
9	Red	5	10.0±0.3	102	13.0±0.2	3.0	5	10.3±0.2	98.1	14.2±0.1	3.9	-
10	grape											
11	cocktail	10	14.6±0.2	98.6	17.4±0.2	2.8	10	15.3±0.2	98.7	19.3±0.3	4.0	-
12												
13		50	55.2±0.3	101	58.0±0.4	2.8	50	55.7±0.4	100	59.7±0.3	4.0	-

*In order to compare two mean values for independent two sample t- and F-tests with equal sample size the statistical t- and F-critical values at 95 % confidence level and 8 degrees of freedom are 2.31 and 6.39, respectively

Table 4 (c) The As(III), As(V) and total As contents of selected water samples (25 mL of water samples, n: 5)

Samples	As(V)				Total As			As(III)	
	Added, $\mu\text{g L}^{-1}$	Found, $\mu\text{g L}^{-1}$	RSD %	*Recovery %	Found, $\mu\text{g L}^{-1}$	RSD %	Recovery %	Added, $\mu\text{g L}^{-1}$	Found, $\mu\text{g L}^{-1}$
	-	**2.30±0.09	3.1	-	**3.10±0.1	3.2	-	-	0.9
Hot spring water	5	7.50±0.1	2.4	103	13.5±0.3	2.4	103	5	6.0
	10	12.7±0.5	1.5	103	22.8±0.4	1.3	98.7	10	10.1
	50	51.8±0.6	1.1	99	103±0.5	0.8	99.8	50	51.1
	-	**1.40±0.06	3.4	-	**2.3±0.2	3.0	-	-	0.9
Cold spring water	5	6.60±0.1	2.1	103	12.6±0.4	2.5	102	5	6.0
	10	11.7±0.3	1.2	103	22.6±0.3	1.3	101	10	10.9
	50	51.8±0.5	0.7	101	103±0.5	0.9	101	50	51.4
	-	**1.20±0.07	3.6	-	**2.10±0.09	3.3	-	-	1.1
Drinking water	5	6.40±0.1	2.2	103	11.9±0.3	2.3	98.3	5	5.5
	10	11.0±0.3	1.4	98.2	22.4±0.5	1.5	101	10	11.4

	50	50.9±0.4	0.8	99.4	103±0.6	0.7	101	50	52.0
	-	**1.10±0.07	3.1	-	**2.3±0.2	3.2	-	-	1.2
Commercial	5	5.9±0.3	2.2	96.7	12.5±0.4	2.4	102	5	6.6
drinking	10	11.4±0.5	1.3	103	22.6±0.5	1.4	101	10	11.2
water	50	50.8±0.6	0.7	99.4	102±0.5	1.0	99.6	50	51.1

* The percent recoveries obtained for five replicate measurements and calculated as: % Recovery = $C_{\text{after spiking}} / (C_{\text{initial}} + C_{\text{spiked}}) \times 100$

** The values found by means of standard addition calibration curves of spiked samples at levels of 5, 10 and 15 $\mu\text{g L}^{-1}$ As(V) before and after pre-oxidation with KMnO_4 in acidic media