Polish Journal of Environmental Studies Vol. 11, No. 6 (2002), 617-623

Validation and Quality Assurance of Arsenic Determination in Urine by GFAAS after Toluene Extraction

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Received: 10 March, 2002
Accepted: 12 July, 2002

Abstract

This paper concerns quality assurance of arsenic determination in urine using the graphite furnace AAS method after toluene extraction. Obtained analytical results are used for validation and quality assurance purposes. The investigated urine samples were acquired from the workers of a large coal-fired Slovak power plant. In the proposed analytical method the following metrological characteristics were calculated: trueness, precision, recovery, the limit of detection and the limit of quantification. The method was satisfactorily used in the intercomparison test. The proposed method is suggested for utilisation in the assessment of occupational or environmental exposure to inorganic arsenic.

Keywords: quality assurance, urine analysis, arsenic determination, GFAAS method, toluene extraction

Introduction

Extensive use of arsenic and its compounds in industry and agriculture has resulted in the risk of humans being in contact with it. High concentrations of arsenic in some natural ground water has also been documented [1]. Monitoring of soluble arsenates is important for aqueous environments. Toxicity of arsenic compounds increases from organic forms through pentavalent arsenate towards arsenite. The premier method for eliminating arsenic compounds from the body is urinary excretion.

A few research workers have attempted to accomplish the direct graphite furnace AAS determination of arsenic in urine. The acidification and dilution of urine has been the most common technique to prepare urine samples before analytical measurement. The direct determination of arsenic in urine using GFAAS is associated with problems such as volatilisation loss, vapour phase and spectral interference, or interaction with the graphite tube [2-6]. Different matrix modifiers including palladium [3], nickel and rhodium salts were used for phosphate interference elimination [2]. An appropriate dilution of the urine samples can reduce the unfavourable influence of the high concentration of phosphate [4].

Several forms of the inorganic arsenic, namely As(III), As(V), and monomethylarsonic (MMA) and dimethylarsinic (DMA) acids, can be determined in aqueous solution by electrothermal atomic absorption spectrometry (ETA AAS) method in the presence or without the presence of potassium iodide [6]. The modified procedure [3] involves acidification of the sample with concentrated HCl, the addition of KI and extraction into toluene. The upper (toluene) layer was first separated and then it was re-extracted by nickel nitrate ($Ni + HNO_3$) water solution. The aqueous phase was finally used for the ETA AAS measurement. As(III), As(V), MMA and DMA were

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extracted if potassium iodide was present. Selective extraction of As(III) was possible in the absence of potassium iodide. Arsenobetaine was not extracted by the applied method [3, 5, 6], therefore the method does not fully indicate total arsenic.

After exposure to inorganic arsenic, the major urinary metabolites are inorganic arsenic, MMA and DMA. The described method, adapted in the way shown in the experimental part, is suggested for the assessment of occupational or environmental exposure to inorganic arsenic.

A set of operating principles that are strictly followed during sample collection, preparation and analysis, enable us to produce data that are accurate, reliable and adequate for the intended purpose. An effective and reliable method of the quality assurance in the analysis of arsenic by a routine multi-level method is presented in this paper.

Experimental Procedures

Material

Urine samples were collected from the workers of the coal-fired plant ENO Nováky (Slovak Republic) into clean polypropylene containers. Then they were frozen and stored at –18 °C. Prior to analyses, the samples were slowly thawed at room temperature.

Method

The procedure used was adapted from Subramanian [3]: 12 mL concentrated hydrochloric acid and 0.4 mL 40% (m/V) potassium iodide solution were added to 2.5 mL of urine in a separatory funnel. Then the mixture was shortly mixed and let stand for 60 min. Subsequently 5 mL of toluene saturated by hydrochloric acid was added and the separatory funnel was shaken for 3 min. The emulsion was decomposed by the addition of 0.4 mL of ethanol. A 3 mL portion of the toluene layer was transferred to the test tube. Arsenic was then re-extracted into 1.5 mL of 0.1% nickel nitrate in 1% HNO_3 during 1 min and the Ni-HNO_3 layer was taken to AAS analysis.

Chemicals

Stock standard solutions of 1g/L As(V), As_2O_5 (Titrisol, No. 1.09939 MERCK), monosodium methylarsonate, MMA, (PS-429, CHEM SERVICE, West Chester), and dimethylarsinic acid sodium salt trihydrate, DMA, (No. 820670, MERCK), were used. Working standard of As was prepared as a mixture of arsenic species in the mass ratio 1As(V):1MMA:2DMA. The standard solution concentrations of 10, 25, and 50 µg As/L were prepared daily by gradual diluting with 0.5 % (V/V) nitric acid.

Toluene was saturated by aqueous phase by shaking 100 mL of toluene (MERCK) with 10 mL of hydrochloric acid in a separatory funnel.

Concentrated hydrochloric acid 30% was obtained from MERCK.

0.1 % solution of nickel nitrate was prepared by dissolving 0.1 g of Ni (MERCK) and adjusted to 100 mL flask with 1 % HNO_3.

40 % solution of potassium iodide was prepared by dissolving of 4 g KI (MERCK) and adjusted to 10 mL with distilled water.

Standard reference material SRM 2670-Toxic Metals in Freeze-Dried Urine, NIST, was prepared for use according to NIST instruction [7].

High purity water, prepared in Ultra High Quality unit (ELGA Ltd, England) from deionised water, was used in all experiments. All chemicals were of analytical reagent grade.

Instrumental

The PERKIN ELMER 4100ZL AAS equipment, with the Zeeman background correction, THGA furnace and an AS-71 autosampler were used for all measurements. Pyrolytic-coated graphite tubes were used. An electrodeless As discharge lamp, operated at 10 W and with a resonance line of 193.7 nm, was used for measuring arsenic absorbance. External modulation from the EDL System 2 Power Supply was applied. Dispensed sample volume of 20 µL was used for analysis. The typical furnace program is given in Table 1.

Table 1. Furnace conditions.

Step	Temp. (°C)	Ramp time (s)	Hold time (s)	Internal flow (mL/min)	Gas type	Read step
1	110	1	20	250	Normal	
2	120	5	50	250	Normal	
3	130	5	50	250	Normal	
4	1300	10	5	250	Normal	
5	2500	0	5	0	Normal	*
6	2500	1	2	250	Normal	

Results and Discussion

AAS Determination

The optimum furnace conditions were found from the corresponding maximum of the pyrolysis – atomization temperature optimization graph (Fig. 1) obtained after the extraction procedure for the urine sample spiked with 25 µg As/L. Either the peak height or the arsenic specific integrated absorbance were measured in the nickel nitrate solution, used as a matrix modifier. The presence of Ni stabilises arsenic at higher temperature of the ashing stage (1300°C). The maximum value of both arsenic specific integrated absorbance (*iA*) and the peak height (*ph*) were obtained at the atomisation temperature of 2500°C.

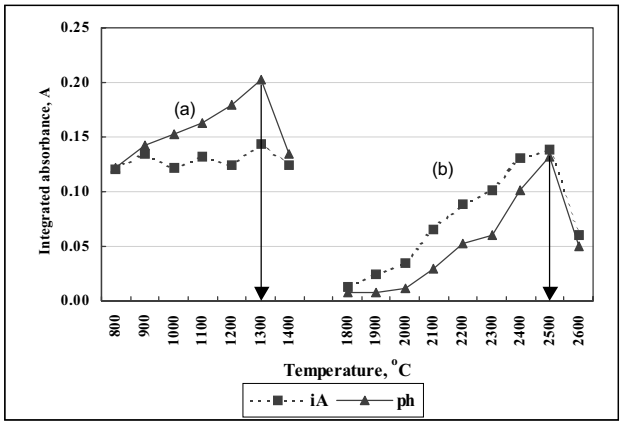

Fig. 1. Pyrolysis (a) and atomization (b) temperature optimization graph for determination of 25 µg/L As in urine. The arsenic specific integrated absorbance is denoted *iA*, peak height is *ph*. The optimum pyrolysis and atomization temperature is at 1300 °C and 2500 °C, respectively.

Signal Evaluation

The arsenic specific integrated absorbance was used for the signal evaluation. Three types of calibration curves were prepared in which the aqueous standards, urine standards and the standard addition technique were used. All standards were treated by the same extraction procedure as the real urine samples. Efficiency and accuracy of the arsenic concentration were evaluated by analyses of the SRM 2670 as well as the internal reference material - *Frozen urine*, spiked with 25 µg As/L (frozen and stored under the same conditions as the real urine samples). The obtained results are collected in Table 2. It is evident that all calibration procedures are appropriate to evaluating the arsenic content in the tested materials. This fact agrees with formerly published results [3] concerning the arsenic species in synthetic urine, natural urine and aqueous standards, respectively, since the responses of As(V), MMA and DMA in the synthetic urine were found to be the same as those of the natural urine samples. In addition, a response identical to the aqueous standards of arsenic species was found if the same extraction procedure was used.

Table 2. The recovery of arsenic[a)] in the SRM-2670 and the internal reference material Frozen urine, related to the chosen type of calibration.

Type of calibration	Recovery, %	
	SRM-2670 480 ± 100 µg/L	Frozen urine 25 µg/L
Standards in the aqueous matrix	109.0 ± 12.0[b)] n=20	103.3 ± 7.7 n =10
Standards in the urine matrix	98.2 ± 15.6 n=16	97.1 ± 13.6 n=12
Standard addition to the urine matrix	100.2 ± 12.6 n=22	98.2 ± 14.2 n=17

[a)] Arsenic working standard as described in Experimental Procedures

[b)] The estimate of the standard deviation

Quality Assurance and Quality Control, QA/QC

The routine analyses of samples were performed in accordance with the principles of Good Laboratory Practice, GLP [8]. The blank was measured in each batch of samples (n = 10–15) by using the described extraction procedure and analysed to check the purity of the used chemicals. The standard solutions needed for calibration purposes were prepared daily.

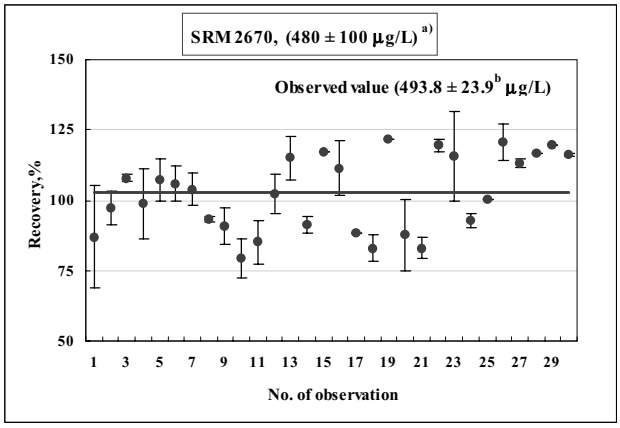

Fig. 2. Control chart of SRM 2670 with [a)] certified value, [b)] the mean and 95% confidence interval. If more than one measurement were performed, the points are represented by the signal means and the corresponding upper and lower confidence limits are connected by vertical lines.

Trueness

Trueness assessment of the method was confirmed by comparing the reference value for the certified reference material SRM 2670 level (480 ± 100 µg/L) with the results

obtained in our laboratory. The obtained laboratory results were characterized by the 95% confidence interval as 493.8 ± 23.9 μg/L As (102.9 ± 4.9 %) for *n*=30 and agreed well with the certified value of SRM 2670. The individual observations are shown in Fig. 2.

Analytical Spike

Precision in the sample loading and the matrix effect on the analyte response were studied by analytical spike. It was made by the addition of the known amount of the analyte to the sample, performed automatically by the QC software controlling the AAS instrument. By rule, the standard solution 25 μg/L As used for the standard addition has to be different than that used for the calibration. The obtained recovery corresponding to *n*=25 analyses of arsenic in urine was characterized by a 95% confidence interval 104.7 ± 3.6 %. The results are given in Fig. 3.

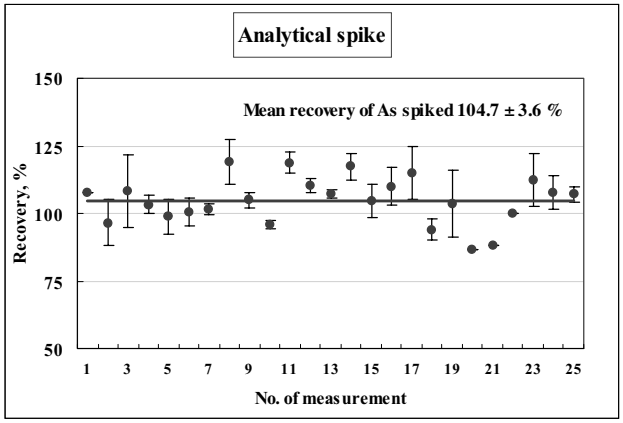

Fig. 3. Control chart of analytical spike made by the addition of 25 μg/L As. If more than one measurement were performed, the points are represented by the signal means and the corresponding upper and lower confidence limits are connected by vertical lines.

Recovery Study

The recovery study was made to confirm the efficiency of the extraction procedure. Randomly selected urine samples were spiked with 20, 25, 80 mg As/L, respectively, before the sample treatment procedure. Then 20 analyses of arsenic in urine were performed and the obtained mean recovery of matrix spike was 99.7 ± 6.3 % (95 % confidence interval). The individual values of the spiked urine samples are reported in Table 3.

Table 3. Recovery study.

Sample No.	Recovery in % for arsenic concentration in μg/L			
	No standard addition	+20 μg As/L	+25 μg As/L	+80 μg As/L
6162	17.4			86.5 / 86.4
6261	ND [a]		27.5 / 110.0	
6262	ND [a]		25.2 / 100.8 [b]	
6247	ND [a]		30.5 / 122 [b]	
6286	< 10.5 [b]		33.3 / 91.2	
6416	26.8		51.6 / 99.2	
Blank urine 1	< 10.5 [b]		28.5 / 112.0	
Blank urine 2	ND [a]		29.1 / 116.4	
6444	15.4		35.5 / 80.4	
Blank urine 3	ND [a]		26.8 / 107.2	
Blank urine 4	ND [a]		26.3 / 105.2	
6471	ND [a]		26.3 / 97.6	
6502	ND [a]		24.4 / 90.5	
6552	27.5		54.8 / 109.2	
6601	< 10.5 [b]		32.8 / 89.2	
6536	24.1	42.2 / 90.5		
6561	18.1	34.5 / 82		
6594	14.1	33.1 / 95		
6608	36.4	60.5 /120.5		
6396	15.4	30.4 / 75		

[a] ND – not detected, below the limit of detection

Internal Reference Material *Frozen urine*

The urine samples, stored at –18 °C for several months, were slowly thawed before treatment at room temperature. The analyses of the internal reference material *Frozen urine* were made in order to test the storage procedure. The *Frozen urine* sample was prepared from the collected urine portions of one volunteer. The working standard solution of As (a mixture of As(V), MMA, DMA - as described in Experimental Procedures) was added into the well-mixed urine sample to get the final concentration 25 μg As/L. The spiked urine sample was divided into 50 mL polypropylene decontaminated flasks and was frozen at –18 °C. For each analysis, one portion of the *Frozen urine* was thawed and treated the same way as the natural samples. The mean recovery, evaluated as the 95 % confidence interval, was 99.6 ± 3.9 % (Fig. 4).

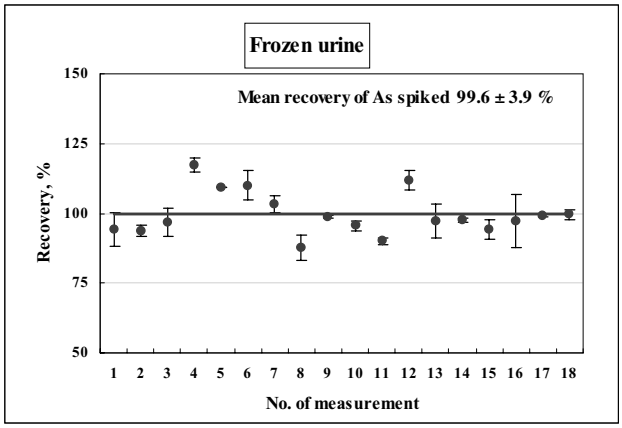

Fig. 4. Control chart of internal reference material *Frozen urine*, to which 25 μg/L As was added. If more than one measurement were performed, the the points are represented by the signal means and the corresponding upper and lower confidence limits are connected by vertical lines.

Intercomparison Test

The applied method was verified in the Intercomparison of Analytical Methods for Arsenic Speciation in Human Urine [9], in which the sum of As(III), As(V), MMA and DMA determination was included. The results given in Table 4 are encouraging. With seven laboratories included into the intercomparison test, the percentage recoveries (ratio of the found mean arsenic to the concentration spiked) ranged from 107.1 to 283.5 % for the sample 1A, and our laboratory result 116.5 % was appreciable. The percentage recoveries improved significantly for samples 2A and 3A, where the samples were spiked with a higher concentration of arsenic, i.e. 28 and 91 μg/L, respectively. The mean percentage recovery of the spiked arsenic for seven tested laboratories was 110 % both for the sample 2A and 3A. The results of our laboratory, 100 % and 113 %, respectively, agreed well with the reported values.

Table 4. Intercomparison test

Sample	Spiked arsenic concentration[a)] [μg/L]	Mean concentrations[b)] included in the test Lab. mean [μg/L] Recovery, % in parentheses	Our results (SIPH B.Bystrica) Lab. mean [μg/L] Recovery, %in parentheses
1A	8.5	11.7 (138)	9.9 (116.5)
2A	28.0	30.9 (110.0)	28.2 (100.7)
3A	91.0	100.0 (110.0)	102.0 (113.3)

[a)] Samples were spiked by the As mixture of As(III), As(V),MMA, and DMA – according to the document [8].
[b)] The sum of As(III), As(V), MMA, and DMA was determined in seven laboratories.

Precision

Duplicate samples were obtained according to the Harmonized Guidelines for Internal Quality Control in Analytical Chemistry Laboratories [10]. Randomly selected samples were divided into two portions and each one was treated by the same extraction procedure. The relative standard deviations were calculated from the duplicate results and were compared with the coefficient of variation CV(%) given by the Horwitz equation [11]:

$$CV(\%) = 2^{(1 - 0.5 \log c)}$$

The Horwitz equation is considered a general expression of between-laboratory precision, since it is based on the evaluation of more then 150 independent collaborative studies, where at least five analytical methods – chromatography, atomic absorption spectrometry, spectrophotometry, polarography, and bioassay were applied. The within-laboratory CV should be in the range between one-half to two-thirds of the between-laboratory CV [11].

In our case, the relative standard deviations of the laboratory duplicate determinations (within-laboratory CV) did not exceed the relative standard deviation values calculated by the Horwitz equation. The individual observations are shown in Fig. 5.

The intra-day and inter-day precision of the applied method was calculated from the duplicate analyses of the SRM 2670 and the internal reference material *Frozen urine*, both performed on the same day and different days, respectively. The evaluation of the repeatability and reproducibility were carried out using the ANOVA 1 test according to the Slovak Technical Norm [12]. The obtained values are given in Table 5.

Table 5. Intra- and inter-day precision of the arsenic GF AAS determination in urine.

Sample concentration [μg/L]	Intra-day precision (repeatability)		Inter-day precision (reproducibility)	
	Standard deviation *Sr* [μg/L]	Relative standard deviation *Sr* [%]	Standard deviation *Sr* [μg/L]	Relative standard deviation *Sr* [%]
Frozen urine + added 25 μg As/L	0.8	3.2	2.7	10.8
SRM 2670 48 ± 10 μg As/L	3.0	6.3	6.5	13.5

Limit of Detection

The limit of detection (LOD) and the limit of quantification (LOQ) were first calculated according to the former traditional IUPAC way [13] by measuring the blank signals and determining the concentration from the calibration curve. The LOD 3.1 μg/L was taken as the concentration, corresponding to the mean blank value plus the 3-fold multiply of the blank signal standard deviation, s_b. Analogically, the found LOQ 10.5 μg/L corresponds to the 10-fold multiply of the s_b.

The LOD and LOQ values were calculated also by the upper limit approach newly recommended by IUPAC [14]. This way is based on the one-sided upper confidence limit of the blank signal, computed by using the critical value of the t-distribution, $t(\nu,1\text{-}\alpha)$ and the residual standard deviation, s_{yx}, found in regression for the straight-line calibration line. The uncertainty of the calibration plot position (i.e. its intercept and slope) as well as that of the mean blank signal has been here considered.

The LOD and LOQ values were evaluated from the three separately measured calibration curves. Each one was designed using nine points equidistantly spaced on the concentration axis and the same number of replicate measurements (triplicates or duplicates) of each point. The average values from all measurements were used for the computation of the LOD and LOQ. The calculated LOD 3.7 μg/L and LOQ 11.2 μg/L were close to the traditionally determined LOD and LOQ, however, they are assumed to be more reliable.

Conclusion

The analyses of arsenic were performed in a routine way and the quality assurance was followed during all steps of the multi-level procedure: collection, sample preparation, handling, and final AAS analysis. The internal reference material *Frozen urine* was prepared, treated and analysed to simulate pretreatment the urine sample. The analytical spike and recovery study automatically offered by the QC software of the AAS instrument were used to compensate the matrix effect on the analyte response. The following method performance characteristics were evaluated: trueness, precision, recovery, limit of detection, and limit of quantification.

In this work, 465 analyses of arsenic in urine were performed in order to achieve information on the sum of As(III), As(V), MMA and DMA, expressing nearly total arsenic. Among all results, the content of arsenic was found below the LOD limit in 12 samples, 88 samples were determined within the range between the LOD and LOQ limits. The maximum, median and mean values of the As content were found 256.9 μg/L, 19.1 μg/L, and 27.1 μg/L,

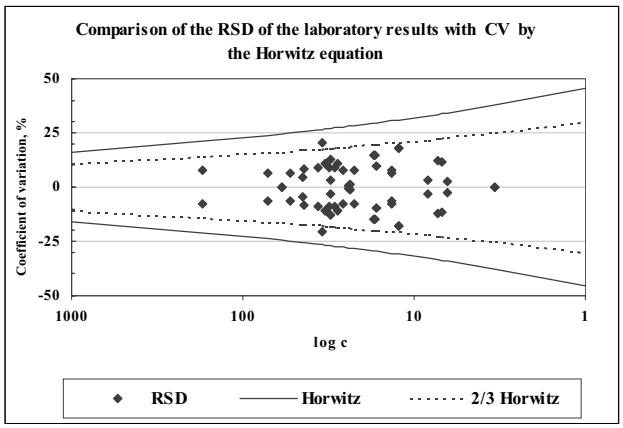

Fig. 5. Comparison of the laboratory RSD results with the CV (coefficient of variation) computed by the Horwitz equation. The CV equals 100 multiply of the relative standard deviation. The concentration of arsenic is in μg/L.

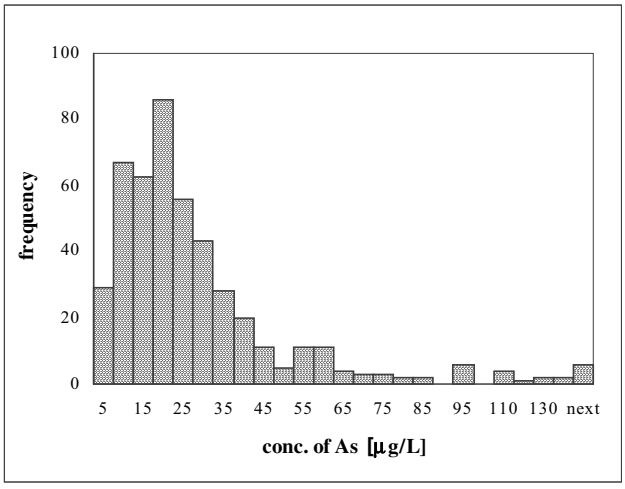

Fig. 6. Histogram of the results of arsenic determination in urine samples.

respectively. The frequency of the results is given in the histogram shown in Fig. 6.

Acknowledgment

This study was partly supported by the Slovak grant VEGA 1/9129/02.

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