Speciation of arsenic (III,V) based on methyltrioctylammonium mercaptobenzoate and centrifuging dispersive liquid-liquid microextraction in water and blood

Ahmad Riahi [a], **Elham Mosafayian Jahromy** [b] **and Bahareh Fahimirad** [C,*]

[a] *Department of Chemistry, Australian Community of Science, Hobart,Tasmania, Australia*
[b] *Islamic Azad University of Pharmaceutical Sciences (IAUPS), Medical Nano Technology Tehran, Iran*
[C,*]*Department of Chemistry, Semnan University, Semnan, Iran*

ABSTRACT

A novel analytical method based on centrifuging dispersive liquid-liquid microextraction (CD-LLME) procedure for pre-concentration of As (III) has been developed prior to determine by hydride generation atomic absorption spectrometry (HG-AAS). In this method, 0.1 g of a task specific ionic liquids (methyltrioctylammonium 2-mercaptobenzoate; TOMAS; TSIL) as the extracting and complexing solvent and acetone as dispersant solvent were rapidly added into the water and blood samples at pH 4.5. The As (V) is simply calculated by difference between total concentration and inorganic forms As (III) in liquid samples. By optimizing parameters, the enrichment factor (EF) was obtained 9.8 and 49.6 for blood and water samples, respectively. The limit of detection (LOD) of 22.4 ngL^{-1} and 4.3 ngL^{-1} were achieved for 10 mL and 50 mL of As(III) in blood and water samples, respectively (RSD<%5). The real samples were validated by certified reference material (CRM) by proposed procedure.

1. Introduction

Analytical methods has important role for determining hazardous heavy metal in different matrixes such as human blood and environmental samples. Analytical methods based on nanotechnology and ionic liquids was used for determination arsenic concentration in the blood, urine and serum samples by different instruments such as ICP-MS, ET-AAS and HG-AAS [1]. Inorganic arsenic compounds are toxic in human body but organic arsenic is usually less harmful [2].

Therefore, speciation and determination of arsenic in human blood and water samples is very important [3]. The toxicity of arsenic compounds is generally linked to the soluble inorganic trivalent forms, which is controlled by pH. Human exposure assessment in workers to arsenic containing substances includes short term (recent or acute exposure) and long term (chronic exposure) tests that can be performed to monitor detoxification efficiencies. Exposure of arsenic can lead to progressive peripheral and central nervous changes, such as, numbness and muscle tenderness. Normal arsenic concentrations in blood and urine are typically below 50 µg L^{-1} and 7 µg L^{-1}, respectively [4]. Threshold limit value

(TLV) of arsenic concentration in human blood is less than 2.5 μg dL^{-1} [5].

So, the sensitive analytical techniques, such as; high performance liquid chromatography coupled to inductively coupled plasma mass spectrometry (HPLC-ICP-MS)[6], inductively coupled plasma atomic emission spectrometry (ICP-AES)[7], Cold vapor/hydride generation atomic absorption spectrometry (HG-AAS)[8], hydride generation and atomic fluorescence spectrometry (HGAFS)[9] and electro-thermal atomic absorption spectrometry (ET-AAS)[10]. Gas Chromatography-Inductively Coupled Plasma-Mass Spectrometry (GC-ICP-MS) or Ion Chromatography Coupled to inductively coupled plasma mass spectrometry (IC- ICP-MS) was required for determination and speciation of arsenic in blood and water samples. Among them, HG-AAS is a conversional instrument which was widely used for arsenic determination in human biological samples and waters. But, sample preparation was needed for preconcentration and separation ions from real samples before using analytical techniques. The ionic liquids (ILs) as green solvent were used for separation and determination metals in liquid phases. The different hydrophobic/hydrophilic ILs can be extracted ions from waters with ligand by spectrometry methods [11-14]. The cadmium, chromium and mercury were removed from water samples by TSILs. The sample preparation procedures based on ionic liquids (ILs) was used for this purposed. Recently, the liquid–liquid extraction (LLE)[15], cluod point extraction (IL-CPE)[16], ionic liquid based on solid phase extraction (IL-SPE) [17] was reported by previous papers. Arsenic speciation and determination by GC-ICP-MS or IC- ICP-MS were too much expensive. On the other hands, the arsenic speciation with conversional instruments needs to prepare difficult samples at low time. In this study, a new analytical method based on TOMAS (C$_{32}$H$_{59}$NO$_2$S) was used for arsenic speciation in water and human blood samples by CD-LLME procedure. Based on results, many advantages such as, low time, efficient extraction and high recovery were obtained.

2. Experimental

2.1. Apparatus
The experiments were performed using a GBC-932 atomic absorption spectrometer equipped with a cold vapor/hydride generation module (HG3000-AAS -AUS). The operating parameters for the metal of interest were set as recommended by the manufacturer. Mercury and arsenic determined by HGAAS respectively. Arsenic hollow cathode lamp based on 8 mA, 193.7 nm and the spectral bandwidth of 1 nm was used. The pH values of the solutions were measured by a digital pH meter (Metrohm 744). In all analysis the deuterium background correction was turn on (Table 1). The instrumental calibration curve was linear between 0.5 –30 μg L^{-1}. All containers (quartz crucibles, plastic tubes) were cleaned with detergent and treated successively by the HNO$_3$(2%) and rinsed with de-ionized water(DW). The pure argon gas (99.99%) was used as a carrier gas for HGAAS analysis. The reduced flame was turn on by HG-AAS.

2.2. Materials
All chemicals of analytical grade such as nitric acid, hydrochloric acid, Polyoxyethylene octyl phenyl ether (TX-100), sodium acetate, sodium hydroxide, and sodium borohydride (NaBH$_4$) were from Merck Germany. Reducing agents (aqueous solution of 0.6% sodium borohydride in 0.5% sodium hydroxide) were prepared freshly and filtered before use. Arsenic standard solutions were prepared from a stock solution of 1000 mg L^{-1} as ultra-trace in 2% nitric acid from Fluka Switzerland (No; 39436). Working standard solutions were prepared by dilution of stock and intermediate standards. Buffer solutions were prepared from 1-2 mol L^{-1} sodium acetate and acetic acid for pH= 3-7. Ultrapure water was obtained from a Water System of Iranian research Institute of Petroleum Industry (Millipore RIPI). Alderich. The TSIL, Ethyltrioctylammonium 2-mercaptobenzoate or Trioctylmethylammonium thiosalicylate (TOMAS, CAS Number 1027004-61-0) was purchased from Sigma Aldrich.

Table 1. Instrumental Conditions for arsenic

Parameter	Arsenic
Wavelength (nm)	193.7
Lamp current (mA)	8.0
Slit (nm)	1.0
LOD ($\mu g\ L^{-1}$)	0.22
Linear Range ($\mu g\ L^{-1}$)	0.5-58
Mode	Peak area

2.3. Sampling

For sampling, the clean glass tubes and container were purchased from Iranian company. The 10 mL of human blood samples were collected from industrial factory of Iran. For sampling, all glass tubes were washed with a 0.5 mol L^{-1} HNO_3 solution for at least 24 h and thoroughly rinsed 10 times with ultrapure water (UPW) before using. As concentrations of arsenic in blood / serum are very low, even minor contamination at any stage of sampling, sample storage and handling, or analysis has the potential to affect the accuracy of the results. For analysis in blood 10 μL, pure heparin (free metals) is added to a 10 mL blood sample. The human blood sample was maintained at –20 °C in a cleaned glass tube. The water prepared in 250 mL of polyethylene bottle (PEB) based on ASTM for sampling and storage by acidifying.

2.4. General procedure

The developed method based on centrifuging dispersive liquid-liquid microextraction (CD-LLME) was used for arsenic speciation in human blood and water samples at pH=4.5. By proposed procedure, the concentrations of As (III, V) in range of 1-5.8 $\mu g\ L^{-1}$ were determined by HG-AAS in human blood and water samples. As (III) can be extracted with TOMAS in liquid phase without any chelating agent at optimized pH. Flame condition tuned based on 1.2 L min^{-1} of fuel with low air flowrate for As by HG-AAS. By CD-LLME procedure, 0.12 g of TOMAS diluted with 0.2 mL of acetone and injected into 10 mL of blood and standard samples which was included the arsenic
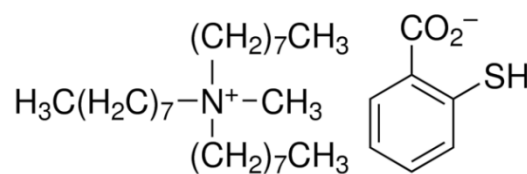
Fig. 1. The schema of TSIL (TOMAS)

concentration of 1-5.8 $\mu g\ L^{-1}$. Then, the pH was adjusted to 4.5 with buffer solution, shaking for 5 min and then transferred to a centrifuge tube. Arsenic (III) was complexed with TSIL (As-TOMAS) and then, the TSIL were separated from liquid phase by centrifuging of turbid solution at 3 min with 3500 rpm. The upper phase of TOMAS, with olive green color layer, was removed with a transfer pipette to PEB (5 mL). The As(III) was back-extracted from TOMATS at acidic pH by 0.5 mL of hydrochloric acid solution (2 M) which was shaken for 1.0 min and diluted with ultrapure water up to 1mL. Then, the TOMAS phase was removed by centrifuging and pipette and aqueous phase was determined by HG-AAS. The same procedure was done on sample blank for water and human serum/blood samples without As(III,V). Finally, the As (V) was reduced to As (III) with KI (1M) and ascorbic acid, the total arsenic (TAs) was determined. In addition, the concentration of As (V) was calculated by subtracting the content of As (III) from total arsenic content. The extraction conditions based on TOMAS were explained in Table 2.

3. Results and Discussion

In proposed method, for increasing higher sensitivity, selectivity and precision, of determining and speciation arsenic (As) in blood and water samples, we studied and optimized thoroughly, the effect of the main parameters, like the type of disperser and extraction solvent, sample volume, sample acidity, amount of TOMAS as chelating agent, and extraction time.

Human sampling based on ethical rules was confirmed by the Ethical Committee of Islamic Azad University, Tehran Medical Sciences (Ethical Code: R.IAU.PS.REC.1399.106)

Table 2. The extraction conditions of As(III) based on TOMAS by CD-LLME method

| Features (Human blood)	Value As
Mean RSD% (blood, n=10)	3.2
LOD of CD-LLME(blood, µg L^{-1})	0.022
Enrichment factor(blood)	9.8
Volume of blood (mL)	10
Linear range of blood, PA (µgL^{-1})	0.05 –5.9
Correlation coefficient of DLLME	R = 0.9965
Features (Water)	**Value As**
Mean RSD% (water, n=10)	2.8
LOD of CD-LLME(water, µg L^{-1})	0.004
Enrichment factor(water)	48.6
Volume of water(mL)	50
Linear range of water, PA (µgL^{-1})	0.01-1.1
Correlation coefficient of DLLME	R = 0.9983

PA = Peak Area

3.1. Instrumental

The repeatability of results was investigated for speciation and determination arsenic in blood and water samples by hydride generation atomic absorption spectrometer (HGAAS) in present of flame. After mixing reagents and samples, the mixture solution moved to reaction coil where the metal hydride is formed and then passed through a gas-liquid separator where the hydride vapor is removed from the bulk liquid using an inert carrier gas. The hydride is then fed into a fused quartz absorption cell. For arsenic determination the cell is mounted over a burner and heated by an air-acetylene flame (HGAAS). Flame conditions were with 1.2 Lmin^{-1} fuel and minimum flow air.

3.2. Effect of pH range

The complexation phenomenon is strongly conditioned by the pH of solutions and subsequently affects the extraction efficiency of the As(III) by complexing of TOMAS. As previously research, ionic liquids such as TOMAS was decomposed in lower pH (less than pH=3). Therefore, the effect of pH was studied and evaluated in the pH range of 3 −11 as a lower limit of quantification (LLOQ) and upper limit of quantification (ULOQ) for 0.05-5.0 µg L^{-1} for As(III). The results show that the high extraction efficiency for As (III) were achieved in pH=4.5 and As(V) had no extraction in optimized pH (less than 5%). So, the procedure was applied to speciation of arsenic in water and blood samples (Fig. 2).
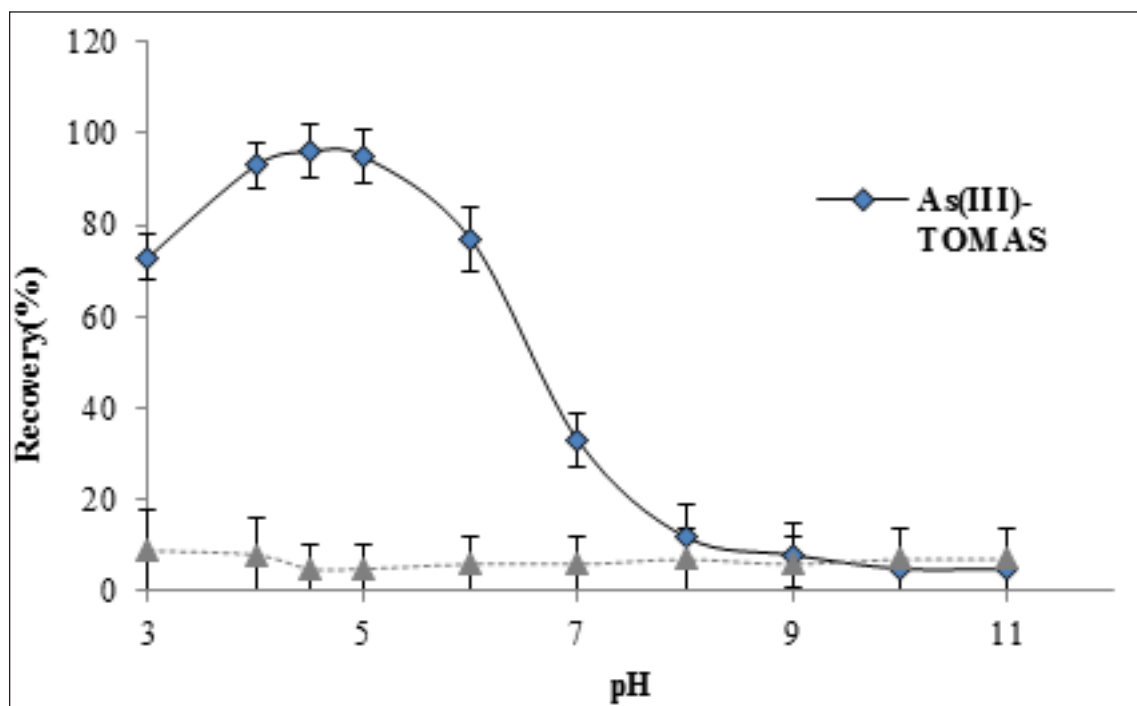
Fig. 2. The effect of pH on As(III) extraction based on TOMAS by CD-LLME method

3.3. Optimization of amount of TSIL and extraction time

The variation of extraction efficiency upon TOMAS amount as TSIL was examined within the range of 0.02-0.2 g for arsenic concentration from 0.05 to 5.0 μg L^{-1} in blood samples. It was observed that the extraction efficiency of the system was remarkably affected by the TSIL amount. Quantitative extraction of As(III) based on TOMAS was observed more than 0.08 g and 0.1 g for 10 mL and 50 mL of blood and water samples, respectively. So, 0.12 g of TOMAS was selected as optimum amount of TSIL for arsenic speciation in both samples (Fig.3). Triton X-100, an emulsifier and anti-sticking agent, was added to the solution in order to raise the efficiency of the extraction procedure. After added TX-100 [1% (w/v)] the quantitative extraction was observed for arsenic by TOMAS in human blood samples. The effectiveness of As(III) extraction under the influence of shaking and centrifugation time was studied. The different shaking and centrifuging times (3500rpm) were studied and optimized by CD-LLME procedure. The results showed us, the 5.0 min of shaking and 3.0 min of centrifuging had efficient extraction for proposed method.

3.4. Effect of sample volume

Sample volume is one of the most important parameter to be studied when real samples are analyzed by a pre-concentration technique, since it conditions the sensitivity enhancement of the method. The effect of sample volume was examined in a range of 1–100 mL for 5.0 μg L^{-1} and 1.0 μg L^{-1} As(III) ions in blood and water samples, respectively. It was found that the As(III) could be quantitatively extracted in blood samples for 12 mL of the sample solution. At higher a volume the recoveries are decreased. It was also noticed that higher sample volumes partially solubilized the TOMAS in liquid phase and lead to non-reproducible results. Therefore sample volume of 10 mL was selected for further experiments for human blood samples. Also, the results showed, the As(III) was efficient extracted based on TOMAS in 60 mL of water samples. So, 50 mL of water samples selected for further study (Fig. 4).
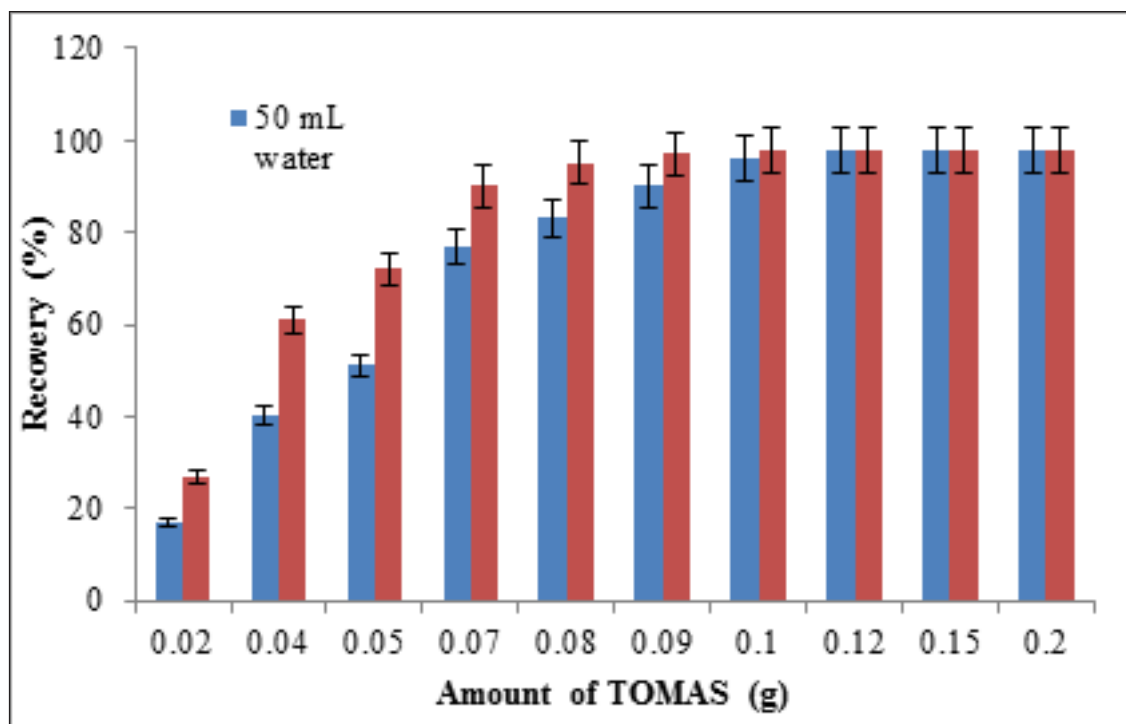

Fig. 3. The effect of amount of TOMAS on As(III) extraction by CD-LLME method
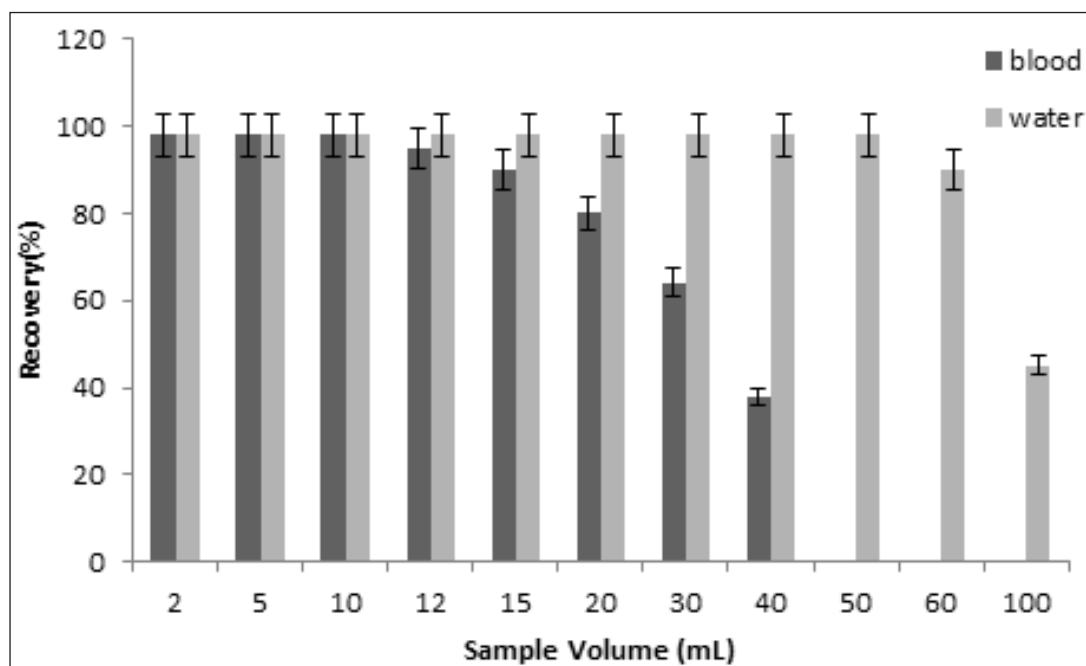
Fig. 4. The effect of sample volume for As(III) extraction by CD-LLME method

3.5. Interferences study.

TOMAS is a TSIL with thiol (HS) group which was acted as a chelating agent for many transition metals. Thus, for extraction of As(III) with TOMAS, the interferences coexisting ions such as mercury, lead, copper, zinc, vanadium, and silver should be considered. The effect of potential interfere occurring in blood and water samples on the determination of As(III) were tested in optimized conditions by CD-LLME procedure. The extraction recovery of TOMAS for 0.05-5.9 μg L^{-1} and 0.01-1.0 μg L^{-1} of As(III,V) was tested in blood and water samples with individual ions interferences. The results showed that many ions could be tolerated up to at least 1-3 mg L^{-1}. Mercury and silver had low tolerated up to at least 80-400 μg L^{-1} by using TOMAS in water and blood samples. High concentrations of alkali metals, alkaline earth metals, CO_3^{2-} and PO_4^{3-} which are usually found in blood samples, were tested by 0.5-1.0 mg L^{-1} and did not effect on extraction recovery of As(III) by CD-LLME procedure (Table 3).

3.6. Method validation

For validation of proposed method, 10 mL and

Table 3. The effect of interferences coexisting ions on extraction of As(III) in human blood and water samples by CD-LLME method

Interfering Ions in blood(M)	Mean ratio $(C_M/C_{As(III)})$	Recovery (%)
	As(III)	As(III)
Cr^{3+}, Al^{3+}, Mn^{2+}, Cd^{2+}, V^{3+}, Pb^{2+}	750	98.8
Zn^{2+}, Cu^{2+}, Ni^{2+}, Co^{2+}, Mo^{2+}	500	95.9
I$^-$, Br$^-$, F$^-$, Cl$^-$	1000	98.5
Na^+, K^+, Ca^{2+}, Mg^{2+}	900	99.1
CO_3^{2-}, PO_4^{3-}, HCO3$^-$	700	96.7
Ag^+	300	98.2
Hg^{2+}	80	96.6
NO3$^-$, SO_4^{2-}	800	97.5
Interfering Ions in water (M)	**Mean ratio** $(C_M/C_{As(III)})$	**Recovery** (%)
	As(III)	As(III)
Cr^{3+}, Al^{3+}, Mn^{2+}, Cd^{2+}, V^{3+}, Pb^{2+}	900	97.7
Zn^{2+}, Cu^{2+}, Ni^{2+}, Co^{2+}, Mo^{2+}	650	97.2
I$^-$, Br$^-$, F$^-$, Cl$^-$	1200	97.4
Na^+, K^+, Ca^{2+}, Mg^{2+}	1000	98.6
CO_3^{2-}, PO_4^{3-}, HCO3$^-$	950	96.6
Ag^+	400	99.2
Hg^{2+}	150	97.3
NO3$^-$, SO_4^{2-}	1000	98.1

Table 4. Validation of the methodology was performed by standard reference material (NIST SRM 2670)

SRM	As(III)	As(V)	Found [a] As(III)	Found [a] As(V)	Recovery As(III)	Recovery As(V)
Urine [b]	2.50 ± 0.2	1.50 ± 0.2	2.52 ± 0.18	1.45 ± 0.11	100.8 %	96.6%
Blood [c]	2.07 ± 0.63	-----	1.98 ± 0.12	-----	95.7%	-----

[a] Mean value ± standard deviation based on three replicate measurements

[b]NIST, SRM 2670, arsenic in frozen dried urine, pH 4.0 ,-20°C

[c] NIST, SRM 955c, Caprine Blood, Level 1

50 mL of blood and water samples were used. Results showed that there are no interferences from major consistent of blood samples, therefore we have explored the feasibility of the methodology using proposed method for the determination of As(III) ions in different matrices. Validation of the methodology was performed by standard reference material (NIST SRM 2670) with certified values for arsenic speciation (Table 4). Also, the spiked of standard arsenic solutions with real human blood and water samples were done to demonstrate the reliability of the method for determination and speciation of As (III) and As (V) (Table 5). In addition, the ability of different methods compared to CD-LLME method in Table 6. The TOMAS based on CD-LLME-HGAAS technique can be used for determination and speciation of arsenic (As^{3+}, As^{5+}) in human samples as compared to HG-AAS or ET-AAS (project Ethical Code: R.IAU.PS.REC.1399.106).

3.7. Comparing to other methods

The figures of merit of the CD-LLME method compared to the alternative methods for arsenic speciation (III, V) in different matrixes (Table 7). Recently, the different techniques for extraction/separation/detemination arsenic spcies in human biological fluids and waters have been reported. Some techniques such as dispersive liquid–liquid microextraction (DLLME), cloud point extraction, and solid phase extraction have already used for extraction and speciation arsenic [18-21]. In this work, the TSIL (TOMAS) based CD-LLME combined with CV-AAS for speciation and determination of As (II, V) in human blood and water samples. The LOD, RSD and linear range values compared to other published method. Wen et al. used cloud point extraction with ICP-optical emission spectrometry for speciation of AS (III, V) with LOD of 0.72 and RSD of 3.5 which was higher than CD-LLME procedure [18]. Based on

Table 5. Evaluation of accuracy and precision of results in human blood and water samples by spiking of arsenic standard (III, V) based on CD-LLME method

Sample	Added As(III)	Added As(V)	Found [a] As(III)	Found [a] As(V)	Found [a] Total As	Recovery (%) As(III)	Recovery (%) As(V)	Recovery (%) Total As
CRM*	-------	-------	2.45 ± 0.14	1.42 ± 0.11	3.87± 0.21	98.0	94.6	96.7
	2.0	-------	4.41 ± 0.23	1.44 ± 0.12	5.85± 0.28	98.1	-------	99.0
	-------	1.5	2.43 ± 0.15	2.95 ± 0.16	5.38± 0.25	-------	102	100.6
Water	-------	-------	0.88 ± 0.04	0.59 ± 0.02	1.47 ± 0.07	-------	-------	-------
	1.0	-------	1.85 ± 0.09	0.61 ± 0.03	2.46 ± 0.13	97.0	-------	98.8
	-------	0.5	0.87 ± 0.05	1.07 ± 0.05	1.94 ± 0.11	-------	95.7	94.5
Blood	-------	-------	1.77± 0.08	ND	1.77± 0.08	-------	-------	-------
	1.5	-------	3.24± 0.14	ND	3.24± 0.14	98.0	-------	98.0
	-------	1.5	1.74± 0.08	1.48± 0.07	3.22± 0.18	-------	98.6	96.5
Urine	-------	-------	1.15± 0.06	1.06± 0.05	2.21± 0.11	-------	-------	-------
	1.0	-------	2.17± 0.08	1.02± 0.05	3.19± 0.15	102	-------	97.9
	-------	1.0	1.13± 0.05	2.03± 0.10	3.16± 0.16	-------	97.0	95.0

[a]Mean value ± standard deviation based on three replicate measurements

*NIST SRM 2670, arsenic in frozen dried urine, pH 4.5,-20°C

Table 6. Comparing of different techniques with CD-LLME method for determination and speciation of arsenic (III, V) in real samples

As species	Sample	HG-AAS*	CD-LLME/HG-AAS*	LC-MS/MS*
Water	As (V)	-----	0.304 ± 0.016	0.296 ± 0.012
	As (III)	-----	0.197 ± 0.011	0.201 ± 0.008
	Total	± 0.027 0.485	± 0.024 0.501	± 0.015 0.497
Blood	As(V)	-----	0.865 ± 0.043	0.844 ± 0.032
	As (III)	-----	2.641 ± 0.127	2.702 ± 0.096
	Total	3.317 ± 0.175	3.486 ± 0.166	3.546 ± 0.121

*Mean value ± standard deviation based on three replicate measurements (N=5, P= 0.95)

Table 7. Comparison of the published methods with the proposed method in this work

Method	Separation	Matrixes	LR*	DL*	RSD%	References
ICP-OES [a]	APDC-CPE [b]	Snow water	2-50	0.72	3.5	[18]
ETAAS	HF-LPME[C]	Hair water	1-50	0.12	8.0	[19]
LC-HG-AFS[D]	1-octyl-3-methylimidazolium chloride(IL)	wine	1-2000	0.81	2.98	[20]
ET-AAS	DDTP -CLLME[H]	plasma urine	0.1-50	0.03-0.05	4.0- 5.7	[21]
HGAAS	TOMAS- CD-LLME	Blood	0.05 – 5.9	0.022	3.2	This work
HGAAS	TOMAS/ CD-LLME	Water	0.01 –1.1	0.004	2.8	This work

* Linear rang (LR, µg L−1); Detection limit (DL, µg L−1)

[a] inductively plasma-optical emission spectrometry(ICP-OES)

[b] Ammonium 1-pyrrolidinedithiocarbamate(APDC) - Cloud point extraction (CPE)

[C] Hollow fiber liquid phase microextraction combined

[D] liquid chromatography - hydride generation atomic fluorescence spectrometry

[H] Diethyldithiophosphoric acid (DDTP)-Centrifuging liquid-liquid microextractio

Table 7, the sensitivity of the developed method is similar to other reported methods. The linear range was perfectly adequate for the analyzed human blood samples. Lower LOD values are related with higher sensitivity of proposed method which was used in this study. Also, some methods have higher PF that analyzed by ICP in water or human blood samples with large sample volume. TSIL (TOMAS) based CD-LLME combined with CV-AAS showed a rapid and easy extraction and speciation of As (III,V) using a user-friendly instruments.

4. Conclusions

The procedure here studied takes advantage of the combination of a very simple, reliable way of pre-concentrating in sea water and blood samples for arsenic determination and speciation with the sensitive TOMAS@HG-AAS technique. The increase in sensitivity resulting of sample pre-concentration,   good sample frequency and possibility of speciation of As (III), As (V) forms of these analytes, means the procedure can be considered an alternative to high-performance liquid chromatography (HPLC) in combination with inductively coupled plasma mass spectrometry (ICP-MS) , Ion Chromatography Coupled to inductively coupled plasma mass spectrometry (IC- ICP-MS) and ICP-MS. The results showed that the quantitative extraction (QE) and enrichment factor (EF) for water samples were more than 95% and 49.6, respectively (RSD<5%). Linear range of arsenic in blood and water samples was obtained 0.05 –5.9 and 0.01-1.1µgL^{-1}, respectively by CD-LLME method. By proposed procedures, the satisfactory results of ultra-trace analysis for arsenic species in blood and water samples were achieved.

5. Acknowledgements

Iran. (EthicalCode:R.IAU.PS.REC.1399.106)

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