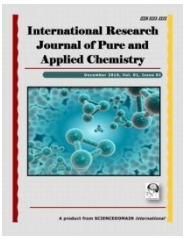

International Research Journal of Pure & Applied Chemistry
4(3): 362-377, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

Selective Extraction and Determination of Arsenic (III) and Arsenic (V) in Some Food Samples by Cloud-Point Extraction Coupled with Hydride Generation Atomic Absorption Spectrometry

Azhar A. Ghali[1], Zuhair A. A. Khammas[1*] and Kasim H. Kadhim[2]

[1]Department of Chemistry, College of Science for Women, University of Baghdad, Jadiyriah, Baghdad, Iraq.
[2]Department of Chemistry, College of Science, University of Babylon, Hillah, Babylon, Iraq.

Authors' contributions

This work was performed in an integrated collaboration between all authors. Authors ZAAK and KHK designed and supervised the work study. Author ZAAK wrote the protocol, helped in analyzing the data statistically and wrote the final draft of the manuscript. Author AAG carried out the most experimental works according to the plan and managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

Received 25th November 2013
Accepted 6th February 2014
Published 27th February 2014

ABSTRACT

Aims: To develop a new selective approach for the extraction and determination of trace amounts of As(III) and As(V) in some food samples by cloud point extraction (CPE) using two new synthesized ligands coupled with hydride-generation atomic absorption spectrometry (HG-AAS).
Study Design: All significant variables for both As (III) and As (V) species that impact the separation and determination steps were studied by one factor-at-a-time (OFAT) optimization.
Place and Duration of Study: Department of Chemistry, College of Science for Women, University of Baghdad, Baghdad, Iraq between October 2012 and May 2013.
Methodology: The method involved the selective complexation of As (III) with 7-(6-

**Corresponding author: Email: dr_zuhair52@yahoo.com;*

BrBTA8HQ) or As (V) with 7-(6-MBTA8HQ) at certain pH values. Then the two complexes were individually extracted into a non-ionic surfactant (Triton X-114) and separated by centrifugation from which the two species were individually determined by means of HG-AAS. The established method was applied to the determination of the two As species in the fish and honey samples with satisfactory results where the detection limits of the method in the sample were of 0.0033 and 0.0065 μg g^{-1} for As (III); 0.0063 and 0.0125 μg g^{-1} or As(V) in fish and honey samples respectively.

Results: Under the optimum established conditions, the enrichment factors of 80 and 73.7 fold were obtained for As (III) and As (V) respectively leading to detection limits of 1.3 ng mL^{-1} of As(III) and 2.5 ng mL^{-1} for As(V) in aqueous solution. The Beer's law was obeyed in concentration range of 1.5-100 ng mL^{-1} for As (III) and 4-150 ng mL^{-1} for As (V). The accuracy of the method was checked by the standard additions procedure. The percent recoveries of As (III) and As (V) were found to be in the range of 98.3-99.3% and 98-101.6% respectively.

Conclusion: The establish method avoids the steps of oxidation/reduction of As species giving good analytical figures of merit and being most suitable for the determination of two As species individually in the selected samples and other food matrices.

Keywords: As (III) and as (V) species; new two ligands; cloud-point extraction (CPE); hydride generation atomic absorption spectrometry (HGAAS).

1. INTRODUCTION

Arsenic mainly occurs in two oxidation states and may form several inorganic and organic species with very different physicochemical properties [1]. Inorganic compounds of As are more toxic than their organic counterpart. The toxicity of As (III) is 10–20 times higher than that of As (V) and almost 70 times more toxic than of the methylated species [2]. Many epidemiological evidences show that arsenic in drinking water causes diseases such as skin cancer and several internal cancers especially lung, bladder and kidney cancers, even when trace arsenic is ingested due to enzyme activity inhibition [3-4]. In fact, recent irregular anthropogenic activities have resulted in worldwide arsenic contamination problems. To protect human life from the exposure to this element, World Health Organization (WHO) has recommended the maximum permissible limit for arsenic in water as 50 μg L^{-1} [5] and the provisional tolerable weekly intake (PTWI) for inorganic arsenic indicated by the Joint Food and Agriculture Organization (FAO) /WHO Expert Committee on Food Additives is 0.015 mg kg^{-1} body mass/week [6]. It was reported that more than 90% of the total arsenic ingested comes from fish. However, less than 3% of the As in fish is present in the inorganic form (arsenite or arsenate) [7]. In light of this information, speciation analysis of arsenic seems exactly to be of prime importance from the point of view of health impact to obtain sufficient information on its toxicity and biotransformation in its different oxidation states.

It was reported that direct determination of As species is absolutely a difficult assignment even if more sophisticated instrumentation are employed due to the lack of selectivity and insufficient sensitivity. Thus separation and preconcentration procedures are needed before their detection by instrumental techniques. The most widely used procedures for separation/preconcentration of the speciation of As (III) and As (V) that coupled with different instrumental techniques involving liquid-liquid extraction [8],solid phase extraction [9-13], liquid-liquid micro extraction [14-15] , ion exchange [16-17], microwave-assisted extraction [18], ion exchange chromatography [19] gas-diffusion [20], ultrasonic extraction [21] and

hydride generation [22-25]. Although some of these procedures gave an acceptable enrichment factor, but the disadvantages such as using toxic organic solvents in large amounts, relatively expensive, time-consuming and unavailable in many laboratories have limited their uses [26-27]. Recently, cloud point extraction (CPE) which uses surfactants as a mediating solvent has emerged as a promised alternative tool compared with the above mentioned extraction methodologies due to its high enrichment factor, versatile, simple, fast, cheap and environmentally-friendly procedure [28-29]. Simply, CPE methodology is based on the separation of hydrophobic analyte between two isotropic phases generated from micellar of non-ionic surfactant solution when heated over its critical cloud point temperature. One phase called a surfactant-rich phase of small volume containing analyte trapped by organized micelles and other phase is called surfactant-poor phase or bulk aqueous phase.

Number of reports has recently been published in the chemical literatures concerning the speciation determination of arsenic in different matrices by using CPE combined with different detection systems [27,28,30-32]. The main limitation for the most reported methods was the extraction and determination of only one As species, while the other species was found by subtraction. To overcome this limitation, the separation and preconcentration of both species by CPE before detection will avoid the use of other methods such as reduction and/or oxidation procedures to convert one species to another.

In the present work, we have attempted to develop a method based on a selective determination of As(III) and As (V) by coupling CPE with hydride-generation atomic absorption spectrometry (HGAAS) using two laboratory-made reagents, each chelating agent is only able to complex one As species, namely 7-(6-Bromo 2-benzothiazolylazo) 8-Hydroxyquinoline [7-(6-BrBTA8HQ)] as chelating agent for As(III) and 7-(6-Methoxy 2-benzothiazolylazo) 8-hydroxyquinoline [7-(6-MBTA8HQ)] for As (V) in aqueous and sample solutions using Triton X-114 as a mediating extractant. The established methods were exploited for a selective determination of As species in fish and bee honey samples.

2. EXPERIMENTALS

2.1 Apparatus

Atomic absorption measurements for arsenic species were carried out using a Double-Beam Atomic Absorption Spectrophotometer AA-6300 (Shimadzu corporation, Kyoto, Japan), equipped with a quartz T-tube cell and hydride vapor generation system HVG-1 (P/N: 206-17143). The HVG-1 is a continuous flow system comprises the peristaltic pump for sending the sample, and reagent solutions to the reaction coil. AAS instrument is provided with background corrector BGC-D_2 and whole system can run with AA WizAArd software in flame mode. The instrumental conditions used for arsenic speciation determination were as follows: wavelength, 193.7 nm; lamp current, 12 mA; slit width, 0.7 nm flame type, Air-C_2H_2 with air and acetylene flow rates at 7.0 and 2.0 L min^{-}1, respectively. Integrated absorbance (peak area) was used for signal evaluation due to give a stable absorbance in almost 30 s. Generated arsine by HVG-1 was carried out according to instruction manual with little modifications. Generated arsines were swept to gas-liquid separator by argon (purity 99.9%) as the carrier gas at a pressure of 250 kPa and then to T-shaped absorption cell heated to approximately 900ºC in an air-acetylene flame. A microprocessor pH meter 211 model (Triup International Corp, Italy) with a combined electrode was used for pH measurements.

2.2 Materials and Reagents

All chemicals used were analytical reagent grade. Deionised water was used in all experiments. non-ionic surfactant Triton X-114 whose chemical formula is *t*-Oct-C_6H_4-$(OCH_2CH_2)_n$ OH, with n equal to 7-8 and an average molecular weight of 537 g/mol, was obtained from ACROS ORGANICS (New Jersey, USA) and was used without further purification. The aqueous solution (10% v/v) of Triton X-114 was prepared by dissolving 10 mL of concentrated solution in 100 mL water. A stock standard solution of As(III) at a concentration of 1000 µg mL^{-1} was prepared by dissolving 0.1320 g of As_2O_3 (BHD, England) in 2.0 mL of 1.0 M NaOH (Fluka, Germany) and to this solution 5 mL of 1M HCl (BHD, England) was added and the volume was completed to 100 mL with water. The stock solution of 1000 µg mL^{-1} As(V) was prepared by dissolving 0.4164 g of Na_2HAsO_4 (Fluka, Germany) with small amount of water and diluted to 100 mL with water. The working standard solutions of both species were prepared freshly by appropriate diluting of the stock standard solution with water. Acetate buffer solutions at different pH were prepared by mixing appropriate amounts of acetic acid and ammonium acetate (BDH, England). The stock chelating agent solutions (3.0×10^{-5} M of 7-(6BrBTA8HQ) and (5.0×10^{-4} M) of 7-(6MBTA8HQ) were prepared by dissolving an appropriate amounts in ethanol (BDH, England). Hydrochloric acid solution, 6 M was prepared by diluting appropriate amount of concentrated HCl (BDH, England) with water. The reductant, a 3.0% (w/v) sodium tetrahydroborate solution, was prepared by dissolving $NaBH_4$ (BDH, England) in 1.0% (w/v) NaOH (Fluka, Germany) and stored in a polyethylene flask under refrigeration. All glassware used were kept in 10% (w/v) HNO_3 for at least 24 h and subsequently washed five times with water.

2.3 Synthesis and Characterization of Reagents

The synthesis and characterization of 7-(6-bromo2-benzothiazolylazo) 8-hydroxyquinoline [7-(6-BrBTA8HQ)] used in work was previously published elsewhere [33]. A 7-(6-Methoxy 2-benzothiazolylazo) 8-Hydroxyquinoline [7-(6-MBTA8HQ)] was synthesized according to general procedure described elsewhere [34]. A 2-amino-6-methoxy benzothiazole (3.6044 g, 0.02 mol) was dissolved in 25 mL of distilled water and 5 mL of concentrated hydrochloric acid and diazotized below 5ºC with(1.38 g, 0.02 mol) sodium nitrite. The resulting diazonium chloride solution was added drop wise with cooling to the solution of (2.903 g, 0.02 mol) 8-hydroxyquinoline dissolved in 50 mL of distilled water and the mixture left in the refrigerator overnight. The mixture was neutralized with dilute hydrochloric acid to (pH = 6.0). The solid product was filtered off, washed with cold distilled water, crystallized twice from hot ethanol and dried over $CaCl_2$ to give red crystals. Yield, 86 %; mp 232-234ºC; anal calcd for $C_{17}H_{12}N_4O_2S$(336.37 g mol^{-1}) C, 60.70;H, 3.39;N, 16.6; S, 9.53; found C, 58.98; H, 3.12; N, 15.33; S, 10.06; IR(KBr) v_{max}/cm^{-1}, 3425, (m, Ar-OH), 3170(w,Ar-H), 2923(w,C-H aliphatic), 1650(s,v C=N), 1458(m,v N=N), 1388(m,v C=C), 1334(m,v C-O), 1265(m,v C-N), 1095(m,v C-S),979(δ Ar-H); ^{1}HNMR(DMSO-*d*6, 298 K,) d/ppm) 3.425 (1H, O-CH_3), 3.723(m,1H, OH), 6.723 (s, H, phenyl), 7.709 (s,3H, pyridyl, phenoyl), 7.994 (s, H, phenyl) , 8.541 (s,2H, pyridyl, phenyl) and 8.860 (s, H, pyridyl). The chemical structure of 7-(6-Methoxy 2-benzothiazolylazo)-8Hydroxyquinoline abbreviated as 7-(6-MBTA8HQ) is shown in Fig. 1.

The reagent is insoluble in water but very soluble in some organic solvent like methanol, ethanol, acetone, Dimethyl sulfoxide (DMSO) and Dimethylformamide (DMF). The sulphur was determined gravimetrically by mineralization of 7-(6-MBTA8HQ) with concentrated

mineral acids (HNO_3, HCl) and sulphate was precipitated as $BaSO_4$, dried, filtered and weighed.

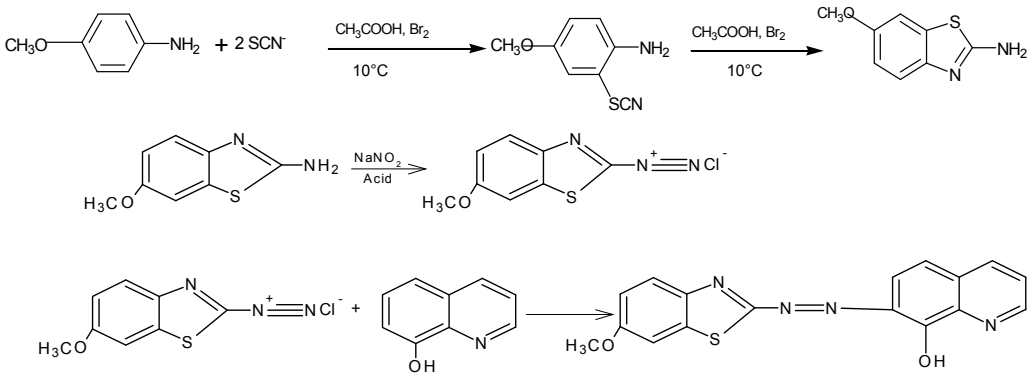

Fig. 1. Synthesis pathway of7-(6-Methoxy 2-benzothiazolylazo) 8Hydroxyquinoline (6-MBTA8HQ)

2.4 General Procedure for CPE

To an aliquot of 2.5 mL of a solution containing known amount arsenic (III) or arsenic(V) standard or in sample solution, 0.3 mL of 3.0 x 10^{-5} mol L^{-1} 7-(6BrBTA8HQ) reagent solution for As(III), or 0.2 mL of 5.0 x 10^{-4} mol L^{-1} 7-(6MBTA8HQ) reagent solution for As(V),1.5 mL of acetate buffer solution (pH = 4.0 for As(III) or 5.0 for As(V)),0.2 mL of Triton X-114 (10%) for As(III) and As(V) were mixed in a 5-mL standard flask and diluted to mark with deionized water. The contents of the flask were transferred into a 10 mL centrifuging tube and the phase separation was induced by heating the contents in a water bath at 60ºC for 10 min. Separation of the phases was accelerated by centrifuging at 3500 rpm for 15 min. Without cooling, the surfactant-rich phases became viscous. Then, the aqueous phase could be separated by using a syringe. Subsequently, a 0.5 ml of 0.1M nitric acid in ethanol was added to the surfactant-rich phase in order to decrease its viscosity and the As (III) content of this solution was determined by means of HGAAS. For As (V), 1mL of 10 % w/v KI plus 0.3% w/v ascorbic acid and 2 mL of concentrated HCl were added to the surfactant-rich phase (this solution was left to stand for 1 h prior to analysis) and then the content of As (V) was determined by using HGAAS.

2.5 Preparation of Fish Samples

Fish sample solution was prepared according to the procedure adopted by Prester and Blanusa [35] using acid digestion in closed tubes. About 4 g of homogenized fish was first digested with 2 mL of concentrated nitric acid in closed borosilicate glass tube overnight at room temperature and then the next day at 80ºC for five hours in a programmed water bath WB 710 model (OPTIMA, Japan). After digestion samples were cooled at room temperature (25±5ºC) and the volume adjusted to 10 mL with water. 0.5 mL aliquot of the final solution were extracted and analyzed for arsenic species according to the prescribed general procedure for CPE and determined by means of HG-AAS.

2.6 Preparation of Honey Samples

The honey samples were slightly heated in a water bath at 40ºC for 2 h to decrease honey viscosity, and homogenization in order to facilitate the handling of sample aliquots [36]. After cooling, aliquots containing 5 g of each sample were weighed directly into PTFE flasks, to which 0.5 mL of HNO_3 + 0.5 mL of H_2O_2 were added and the mixture allowed to stand for 12 h. Subsequently, the flasks were closed with screw caps and heated to 100ºC for 3 h. After cooling at room temperature, the flasks were opened, the resulting solution transferred to graduated polypropylene vials and the volume brought to 25 mL by adding 0.5 M HCl. The aliquots of the final solution were extracted and analyzed for arsenic species according to the prescribed general procedure for CPE and determined by means of HG-AAS.

2.7 Statistical Analysis

All mathematical and statistical computations were made using Excel 2007 (Microsoft Office) and Minitab version 14 (Minitab Inc., State College, PA, USA).

3. RESULTS AND DISCUSSION

3.1 Optimization of CPE Procedure

The influence of different analytical parameters which impact the CPE procedure for the two species such as, pH, concentration of reagents, amount of non-ionic surfactant, temperature and incubation time were studied using one factor-at-a-time (OFAT) strategy in searching of the optimum conditions to maximize recovery percentage and other figures of merit such as sensitivity and detection limit of both species in the selected matrices. Each experiment of the above factors was conducted followed the general CPE procedure (2.4). The pH of solution is one of the most important factors affecting the formation of complexes and their subsequent extraction in the micelle-mediating solvent [37]. In order to evaluate the impact of pH on absorbance of complex formation of As (III) with7-(6-BrBTA8HQ) or As (V) with7-(6-MBTA8HQ), the experiments were performed by varying the pH from 3 and 9. It can be seen that the maximum absorption signal obtained at pH 4.0 and 5.0 for As (III) and As (V) complexes were at pH 4.0 and 5.0 Fig. 2. Accordingly, pH 4.0 and 5.0 were selected as the optimum for each complex.

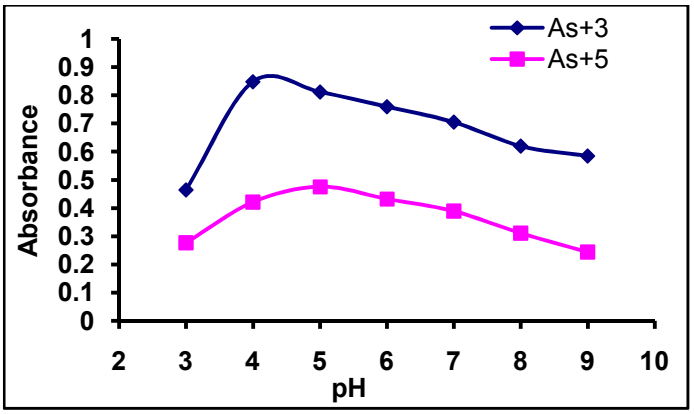

Fig. 2. Effect of pH on the formation of 7-(6-BrBTA8HQ) complex formed with As (III) and 7-(6-MBTA8HQ) complex formed with As (V)

The effect of 7-(6-BrBTA8HQ) and 7-(6-MBTA8HQ) concentrations on absorption signals of 50 ng mL^{-1} of As(III) and As(V) were studied by varying the volume of each reagent from 0.1-0.6 mL of $3x10^{-5}$ of 7-(6-BrBTA8HQ) and $5x10^{-4}$ of 7-(6-MBTA8HQ). It was shown Fig. 3 that the maximum absorbance of both As (III) and As (V) were optimum at 0.2 mL. Beyond this volume, any excessive amount of chelating reagent was not necessary. Therefore, 0.2 mL of $3x10^{-5}$ of 7-(6-BrBTA8HQ) and $5x10^{-4}$ of 7-(6-MBTA8HQ) were used for further experiments.

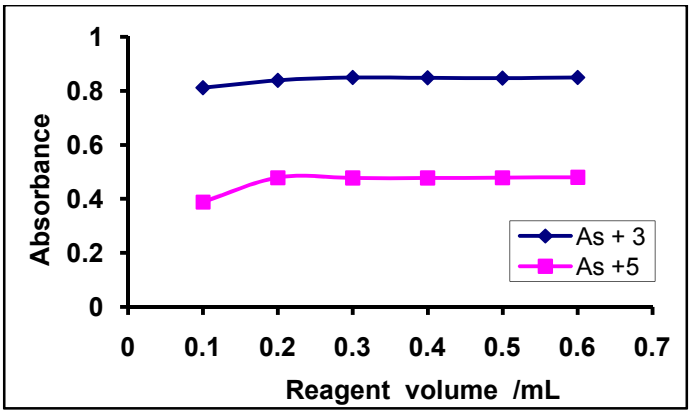

Fig. 3. Effect of concentration of 7-(6-BrBTA8HQ) / 7-(6-MBTA8HQ) on the CPE of As (III)/As (V)

The influence of Triton X-114 amount on the CPE for two complexes were investigated within the surfactant volume range of 0.05 – 0.4 of 10% (v/v). It can be seen Fig. 4 that the optimum quantity of both complexes species was extracted at the volume 0.2 mL of 10% (v/v) Triton X-114 and thus chosen for further experiments.

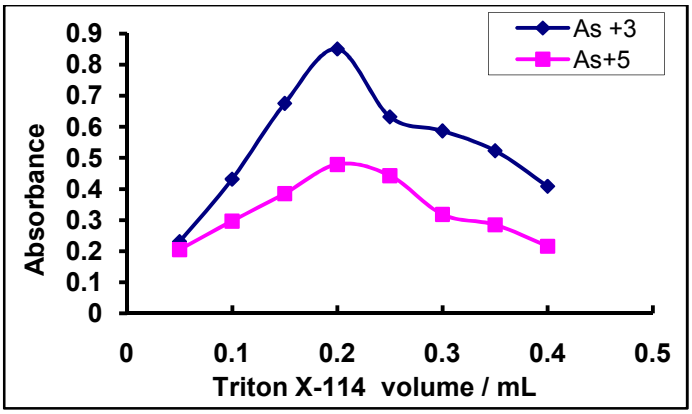

Fig. 4. Effect of amount of Triton X-114 on the CPE of As (III)/As (V)

Fig. 5 shows the effect of equilibration temperature on the absorption signal of both complexes species by varying temperature from 30 to 80ºC at incubation time of 15 min. The experimental results showed that the maximum signal intensity for As (III) and As (V) was attained in the range of 50 – 70ºC. Thereafter, the CPE efficiency of the analytes was

decreased by increasing temperature. Thus, an equilibration temperature of 60ºC for maximum extraction of As (III) and As (V) was chosen as optimal. The influence of the incubation time was also studied in the range of 5 – 30 min at 60ºC. It was also observed that the incubation time of 15 min is sufficient for the maximum absorption signal for the two complexes Fig. 6.

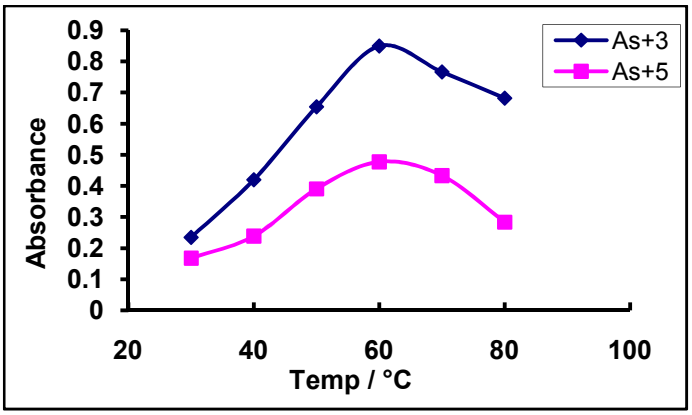

Fig. 5. Effect of equilibration temperature on the CPE of As (III)/As (V)

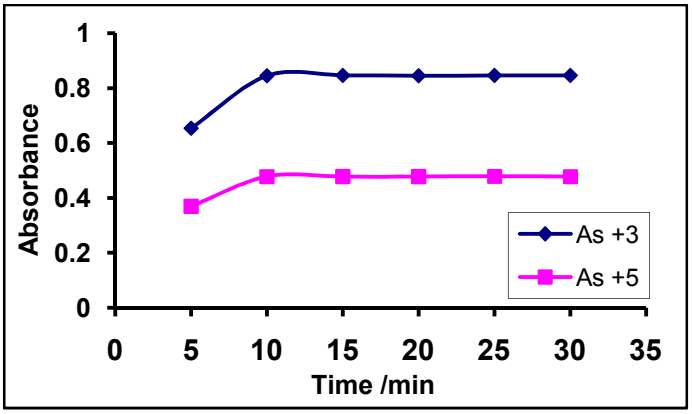

Fig. 6. Effect of incubation time on the CPE of As (III)/As (V)

The effect of centrifugation rate and time was also investigated on extraction efficiency. A centrifuging time of 10 min at 3500 rpm was selected for the entire CPE procedure as being optimum and beyond this time no confirmation was observed for improving extraction efficiency. It is worth noting that the absorbance signal obtained from As (III)- 7-(6-MBTA8HQ) complex is higher than those of As(V)- 7-(6-BrBTA8HQ) in all the aforesaid CPE optimization procedures. This may be ascribed to the possibility of As (III) ions to form more stable complex with 7-(6-MBTA8HQ) at its specific pH than those of As (V) with7-(6-BrBTA8HQ). Additionally, there might be some lose of As (V) or incomplete conversion to As (III) when reaction of As (V) complex with KI and ascorbic acid in the treatment of surfactant-rich phase before its injection to HG system leading to poor sensitivity. However, more optimization is needed for As (V) with KI and ascorbic acid at this stage to ensure the complete conversion to As (III) before subjected to hydride generation process.

3.2 Analytical Figures of Merit

Under the optimized conditions, the calibration graphs were constructed by plotting the absorbance signal against the concentrations of each analyte species subjected according to the general procedure for CPE. The statistical evaluation for the calibration graphs has shown that a strong correlation between signal and As (III) or As (V) concentration may exist (r = 0.9998 or 0.9997). On the other hand, the analysis of variance (ANOVA) also proved the linear regression equations [y= 0.0034±0.0069) + (16.842± 0.1235) x] and [(0.072± 0.0058) + (7.8331± 0.0913) x] for As (III) and As (V) respectively were statistically valid. This because of the ratio (MS_{reg}/MS_{error}) for 1 and 9 dof and 1 and 7 dof, larger than critical value ($F_{1,9}$ = 5.12 and $F_{1,7}$ = 5.59 at 95% CI) indicating that the predication based on the regression line is satisfactory Table 1.

Table 1. Analysis of Variance table of regression line for As (III) and As (V)

Ion	Source	dof	SS	MS	F	P
AS(III)	Regression	1	3.55053	3.55053	59555.57	0.000
	Residual Error	9	0.00054	0.00006		
	Total	10	3.55107			
As(V)	Regression	1	1.29505	1.29505	10841.88	0.000
	Residual Error	7	0.00084	0.00012		
	Total	**8**	**1.29589**			

dof=degrees of freedom, SS: sum of squares, MS: mean of squares, F (Fisher F-test), p (probability)

The statistical analytical results for the calibration data for both species are summarized in Table 2.

Table 2. Method validation of the determination of As (III) - 7-(6-BrBTA8HQ) and As (V) - 7-(6-MBTA8HQ) using CPE-HG-AAS

Parameter	As(III)	As(V)
Regression equation	y= 16.842 x + 0.0034	y= 7.8331x + 0.072
Correlation coefficient(r)	0.9998	0.9997
C.L. for the slope (b± tsb*)* at 95%	16.842±0.1235	7.8331± 0.0913
C.L. for the intercept (a± tsb*)* at 95%	0.0034± 0.0069	0.072± 0.0058
Concentration range (ng mL^{-1})	1.5-100	4-150
Limit of Detection (ng mL^{-1})	1.3	2.5
Limit of Quantitation (ng mL^{-1})	4.4	3.1
RSD% (n=7) at 10 ng As(III) mL^{-1}	1.17%	1.09%
Recovery%	102	97
Preconcentration factor(PF)*	55.5	50.0
Enrichment factor(EF)**	80.0	73.3
Molar absorptivity ($L.mol^{-1}.cm^{-1}$)***	$3x10^6$	$2x10^6$

**PF is calculated as the ratio of volumes of aqueous phase to that of surface-rich phase ** EF is calculated as the ratio of slope of calibration curves obtained with and without CPE ***determined by UV-Vis Spectrophotometry*

The proposed method has achieved an enrichment factor of 80 and 73.3 fold, allowing the limit of detection of 1.3 and 2.5 ng mL^{-1} has to be obtained for As (III) and As (V) in aqueous solution respectively. This was revealed that the prepared ligands in this work beside CPE-HG-AAS gave satisfactory analytical figures of merit for As (III) which was more comparable

or better with those obtained by some previous studies Table 3. While the limit of detection of As (V) species in aqueous solution obtained in this study was almost comparable to that obtained by Ulusoy et al. [27] who employed the CPE coupled with Flame atomic absorption spectrometry (FAAS) based on the formation of ion-paring complex of As (V) with Pyronine B in the presence of cetyl pyridinium chloride (CPC) at pH 8.0 and extracted into the non-ionic surfactant Triton X-114.

Table 3. Comparison of the proposed method for As (III) with some reported methods published in chemical literatures

Sample	Technique	Linear range ($ng\ mL^{-1}$)	Detection limit ($ng\ mL^{-1}$)	EF	Ref.
Fish , honey	CPE-HGAAS	1.5-100 4-150	1.3 2.5	80 73.3	This work
Plastic	HG-ICP-OES	5–100	1.4	-	[38]
Natural water	GFAAS	up to 200	24	50	[13]
water	Electrochemical FIA	50-60000	1.0	-	[39]
Aqueous solution	GD-FIA-CL	0.6-25000	0.6	-	[20]
Ground water	HPLC–HG–AAS	Up to-500	7.8	-	[40]
River water	CCSA	100-300	3.0	-	[41]
environmental waters	PFIA	0.25-2000	0.18	-	[42]
Soil, plants	ICP-OES-MS	1-100	0.1-0.3	-	[16]
Lake,river water	CPE-GFAAS	0.1-20	0.04	36	[29]
Water , beer	HPLC-AFS	Up to 500	0.12	-	[43]
Drinking ,tap water	CPE-FAAS	0.03–4.00	0.008	60	[27]

HG-ICP-OES: hydride generation-inductively coupled plasma-optical emission spectrometry; GFAAS: graphite furnace atomic absorption spectrometry; FIA: flow injection analysis; GD- FIA-CL: gas-diffusion -flow injection analysis –; HPLC–HG–AAS; high performance liquid chromatography- hydride generation-atomic absorption spectrometry; CCSA; Constant current stripping analysis; PFIA ; pervaporation-flow injection; ICP-OES-MS: inductively coupled plasma-optical emission spectrometry-mass spectrometry; HPLC-AFS: high performance liquid chromatography-atomic fluorescence spectrometry.

There are very few of reported methods in chemical literatures have determined both arsenic species selectively, while the majority of these methods have assigned only one species, and the another form calculated after conversion (reduction or oxidation) by the difference after total arsenic determination. By virtue of our knowledge, in the course of this research work, only one paper has been appeared in 2011 by Pakistan's workers [32] who have used two ligands such as ammonium pyrrolidine dithiocarbamate (APDC) and molybdate with As (III) and As (V) respectively, in an attempt to determine both inorganic species sequentially by CPE using Triton X-114 as an extracting medium and GFAAS with modifier (Pd + Mg $(NO_3)_2$ as detector. They found that the limit of detection of 0.04 and 0.20 ng L^{-1} for As(III) and As(V) respectively, which was better than our findings. This can be ascribed to use more sensitive detection like electrothermal atomization AAS than HGAAS and/or the later might be subjected to more interferences than the former. However, by considering a limit of detection (LOD) of 1.3 µg L^{-1} for As (III) and 4 g of fish in 10 mL or 5 g of honey sample in 25 mL, the LOD of the method would be 0.0033 and 0.0065 µg g^{-1} respectively. Similarly, the detection limit of 2.5 µg L^{-1} for As (V) and 4 g of fish in 10 mL or 5 g of honey in 25 mL, the LOD of the proposed method would be of 0.0063 and 0.0125 µg g^{-1} respectively. On that basis the proposed method has successfully exploited for the determination of both species

in various samples of fish and honey that selected randomly from Iraqi markets in order to test the applicability and reliability of the method.

3.3 Accuracy of the Proposed Method

Accuracy was examined through recovery percent studies by spiking (standard additions) four samples of fish and/or honey selected randomly at three concentration levels of 2.5, 5.0 and 10.0 µg L^{-1} standard solutions followed the general CPE procedure and detected by HGAAS. The results are tabulated in Tables 4 and 5. As can be seen that the recoveries percent were within of very acceptable levels, indicating that the determination of As (III) and As (V) using the developed procedures do not highly affected by the presence of the other metals in the selected sample matrices thus the determinate errors were completely absent.

Table 4. Representative recovery percentages for analysis of As (III) and As (V) in fish samples by CPE-HGAAS

Type of species	Amount added (µg L^{-1})	Amount found (µg L^{-1})	Recovery (%)	Relative Error (%)	Mean Recovery ± S_d
As(III)	0.0	36.4*	-		
	2.5	38.8	96	-4	98.33±3.21
	5.0	41.5	102	2	
	10.0	46.1	97	-3	
	0.0	66.1**	-		
	2.5	68.7	104	4	99.33±4.16
	5.0	71.0	98	-2	
	10.0	75.7	96	-4	
As(V)	0.0	57.2***	-		
	2.5	59.8	104	4	99.33±4.16
	5.0	62.0	96	-4	
	10.0	67.0	98	-2	
	0.0	60.2**	-		
	2.5	62.8	104	4	99.00±4.35
	5.0	65.0	96	-4	
	10.0	69.9	97	-3	

**Iraqi (1), ** Indonesian ***Iraqi (5)*

3.4 Applications

To test the applicability of the proposed CPE method, five local and four imported fishes beside three Iraqi and six imported honey samples available in the Iraqi markets were analyzed for both As species. The results are tabulated in Tables 6 and 7. The results in Table 6 have revealed that the value for As (III) obtained in all fish samples were in the range 0.030- 0.075 µg g^{-1} with mean value of 0.0458± 0.012 µg g^{-1}, while the As (V) was in the range of 0.0363-0.0961 µg g^{-1} with mean value of 0.0598± 0.014 µg g^{-1} at 95% confidence interval (t_{crit} =2.31dof=8). Likewise, the results in Table 7 have shown that that the value for As (III) obtained in all honey samples were in the range 0.036- 0.090 µg g^{-1} with mean value of 0.0651± 0.0136 µg g^{-1}, while the As (V) was in the range of 0.066-0.0841 µg g^{-1} with mean value of 0.0737± 0.0046 µg g^{-1} at 95% confidence interval (t_{crit} =2.31dof=8). From the results in Tables 5 and 6, we can conclude that the total mean of arsenic species were of 0.106 and 0.139 µg g^{-1} in fish and honey samples respectively. Arsenic amounts determined in all selected fish and honey sample were below the maximum permissible limit of 1.0 µg g^{-1} specified by WHO/FAO [44-45].

Table 5. Representative recovery percentages for analysis of As (III) and As (V) in honey samples by CPE-HGAAS

Type of species	Amount added (μg L^{-1})	Amount found (μg L^{-1})	Recovery (%)	Relative Error (%)	Mean Recovery ±S_d
As(III)	0.0	45.3*	-		
	2.5	47.7	96	-4	98.00±3.464
	5.0	50.4	102	2	
	10.0	54.9	96	-4	
	0.0	54.4**	-		
	2.5	57.0	104	4	101.66±2.516
	5.0	59.5	102	2	
	10.0	64.3	99	-1	
As(V)	0.0	75.2*	-		
	2.5	77.8	104	4	99.33±4.163
	5.0	80.0	96	-4	
	10.0	85.0	98	-2	
	0.0	69.1**	-		
	2.5	71.5	96	-4	99.00±4.358
	5.0	74.3	104	4	
	10.0	78.8	97	-3	

**Iraqi (1), ** Spain*

Table 6. Determination of As (III) and As (V) in fish samples by CPE- HGAAS

Sample No.	Grade	Concn. of As(III) (μg g^{-1})	Concn. of As(V) (μg g^{-1})
1	Iraqi 1*	0.0364±0.0017	0.0961±0.0030
2	Iraqi 2*	0.0300±0.0010	0.0450±0.0017
3	Iraqi 3*	0.05100±0.002	0.0363±0.0010
4	Iraqi4*	0.0331±0.0010	0.0512±0.0015
5	Iraqi5*	0.0364±0.0017	0.0572±0.0017
6	Iranian	0.0390±0.0023	0.0600±0.0026
7	Morocco	0.0452±0.0017	0.0810±0.0026
8	Jordanian	0.0750±0.0034	0.0510±0.0017
9	Indonesian	0.0661 ±0.0030	0.0602±0.0020

Table 7. Determination of As (III) and As (V) in bee honey samples by CPE-HGAAS

Sample No.	Grade	Concn. of As(III) (μg g^{-1})	Concn. of As(V) (μg g^{-1})
1	Iraqi 1*	0.04530±0.002	0.0752±0.0021
2	Iraqi 2*	0.0360±0.0017	0.0690±0.0030
3	Iraqi 3*	0.0600±0.0027	0.0662±0.0017
4	Iranian 2	0.0812±0.0031	0.0750±0.0017
5	Saudi Arabia 1	0.0751±0.0030	0.0690±0.0026
6	Saudi Arabia 2	0.0660±0.0010	0.0810±0.0017
7	Germany 1	0.0900±0.0042	0.0841±0.0026
8	Germany 1	0.0780±0.0021	0.0750±0.0030
9	Spain	0.0544±0.0020	0.0691±0.0026

4. CONCLUSIONS

In this work, we have exploited of using two laboratory-made azo dyes ligands instead of the commercial thiazolylazo dyes such as 1-(2-thiazolylazo)-2-naphthol(TAN), 4-(2-thiazolylazo) resorcinol(TAR) and 2-(2-thiazolylazo)-p-cresol(TAC), for the first time, for cloud point extraction in the selective separation and enrichment of arsenic species as a prior step for determination of ultra-trace quantities in real samples by means of HGAAS. This study has shown that each chelating agent prepared is only able to complex one As species. Therefore, it was not able to use one of these reagents to the estimation of the two arsenic species simultaneously. However, the steps of oxidation and/or reduction among the two arsenic species by using oxidizing or reducing agents were eliminated in the proposed method. Moreover, the enrichment factors and detection limits for both species were a satisfactory compared to those reported in chemical literatures especially with those using sophisticated and expensive instrumentation such as HG-ICP-OES, HPLC–HG–AAS, GFAAS.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Rivas RE, López-García I, Hernández-Córdoba M. Speciation of very low amounts of arsenic and antimony in waters using dispersive liquid–liquid microextraction and electrothermal atomic absorption spectrometry. Spectrochim. Acta Part B. 2009;64(4):329–333.
2. Squibb KS, Fowler BA. The toxicity of arsenic and its compounds. In: B.A. Fowler (Ed.). Biological and Environmental Effects of Arsenic. Elsevier Science Publishers B.V., New York. 1983;233–269.
3. Smith AH. Lopipero PA, Bates MN, Steinmaus CM. Arsenic Epidemiology and Drinking Water Standards. Science. 2002;296(5567):2145-2146.
4. Wang YC. Food safety. Hua Hsiang Yuan Publishing Co Taipei. 1989;116-143.
5. Revanasiddappa HD, Dayananda BP, Kumar TNK. A sensitive spectrophotometric method for the determination of arsenic in environmental samples. Environ Chem Lett. 2007;5(3):151-155.
6. FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series 759. WHO, Geneva, Switzerland; 1989.
7. Ysart G, Miller P, Croasdale M, Crews H, Robb P, Baxter M, De l'Argy C, Harrison N. 1997 UK total diet study dietary exposures to aluminium, arsenic, cadmium, chromium, copper, lead, mercury, nickel, selenium, tin and zinc. Food Addit Contam. 2000;17(19):775-786.
8. Paik MK, Kim MJ, Kim WI, Yoo JH, Park BJ, Im GJ, Park JE, Hong MK. Determination of Arsenic Species in Polished Rice Using a Methanol-water Digestion Method. J Korean Soc Appl Biol Chem. 2010;53(5):634-638.
9. Huang C, Xie W, Li X, Zhang J. Speciation of inorganic arsenic in environmental waters using magnetic solid phase extraction and preconcentration followed by ICP-MS. Microchim Acta. 2011;173(1-2):165–172.

10. Deng F, Dong R, Yu K, Luo X, Tu X, Luo S, Yang L. Determination of trace total inorganic arsenic by hydride generation atomic fluorescence spectrometry after solid phase extraction-preconcentration on aluminium hydroxide gel. Microchim Acta. 2013;180(5-6):509-515.
11. Erdoğan H, Yalçınkaya Ö, Türker AR. Determination of inorganic arsenic species by hydride generation atomic absorption spectrometry in water samples after preconcentration/separation on nano ZrO_2/B_2O_3 by solid phase extraction. Desalination. 2011;280(1-3):391–396.
12. Anthemidis AN, Martavaltzoglou EK. Determination of arsenic (III) by flow injection solid phase extraction coupled with on-line hydride generation atomic absorption spectrometry using a PTFE turnings-packed micro-column. Anal Chim Acta. 2006;573-574:413–418.
13. Liang P, Liu R. Speciation analysis of inorganic arsenic in water samples by immobilized nanometer titanium dioxide separation and graphite furnace atomic absorption spectrometric determination. Anal Chim Acta. 2007;602(1):32–36.
14. Liang P, Peng L, Yan P. Speciation of As (III) and As (V) in water samples by dispersive liquid-liquid microextraction separation and determination by graphite furnace atomic absorption spectrometry. Microchim Acta. 2009;166(1-2):47–52.
15. Rabieh S, Bagheri M, Planer-Friedrich B. Speciation of arsenite and arsenate by electrothermal AAS following ionic liquid dispersive liquid-liquid microextraction. Microchim Acta. 2013;180(5-6):415-421.
16. Chen Z, Akter KF, Rahman MM, Chen RNZ, Naidu R. The separation of arsenic species in soils and plant tissues by anion-exchange chromatography with inductively coupled mass spectrometry using various mobile phases. Microchem J. 2008;89(1):20–28.
17. Ben Issaa N, Rajakovic-Ognjanovic VN, Jovanovic BM, Rajakovic LV. Determination of inorganic arsenic species in natural waters—Benefits of separation and preconcentration on ion exchange and hybrid resins. Anal Chim Acta. 2010;673(2):185–193.
18. Garcıa Salgado S, Quijano Nieto MA, Bonilla Simon MM. Determination of soluble toxic arsenic species in alga samples by microwave-assisted extraction and high performance liquid chromatography–hydride generation–inductively coupled plasma-atomic emission spectrometry. J Chromatogr A. 2006;1129(1):54–60.
19. Leufroy A, Noël L, Dufailly V, Beauchemin D, Guérin T. Determination of seven arsenic species in seafood by ion exchange chromatography coupled to inductively coupled plasma-mass spectrometry following microwave assisted extraction: Method validation and occurrence data. Talanta. 2011;83(3):770–779.
20. Lomonte C, Currell M, Morrison RJS, McKelvie ID, Spas D, Kolev SD. Sensitive and ultra-fast determination of arsenic (III) by gas-diffusion flow injection analysis with chemiluminescence detection. Anal Chim Acta. 2007;583(1):72–77.
21. Paula JFR, Froes-Silva RES, Ciminelli VST. Arsenic determination in complex mining residues by ICP OES after ultrasonic extraction. Microchem J. 2012;104:12–16.
22. Petrov PK, Serafimovski I, Stafilov T, Tsalev DL. Flow injection hydride generation electrothermal atomic absorption spectrometric determination of toxicologically relevant arsenic in urine. Talanta. 2006;69(5):1112–1117.
23. Yang LL, Zhang DQ. In situ preconcentration and determination of trace arsenic in botanical samples by hydride generation-graphite furnace atomic absorption spectrometry with Pd–Zr as chemical modifier. Anal Chim Acta. 2003;491(1):91–97.

24. Sun H, Qiao F, Suo R, Li L, Liang S. Simultaneous determination of trace arsenic (III), antimony (III), total arsenic and antimony in Chinese medicinal herbs by hydride generation-double channel atomic fluorescence spectrometry. Anal Chim Acta. 2004;505(2):255–261.
25. Correia CLT, Gonçalves RA, Azevedo MS, Vieira MA, Campos RC. Determination of total arsenic in seawater by hydride generation atomic fluorescence spectrometry. Microchem J. 2010;96(1):157–160.
26. Saçmac S, Kartal S, Dural S. Dispersive Liquid-Liquid Microextraction Procedure for the Determination of Palladium by Flame Atomic Absorption Spectroscopy. J Braz Chem Soc. 2012;23(6):1033-1040.
27. Ulusoy HI, Akcay M, Gürkan R. Development of an inexpensive and sensitive method for the determination of low quantity of arsenic species in water samples by CPE–FAAS. Talanta. 2011;85(3):1585–1591.
28. Ulusoy HI, Akcay M, Ulusoy S, Gurkan R. Determination of ultra trace arsenic species in water samples by hydride generation atomic absorption spectrometry after cloud point extraction. Anal Chem Acta. 2011;703(2):137-144.
29. Anawar, MHmd. Arsenic speciation in environmental samples by hydride generation andelectrothermal atomic absorption spectrometry. Talanta. 2012;88:30-42.
30. Shemirani F, Baghdadi M, Ramezani M. Preconcentration and determination of ultra trace amounts of arsenic (III) and arsenic (V) in tap water and total arsenic in biological samples by cloud point extraction and electrothermal atomic absorption spectrometry. Talanta. 2005;65(4):882–887.
31. Tang A, Ding GS, Yan XP. Cloud point extraction for the determination of As (III) in water samples by electrothermal atomic absorption spectrometry. Talanta. 2005;67(5):942–946.
32. Baig JA, Kazi TG, Arain MB, Shah AQ, Kabdhro GA, Afridi HI, Khan S, Kolachi NF, Wadhwa SK. Inorganic arsenic speciation in groundwater samples using electrothermal atomic spectrometry following selective separation and cloud point extraction. Anal Sci. 2011;27(4):439-445.
33. Khammas ZAA, Ghali AA Kadhim KH. Cloud Point extraction procedure for the determination of mercury by spectrophotometry using a new synthesized ligand. Iraqi Natl J Chem. 2013;49:27-37.
34. Korn MGA, Ferreira AC, Teixeira LS, Costa ACS. Spectrophotometric Determination of Zinc Using 7-(4-Nitrophenylazo)-8-Hydroxyquinoline-5-Sulfonic Acid. J Braz Chem Soc. 1999;10(1):46 -50.
35. Prester LJ, Blanusa M. Decomposition of fish samples for the determination of mercury. Arh Hig Rada Toksikol. 1998;49(4):343-348.
36. Ioannidou MD, Zachariadis GA, Anthemidis AN, Stratis JA. Direct determination of toxic trace metals in honey and sugars using inductively coupled plasma atomic emission spectrometry. Talanta. 2005;65(1):92–97.
37. Stalikas D. Micelle-mediated extraction as a tool for separation and preconcentrationin metal analysis. Trends Anal Chem. 2002;21(5):343–355.
38. Mihaltan AI, Frentiu T, Ponta M ,Petreus D, Frentiu M, Darvasi E, Marutoiu C. Arsenic and antimony determination in non- biodegradable materials by hydride generation capacitively coupled plasma microtorch optical emission spectrometry. Talanta. 2013;109:84-90
39. Rupasinghea TWT, Cardwell TJ, Robert W, Cattrall RW, Kolevb SD. Determination of arsenic in industrial samples by pervaporation flow injection with amperometric detection. Anal Chim Acta. 2009;652(1-2):266–271.

40. Niedzielski P. The new concept of hyphenated analytical system: Simultaneous determination of inorganic arsenic (III), arsenic (V), selenium (IV) and selenium (VI) by high performance liquid chromatography–hydride generation–(fast sequential) atomic absorption spectrometry during single analysis. Anal Chim Acta. 2005;551(1-2):199–206.
41. Vancara IS, Vytras K, Bobrowski A, Kalcher K. Determination of arsenic at a gold-plated carbon pasteelectrode using constant current stripping analysis. Talanta. 2002;58(1):45–55.
42. Rupasinghe TWT, Cardwell TJ, Cattrall RW, Potter ID, Kolev SD. Determination of arsenic by pervaporation-flow injection hydride generation and permanganate spectrophotometric detection. Anal Chim Acta. 2004;510(1-2):225–230.
43. Melo Coelho NM, Parrilla C, Cervera ML, Pastor A, De La Guardia M. High performance liquid chromatography—atomic fluorescence spectrometric determination of arsenic species in beer samples. Anal Chim Acta. 2003;482(1):73–80.
44. Affum AO, Shiloh DO, Adomako D. Monitoring of arsenic levels in some ready-to-use anti-malaria herbal products from drug sales outlets in the Madina area of Accra, Ghana. Toxicology. 2013;56:131–135.
45. WHO. Evaluation of certain contaminants in food. Seventy-second Report of the JointFAO/WHO Expert Committee on Food Additives. World Health Organization, Geneva. 2011;105

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=405&id=7&aid=3860