Bulgarian Chemical Communications, Special Issue J, (pp. 315 - 322) 2017

Pre-concentration, speciation and determination of As and Sb by optimized experimental design DLLME combined with GF-AAS

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Submitted May 15, 2017; Revised August 21, 2017

Determination of heavy metals is more important than past with the increasing their concentration in environment. Arsenic and antimony have various forms in nature but only some forms of them are toxic. Speciation of them is impossible with common atomic absorption spectroscopy procedure. Dispersive liquid-liquid microextraction has been used for speciation includes complex formation with special ligands, extraction in organic phase and then determination with atomic absorption spectroscopy. Many factors effect on dispersive liquid- liquid microextraction procedure such as volume of disperser solvent, volume of extraction solvent, concentration of chelating agent, coexisting ions, time of extraction, pH, sample volume and salting out effect. All factors were optimized by experimental design and optimum values obtain for each other. Under the optimum condition the enrichment factor equal to 187 and detection limit 0.02 and 0.4 μgL-1 was obtained for Arsenic and Antimony respectively.

Keywords: DLLME, Experimental design, Speciation, Arsenic, Antimony

INTRODUCTION

Arsenic (As) and antimony (Sb) are two toxic elements that often have two oxidation states and are included in many inorganic and organic species with different physicochemical properties. Inorganic species of As and Sb are more toxic than organic species and were found in ground and surface water. Toxicity of As (III) and Sb (III) are 10-20 times more than As (V) and Sb (V) and cause several type of cancers [1, 2].

Arsenic was utilized as poisons, insecticides and wood preservatives in vast level. It is banned or limited in some fields however is used in many of others[3]. Antimony causes headaches, dizziness, and depression. Large doses of antimony such as prolonged skin contact may cause dermatitis, damage the kidneys and the liver, cause violent and frequent vomiting, and lead to death in a few days[4].

For finding sufficient information about toxicity and biotransformation of these elements, it is enough not only to determine the total concentration of them but also to speciation their species, and it is necessary to determine their concentration of each oxidation states.

Inductively coupled plasma atomic emission spectroscopy (ICP-AES),[5] Inductively coupled plasma mass spectrometry (ICP-MS) [6,7], electrothermal atomic absorption spectrometry (ET-AAS)[8] and hydride generation atomic absorption spectrometric (HG-AAS) [9,10] have been suggested as the sensitive and powerful analytical techniques for these kind of determinations. Today all of the mentioned analytical instrument can analyzed samples quickly by acceptable accuracy and precision, but because of the trace amounts of these elements in surface water and the importance of trace analysis of toxic species usually a preparation and pre-concentration step is needed before instrumental analysis.

Sample preparation and pre-concentration are very important for trace elements analysis especially in terms of sample cleaning and time consuming. Also, it has been observed that lower limit of detection is obtained with complete separation of analyte. The purpose of sample preparation is the interfering compounds elimination and the sensitivity enhancement. The classic liquid- liquid extraction (LLE) method is time consuming, prone to contamination and includes several steps. The new methods of extraction solve very problematic defects of LLE. Among different advanced LLE methods, dispersive liquid- liquid microextraction (DLLME) has been categorized as a developed liquid phase microextraction method (LPME) and advantages of it are as following: Rapidness, low cost, simplicity or easy to operation, solvent free or solventless, miniaturized sampling devise and high enrichment factor [11]. A constant and repeatable sedimented phase in each test is one of the most important points in DLLME. In addition, this method can eliminate some main disadvantages of LPME, for example elements and their species with weak partial coefficient by diffusion process cannot be extracted in LPME, but in DLLME by choosing

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strategy of complex formation and derivation coupled with atomic spectroscopy (include ICP and AAS) and its relative methods can determine trace or ultra-trace elements in real samples including environmental and biological samples. A disadvantage of all of LPME methods including DLLME is that these methods need to sample preparation for determination of elements in complex matrix such as blood, milk and other biologic samples. Therefore, almost most of determination by LPME method is carried out in simple matrix such as water solutions. Also, aqueous samples with high salt content such as sea water have a low enrichment factor.

Some affecting parameters on extraction process that must be optimized are: volume of extraction solvent, concentration of chelating agent, coexisting ions, time of extraction, pH, sample volume and salting out effect. One method for obtaining the best condition is using experimental design method whose goals are as follows: (1) Investigation the effect of different factors can influence on enrichment factor in the extraction procedure. (2) Identification of the factors that have higher impact on the extraction results. (3) Obtaining a better insight about the method that helps us to find optimized conditions considering the interactions between factors [12].

This study has focused on speciation and determination of As and Sb species using DLLME as an extraction and pre-concentration step followed by ETAAS method. To achieve the best result, the optimized conditions of DLLME have been obtained by using experimental design methods. Two experimental design methods called Plackett-Burman and Box-Behnken design has been applied in this work. Performing this method ensures us about achieving maximum efficiency and sensitivity of DLLME for determination and speciation of As and Sb by DLLME-ETAAS.

Literatures have suggested some compounds as the matrix modifier such as Mg, Pd, Ni and some other ones [13]. But it is including two injection solutions, first one is extracted organic phase and second one is aqueous solution involve matrix modifier. Other way is using long-term permanent chemical modifier. In this method salt of W, Ir and Mo was considered for the modification purpose [14]. Using sodium tungstate as a modifier and pyrolysing in 400 °C lead to well defined absorbance time and low background effect. Chemical modification of graphite furnace with sodium tungstate has impressive enough for over analysis. Effect of atomization temperature is considered in 1600-2200 °C. There is no significant difference between analyte signals. However time-absorbance profile disturbed to noise below 1700 °C. So 1900 and 2000 °C are chosen for As and Sb respectively.[14]

EXPERIMENTAL

Reagents and chemicals

All used reagents were of analytical grade purity purchased from Merck (Germany). Stock solution of arsenic (1000 mgL^{-1}) was prepared by dissolving 1.32 g As_2O_3 in KOH 20 % and neutralized by H_2SO_4 20 % and then diluted to 1 L. Stock standard solution of antimony was prepared by dissolving 2.743 g of potassium antimonyl tartrate hemihydrates in ultrapure water. Working standard solutions were prepared daily by stepwise diluting of stock standard solutions.

Ammonium pyrrolidine dithiocarbamate (APDC) was dissolved in methanol as the chelating agent (0.2 gL^{-1}). Methanol and carbon tetrachloride were used as the disperser and the extraction solvents, respectively. Sodium thiosulphate was employed as the reduction agent to reducing of pentavalent species of As and Sb.

Apparatus

An atomic absorption spectrometer (Unicam AA929) equipped with continuous source background correction (deuterium lamp) and graphite furnace atomizer (GF90) were employed. Hallow cathode lamps were utilized as the radiation source for each elements. Instrumental parameters for Arsenic and Antimony are shown in Table 1. The pH was measured by using a pH meter (Metrohm 691 pH meter).

Preparation of graphite furnace which no need to matrix modifier

In this study, sodium tungstate was employed as the permanent matrix modifier. Cuvettes were immersed in 100 ml of a solution containing 1 gL^{-1} $Na_2WO_4.2H_2O$ for 12 h. After this time cuvettes were dried in 120 °C for about 4 h. Then tubes were installed on instrument and submitted to the temperatures 120, 200, 1200 and 2400 °C for 120, 120, 30 and 6 seconds, respectively, as conditioning temperature program of graphite atomizers.

Table 1: Instrumental parameters for arsenic and antimony

Instrumental parameters	Arsenic	Antimony
Lamp current (mA)	(As HCL)	(Sb HCL)
Wavelength (nm)	193.7	217.6
Bandwidth	0.5	0.5
Atomizer type	Electrographite	Electrographite
Injected sample volume (µL)	20	20
Background correction	D_2	D_2

Furnace heating program

Step	Temperature As (°C)	Temperature Sb (°C)	Hold (S)	Ramp (°C.S^{-1})
Dry	120	120	30	10
pyrolysis	1200	1200	20	50
Atomization	2600	2200	3	0
Clean	3100	2700	5	0

DLLME procedure

A sample solution containing desired analytes was adjusted in appropriate pH and placed in conical bottom propylene test tube. Then extraction and disperser solvents contain concentration of ligand was rapidly injected into the sample solution and then cloudy solution was centrifuged to remove sedimented extraction phase at the bottom of conical test tube and finally, it was placed in the graphite furnace cuvette by using a 20 microlitre Hamilton syringe to analyze.

RESULTS AND DISCUSSION

Optimization step

Some factors have influenced on determination of As and Sb and should be optimized. Therefore, enrichment factor (EF) was chosen as an analytical response under different conditions.
The enrichment factor as a response is defined in Eq. (1):

$$EF = \frac{C_{sed}}{C_0} \qquad (1)$$

Where, EF is enrichment factor and C_{sed} and C_0 are analyte concentration in sedimented phase and primary analyte concentration in aqueous phase, respectively. C_{sed} was obtained from conventional LLE-ETAAS calibration curve (extraction condition: 10 ml of standard water sample, 0.2 g L^{-1} APDC, 10 ml CCl_4 and pH at 3.1).

Extraction and disperser solvent type

Type of extraction solvent is very important and critical in DLLME process. Extraction solvent should has some properties including, density more than water, low solubility in water, low volatility, able to formed cloudy solution, able to analyte extraction and no interfering with analyte determination technique. Accordingly, to optimize the extraction solvent, several solvents such as chloroform, carbon tetrachloride and dichloromethane were considered and according to Fig. 1 chloroform was selected as extraction solvent. Another important variable in DLLME is disperser solvent type. Disperser solvent should has some properties including, soluble in aqueous and organic phase, low toxicity, low cast and high analyte signal. Methanol, tetra hydro furan (THF), acetone, acetonitrile were tested to obtain the highest analyte signal, or in other hand to obtain the highest EF. In addition, any possible combination of extraction and disperser solvents was examined and finally, methanol was selected as disperser solvent because it shows the highest enrichment factor according to the results shown in Fig. 1.

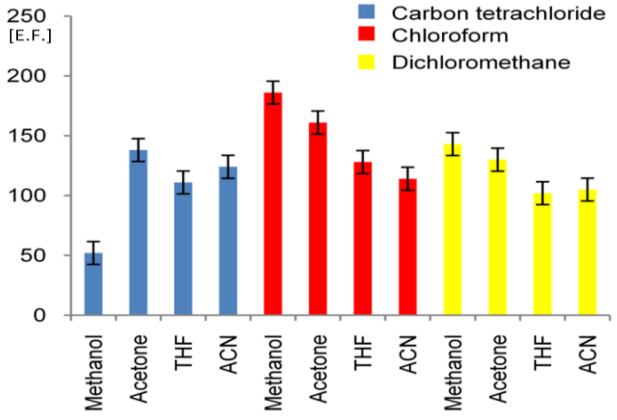

Fig. 1 Selection of extraction and disperser solvent. Methanol, tetra hydrofuran (THF), acetone, acetonitrile (ACN) were tested for highest analyte signal or in other hand to obtain highest EF.

Experimental design: Placket- Burman design

Many factors effect on DLLME such as volume of extraction solvent (A), volume of disperser solvent (B), concentration of chelating agent (C), coexisting ions (D), time of extraction (E), pH (F), sample volume (G) and salting out effect (H).The experimental design method was employed for optimization of these parameters[14]. Plackett-Burman design is a kind of screening tools that can be reduced the number of factors by examining their main effects. In this work, a low and a high value were selected for every possible effective factors and a Plackett-Burman design with 12 runs was constructed to find out main parameters ignoring their interactions. The main effects are determined based on ANOVA results. The normalized obtained results were analyzed by standardized Pareto chart at 5% significance, as shown in Fig. 2. The standard effect was estimated for computing the t statistic for each effect. The bars that are extended beyond the vertical line are significant at the 95% confidence limit statically. Some factors have positive rules and they increase signal by increasing their value and some factors have negative rules inversely, they are shown in hatched and smooth bars in Fig. 2, respectively. As shown in Fig. 2, extraction solvent volume is most effective factor with a negative rule. It's clear that because the concentration of analyte in sedimented phase will be decreased by increasing in extraction solvent volume, it has a negative rule. The next significant important having a negative effect is disperser solvent volume, because the obtained volume of sedimented organic phase will be decreased by increasing this factor and result shows that by using more than 1ml of disperser solvent volume, no sedimented phase volume was obtained. It is appeared that pH has a negative rule. It is because of the fact that complex formation is strongly a pH depending process and in high pH values almost complex formation is terminated. According to Pareto chart, sample size has a positive effect because when this factor is passing to higher value, there is more analyte amount in sample for extraction process and it leads to a higher enrichment factor. Ligand concentration has a positive effect; its influence was investigated in the range of 0.05-0.5 gL^{-1} and results show that the enrichment factor increases until 0.2 gL^{-1} of ligand concentration, up to this value no significant change is observed in enrichment factor. Coexisting ion that has a negative effect was surveyed according to Table 2 for 1μgL^{-1} of analytes. Both of time and salting effect parameters have positive and not very significant effect. Commonly, extraction time is one of the most important factors in the extraction procedure. In DLLME, extraction time is defined as the time between injecting the mixture of disperser and extraction solvents, and starting to centrifuge,[15] but in DLLME experiments show that extraction time has no effect on extraction efficiency. It has been illustrated that this founding due to the very large contact surface between the extraction solvent and aqueous phase, which it is consequence very rapid process of complexation and extraction.

Table 2: Tolerance limit of some coexisting ion

Ion	Tolerance limit $C_{ion}/C_{Analyte}$
Na^{+}	100000
K^{+}	60000
Ca^{+}	5000
Mg^{2+}	5000
Fe^{3+}	100
Cu^{2+}	1000
Zn^{2+}	200
Cl^{-}	100000
$NO3^{-}$	60000
$SO4^{2-}$	12000

For salting addition had been seen two effects as follows: Increasing in extraction solvent volume causes a reduction in enrichment factor and salting-out effect causes an increase in enrichment factor.
Considering the result of Plackett-Burman design, four variables were fixed at suitable values (chelating agent concentration of 0.2 $gL^{-1`}$, salting addition 2 gL^{-1}, no extra time and no coexisting ion).

Box-Behnken design

After finding the main significant variables, next step is using a Box-Behnken experimental design to optimize four factors (pH, volume of extraction

solvent, volume of disperser solvent and sample size) that are selected from Plackett-Burman results. The optimized step can be expressed as Eq. (2) that is a second order polynomial fit, where, y is EF, b_0 is the intercept, b_1-b_{14} are regression coefficients that will be calculated by multivariate linear regression (MLR) techniques and x_1-x_4 are desire factors that must be optimized. The number of run with 4 central points is 28.

$$y = 121.000 - 7.66667V_{dis}\,22.0833V_{ex} + 34.1667V_s - 31.4167pH - 18.6667\,V_{dis}^{\,2} - 19.5417\,V_{ex}^{\,2} + 3.58333V_s^{\,2} - 33.0417pH^2 + 7.50000V_{dis}.V_{ex} - 15.0000V_{dis}.V_s - 22.0000V_{dis}.pH - 6.25000V_{ex}.V_s + 12.5000\,V_{ex}.\,pH - 3.75000\,V_s.pH \quad (2)$$

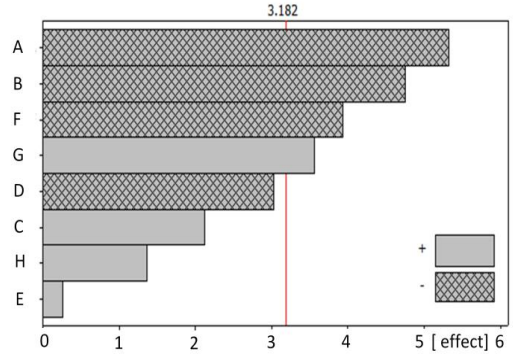

Fig. 2 Standardized (P = 0.05) Pareto chart, representing the estimated the most effective parameters, volume of extraction solvent (A), volume of disperser solvent (B), concentration of chelating agent (C), coexisting ions, (D), time of extraction (E), pH (F), sample volume (G) and salting out effect (H).

With R^2 value = 0.9 that is acceptable in 95% confidence limit, three dimensional plots called response surface also were prepared that show the relation between two variables and the response (EF). Six surface plots that can be explained the optimal condition are shown in Fig. 3. Using obtained result the optimal condition was calculated as followings: volume of extraction solvent (V_{ex}): 31 µL, volume of disperser solvent (V_{dis}):730 µL, pH: 3.1 and sample size volume (V_s):10 mL.

Extraction in optimized conditions leads to improvement in performance of extraction procedures and obtaining higher sensitivity.

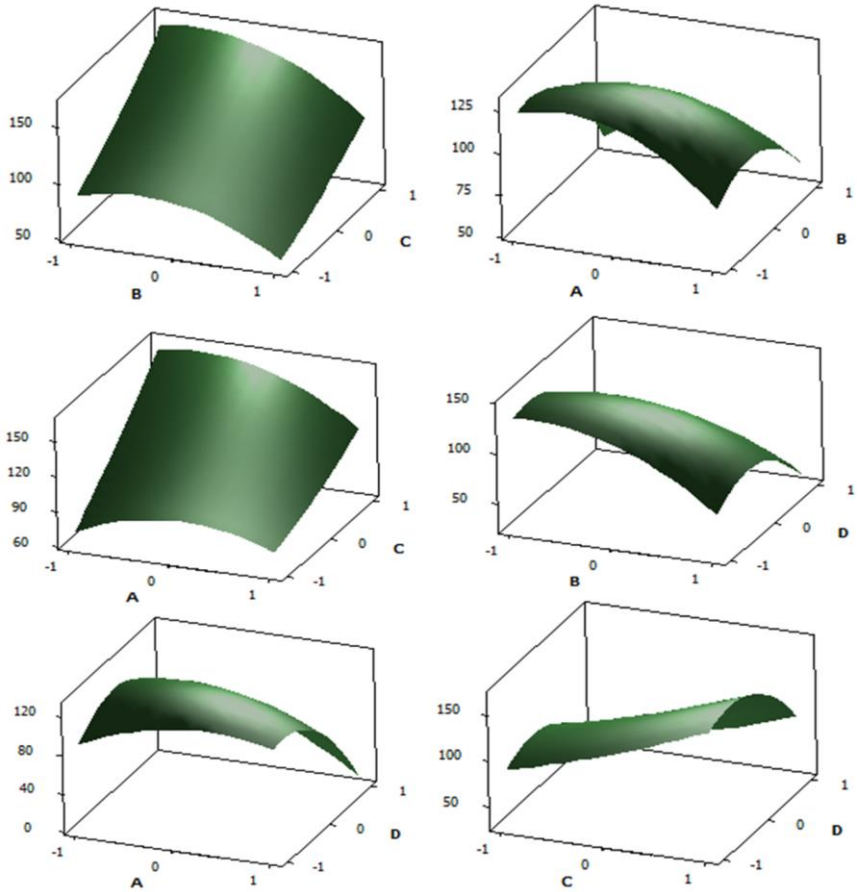

Fig. 3 Six surface plots that can be explained the optimal conditions.

Determination of As and Sb

For determination and speciation of As and Sb, six series of water solutions were prepared including, two series solutions of As(III) and As(V) at 0.2, 0.4, 0.6, 0.8 and 1 μgL^{-1}, and two series solutions of Sb(III) and Sb (V) solutions at 1.4, 1.6, 1.8 and 2 μgL^{-1} and two series of solutions for their mixtures.

Since arsenic (V) and antimony (V) do not react with APDC and therefore, they do not extracted in sedimented phase, the absorbance signals of the solutions that are containing only arsenic (V) and antimony (V) are almost equal to zero (Fig. 4A(a) and 4B(a)). The results of absorbance signal are different for As(III) and Sb(III) solutions because they can extracted by APDC easily. Fig. 4A(b) and 4B(b) show the obtained signals for these two solutions. The same results have been perceived for the mixtures of As and Sb species (Fig. 4A(c) and 4B(c)). Theoretically if the Sb(V) and As(V) in mixture solutions were reduced to Sb(III) and As(III) by a reduction agent, the absorbance signal height must be increased. In this work, this fact was confirmed by the reduction of pentavalent species of these two elements using sodium thiosulphate (0.4M). According to result shown in Fig. 4 A(d) and Fig. 4 B(d),an obvious increase in signal intensity of mixture solution is observed after reduction. Total amounts of As and Sb were determined after reducing the pentavalent species by sodium thiosulphate (0.4M). Then, pentavalent concentration was obtained from difference between trivalent and total amount of them. Results show that pentavalent concentrations equal to added values. In optimized condition, calibration curve is linear in the range of 0.2-2 $\mu gL^{-1}$for As according to $A_{int}=0.012 +0.236C_{As}$ by $R^2=0.9991$. Also, Sb regression equation is $A_{int}=0.005 +0.138C_{Sb}$, and it is linear in the range of 0.4-4 by $R^2= 0.9994$, other detailed result of this method is listed in Table 3.

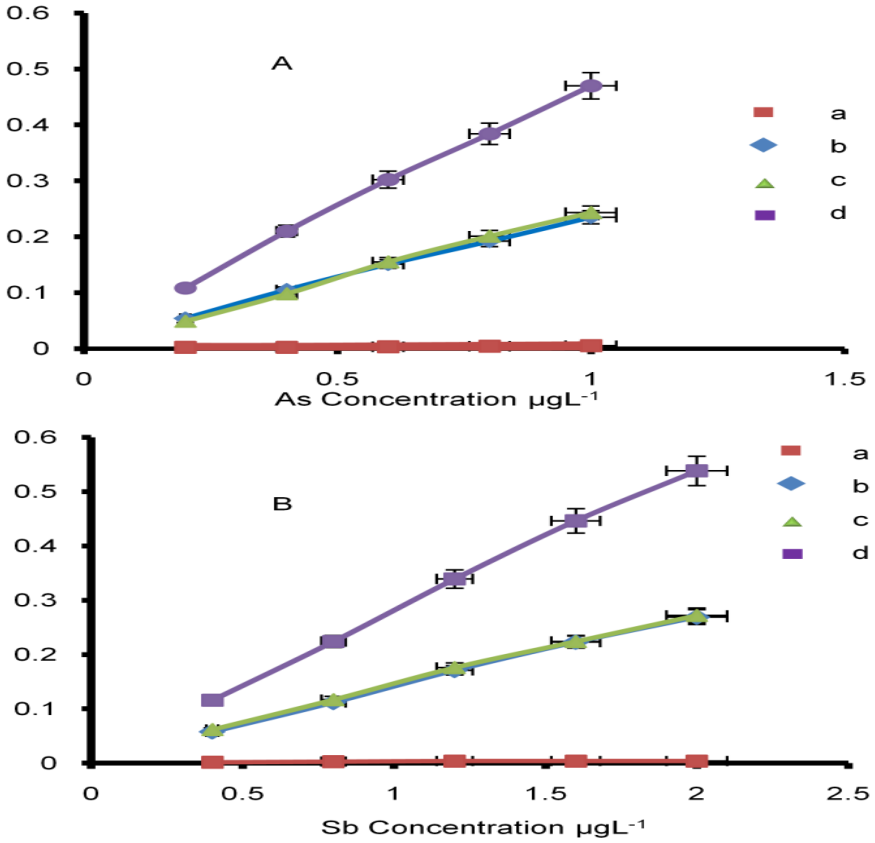

Fig. 4 Absorbance signal of the solutions that containing: pentavalent arsenic (A(a)), pentavalent antimony (B(a)), trivalent arsenic (A(b)), trivalent antimony (B(b)), mixture solution of trivalent and pentavalent arsenic (A(c)), mixture solution of trivalent and pentavalent antimony (B(c)), mixture solution of trivalent and pentavalent arsenic after reduction (A(d)), mixture solution of trivalent and pentavalent antimony after reduction (B(d)).

In Table 4, this method has been compared with other speciation methods of As and Sb in literatures. In comparison with other methods, pre-concentration using DLLME followed by ETAAS method has considerable low LOD and high enrichment factor. Other features of this method are low cost, fast, simple and no requirement of further instruments and can be used as a alternative techniques to more expensive determination and speciation methods of As and Sb in trace levels.

Table 3. Comparison of some reported determinations

Method	Analyte	Detection Method	Sample volume	Enrichment factor	Detection limit, μgL^{-1}	Reference
CPE	Sb	FI--ICP	100	872	0.09	[16]
CPE	Sb	FAAS	10	45	1.82	[17]
SPE	Sb	ETAAS	10	25	0.18	[18]
SPE	Sb	ETAAS	100	60	0.14	[19]
LC	Sb	SFI-HGAAS	20	10	0.06	[20]
SDME	Sb	ETAAS	5	96	0.008	[21]
DLLME	Sb	ETAAS	5	115	0.05	[22]
DLLME	Sb	ETAAS	10	187	0.02	This work
CPE	As	ETAAS	10	53	0.01	[23]
SPE	As	FI-HGAAS	20	10	0.05	[24]
LLE	As	FI-HGAAS	250	33	0.008	[25]
LC	As	ICP-AES	3	200	0.15	[26]
LPE	As	ETAAS	1.8	150	0.05	[27]
Anion exchange resin	As	ICP-AES	20	10	0.1	[28]
DLLME	As	ETAAS	5	115	0.01	[22]
DLLME	As	ETAAS	10	187	0.004	This work

Table 4. Figure of merit.

Characteristic	Sb(III)	As(III)
Working range, μgL^{-1}	0.4-4	0.2-2
Enrichment factor	187	187
Sample size, ml	10	10
0.2 gL^{-1} APDC in methanol, ml	0.730	0.720
Carbon tetrachloride, ml	0.03	0.5
Detection limit (3s)[a], μgL^{-1}	0.04	0.02
Precision (RSD, n=7), %	3.5	3.2
Calibration function (5 standard, n=3, μgL^{-1})	$A_{int}=0.005+0.138C_{Sb}$	$A_{int}=0.012+0.236C_{As}$
Correlation coefficient	0.9994	0.9991

a) Calculated on the basis of three times the standard deviation for 10 replications of the blank.

CONCLUSIONS

In current study, employing of experimental design caused to increasing the enrichment factor by the unfolded factor optimum values. On the other hand, LOD was decreased and determination of analytes was possible in lower level of concentration. A comparison of this method with some other methods shows that DLLME has a higher enrichment factor, lower LOD, lower sample and organic solvent consumption and shorter extraction time (less than 3 min). In addition, this method is a simple, low cost, and reproducible technique and with no need to further instrumentation. Therefore, the represented method is proposed as a proper alternative to more expensive instrument for determination of trace amount of As and Sb.

Acknowledgements. *Authors are grateful to University of Kashan for supporting this work by Grant NO. 463609/2.*

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