GFAAS Determination of Arsenic Levels in Biological Samples of Workers Occupationally Exposed to Metals: An Application in Analytical Toxicology

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ABSTRACT

Arsenic exposure in humans has been associated with adverse health effects such as neurological and cardiovascular effects, diabetes mellitus, skin lesions, skin, lung, kidney and liver cancers. Occupational exposure to arsenic usually occurs with inhalation of arsenic-containing particles in the mining industry. A simple and sensitive method was developed and validated for the determination of arsenic levels in biological samples by graphite furnace atomic absorption spectrometry (GFAAS), equipped with a Zeeman background correction system. Blood, urine, and hair samples are known to be the best biomarkers to assess arsenic exposure in humans. Samples were collected from 95 metal workers who were admitted at the Ankara Occupational Diseases Hospital in Turkey. Prior to analysis, the samples were pre-treated with an acid digestion procedure. The method showed linearity in the range of 0–100 µg/L, with a detection and quantification limit of 0.37 µg/L and 1.1 µg/L, respectively. The calibration curve was characterized by a high correlation coefficient (r=0.9991). Validation of the method was performed in terms of precision and accuracy with the use of reference materials. The method was applied to the analysis of certified reference material samples with satisfactory results (96.77–97.50%). The arsenic levels of the biological samples of the metal workers ranged between 3.83–52.44 µg/L in blood; 1.26–27.54 µg/L in urine; and 0,06–7.90 mg/kg As in hair. The mean arsenic levels in the blood, urine, and hair samples of the silver metal workers were found at 21.25±12.47 µg/L, 6.43±4.99 µg/L, and 1.81±1.79 mg/kg As, respectively.

INTRODUCTION

Arsenic (As) is an extremely poisonous element and has been classified as a human carcinogenic substance, group 1, by the International Agency for Research on Cancer (1). Arsenic exposure in humans is generally associated with the consumption of drinking water contaminated from natural, geological sources of inorganic arsenic. Chronic exposure to arsenic in humans has been related to the development of adverse health effects such as cardiovascular and peripheral vascular diseases, neurological disorders, diabetes mellitus, and various forms of cancer (2–4). Arsenic is found in inorganic and organic forms with different valence or oxidation states in the environment. Unlike inorganic arsenic, organic arsenic compounds in the pentavalent oxidation state are much less toxic because consumption of these organic arsenicals are not immediately accepted into the cells, and meet with limited metabolism (5, 6). Some important arsenic species are listed in Figure 1.

Inorganic arsenic occurs naturally in soil and many kinds of rock, especially in minerals and ores that contain copper, lead, cobalt, silver, and gold. Arsenic trioxide is volatilized during smelting and accumulates in flue dust, which may contain up to 30% arsenic trioxide (8). Thus, inhalation of industrial soil and dust causes arsenic exposure in metal workers. Occupational exposure to chemicals occurs most commonly via inhala-

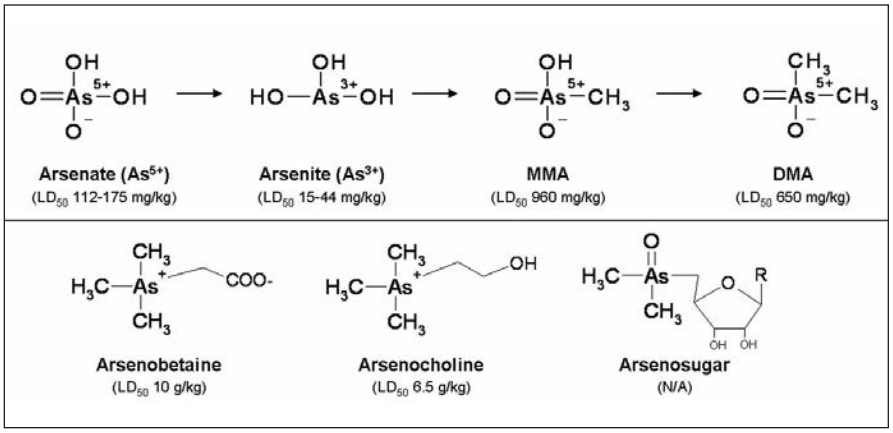

Fig 1. (Yüksel et al.) Some important arsenic species. Common inorganic arsenicals and their metabolites are listed in top row, while organic arsenicals found in seafood are listed in bottom row.

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tion. It can be possible to gather a more accurate prediction of total dose by using biomarkers (9) such as blood, urine, and hair. Since the main route of arsenic excretion takes place in the kidneys, the level of arsenic in urine can be used to predict exposure (10). Blood-arsenic is peculiarly employed only as a sign of very current or comparatively high-level exposure because inorganic arsenic is quickly eliminated from the blood (11). Hair has a unique potential to reveal retrospective information about the exposure of subjects (12). Additionally, once incorporated into keratin, arsenic has limited mobility, so it is known to be deposited in nails and especially in hair (13). Arsenic levels in blood, urine, nail, and hair of unexposed human adults are usually below 1 µg/L, 100 µg/L, 1 mg/kg, and 1 mg/kg, respectively (8).

The determination of arsenic levels in biological samples can be performed by methods such as neutron activation, X-ray fluorescence, atomic absorption and fluorescence spectrometry, and inductively coupled plasma atomic emission and mass spectrometry (ICP-AES and ICP-MS) (14). In recent years, graphite furnace atomic absorption spectrometry (GFAAS), hydride generation atomic absorption spectrometry (HGAAS), and ICP-MS have become the leading techniques. ICP-MS (15-17) is widely used because of its multi-element capabilities, but it is also one of the most expensive instruments (18). Graphite furnace atomic absorption spectrometry is more economical and is a good choice due to its selectivity and sensitivity in the detection of a wide range of metals and non-metals, including arsenic (19).

The Zeeman effect (20) is based on the shift of energy of atoms and molecules in a magnetic field. If a magnetic field is generated at the atomizer (graphite furnace), the absorption lines of the analyte atoms are split into three components. Two of these components (σ–components) are shifted to slightly lower and higher wavelenghts, repectively, whereas the third component (π–component) remains largely unchanged. The π–component can be removed from the spectrum using a polarizer (Figure 2).

The main goal of this study was to develop and validate a sensitive method with graphite furnace atomic absorption spectrometry, equipped with a Zeeman-effect background correction system, to determine arsenic concentrations in biological samples for routine toxicological analytical application. The method developed for the determination of arsenic was applied to blood, urine, and hair samples obtained voluntarily from metal workers. The samples were taken from them at the Ankara Occupational Diseases Hospital, Turkey (21).

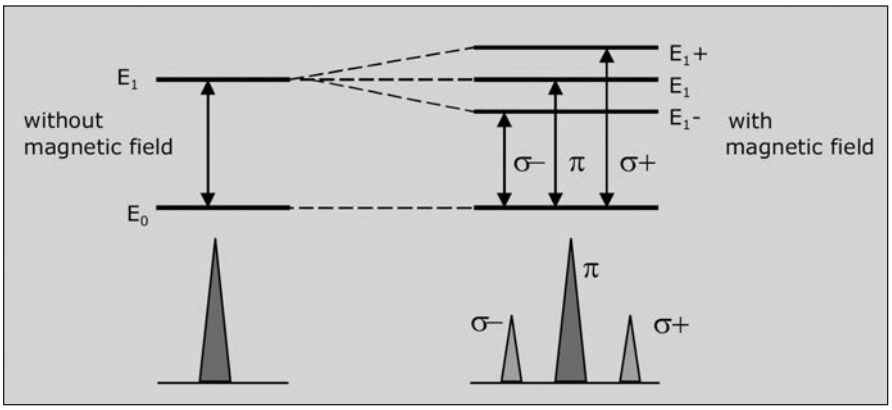

Fig. 2. (Yüksel et al.) Schematic diagram of Zeeman effect.

EXPERIMENTAL

Instrumentation

The measurements for arsenic determination were performed using a Varian AA240Z atomic absorption spectrometer (Varian, Victoria, Australia), equipped with a Zeeman background correction system. A boosted-discharge hollow cathode lamp (Agilent, Australia) was used as the excitation source for arsenic. The digestion procedure for the blood and hair samples was carried out using a Mars Xpress microwave system (CEM, Matthews, NC, USA) with PTFE microwave digestion vessels. The operating parameters for the GFAAS system are listed in Table I.

Standard Solutions and Reagents

A 1000-µg/mL arsenic stock solution was obtained from SCP Science (Courtaboeuf, France). Triton® X-100, polyethylene glycol mono (p-1,1,3,3-tetramethylbutylphenyl) ether, was obtained from Scharlau (Barcelona, Spain). Nitric acid (HNO_3, 65%) was purchased from Merck (Darmstadt, Germany). All chemicals used were of analytical reagent grade unless otherwise specified. Ultrapure water (Human UP 900 Scholar-UV, Korea), with a resistivity of 18 MΩ·cm, was used to prepare the solutions for the experimental process. Argon gas with a purity of 99.999% was purchased from a local supplier (Vasak Gaz, Ankara, Turkey). The reference materials used were BCR-CRM 397 Human Hair Powder (Community Bureau of Reference BCR, Institute for Reference Materials and Measurement, Belgium) and Seronorm™ Trace Elements Whole Blood L-2 (Sero AS, Billingstad, Norway).

Sample Collection

Blood, urine, and hair samples were collected from 95 metal workers (volunteers) at the Ankara Occupational Diseases Hospital, Turkey. The patients ranged in age from 18–61 years. This study was ethically approved by the Research Ethics Committee of the Medical Faculty, Ankara University (Decision Number:11-343-12/25.06.2012). Each volunteer was given a written informed consent form in accordance with the principles as established in The Declaration of Helsinki (World Medical Association, Declaration of Helsinki, 1964). The blood, urine, and hair samples were stored separately at 4 °C in vacutainer blood collection tubes, polypropylene tubes, and polyethylene lock bags, respectively, until the day of analysis.

TABLE I
Operating Parameters for GFAAS System

Element	As
Matrix	Blood, Urine and Hair
Instrument	Zeeman
Concentration Unit	µg/L, µg/kg
Instrument Mode	Absorbance
Sampling	Auto-Mix
Calibration Mode	Concentration
Measurement Mode	Integrated
Replicates Standard	3
Replicate Sample	3
Expansion Factor	1.0
Wavelength	193.7 nm
Slit Width	0.5 nm
Gain	59%
Current	10.0 mA
Background correction	ON
Standard 1	3.0 µg/L
Standard 2	6.0 µg/L
Standard 3	9.0 µg/L
Standard 4	12.0 µg/L
Standard 5	15.0 µg/L
Reslope Standard	Standard 2
Recalibration Rate	50
Calibration Algorithm	Linear

Procedure

In order to prepare calibration standards at the concentrations of 3, 6, 9, 12, and 15 µg/L, a 1000-µg/mL arsenic stock solution was diluted in 5% (v:v) HNO_3. All glassware was kept in 10% (v:v) nitric acid for at least one night prior to each experimental work.

Prior to analysis, the biological samples (except for urine) were pre-treated using the acid digestion procedure. One milliliter of each blood sample was taken into the Teflon® tubes. The microwave system (CEM Mars Xpress) was utilized for digestion of the samples with 5 mL of 65% HNO_3 solution. Similarly, 100-mg amounts of hair samples were taken and washed with Triton®-X, rinsed, and left standing to air-dry. This microwave digestion procedure was also applied to the hair samples. For the urine samples, 1-mL amounts were mixed with 5 mL of 65% HNO_3 (21, 26). All biological samples were diluted with ultra-pure water to 10 mL. The microwave temperature program is listed in Table II.

Optimization and Sample Treatment

Important parameters were adjusted to obtain the best performance from this spectrometric analysis. Selection of the digestion technique, choice of the appropriate wavelength for the biological matrix, calibration concentration range in accordance with element concentration in real samples, assessing the best furnace program and establishing the linearity, were the major criteria for developing and optimizing this atomic absorption spectrometry method. Preliminary studies were performed under these subheadings to establish the best methodology for accurate measurements (22). The graphite furnace temperature program for arsenic determination in biological samples is listed in Table III.

Detection was performed at the 193.7-nm arsenic line. This wavelength was selected due to a higher signal-to-noise ratio in the spectrum of the sample matrices than at the 197.2-nm and 189.0-nm lines. The

TABLE II
Temperature Program For Microwave Digestion

Max. Power (W)	Power (%)	Ramp (min)	Pressure	Temperature (°C)	Hold (min.)
1600	100	10:00	Maximum	210	10:00

TABLE III
Graphite Furnace Temperature Program for Arsenic Determination in Biological Samples

Step	Temperature (°C)	Time (s)	Flow (L/min)	Signal Collection		Reading	
1	85	5	0.3	×	No	×	No
2	95	40	0.3	×	No	×	No
3	120	10	0.3	×	No	×	No
4	800	5	0.3	×	No	×	No
5	800	1	0.3	×	No	×	No
6	800	2	0.0	√	Yes	×	No
7	2450	0.9	0.0	√	Yes	√	Yes
8	2450	2	0.0	√	Yes	√	Yes
9	2450	2	0.3	√	Yes	×	No

proposed method showed linearity in the range of 1–100 µg/L and good repeatability not exceeding 3% for As. On the other hand, the average arsenic levels in the real hair sample solutions without using dilution factors was measured roughly as 2 µg/kg. Hence, for calibration purposes, five calibration standards (namely, 3, 6, 9, 12, and 15 µg/L) were prepared. The calibration graph showed good linearity in the concentration range examined (Figure 3). The correlation coefficient and equation of the calibration curve were, respectively, found to be r=0.9991 and Abs=0.0071C+0.0018, where Abs stands for integrated absorbance and C for the arsenic concentration in µg/L.

Method Validation

In order to validate the method in terms of accuracy and precision, BCR-CRM 397 Human Hair Powder and Seronorm™ Trace Elements Whole Blood L-2 were analyzed for arsenic. Each certified reference material was analyzed 10 times with triplicate measurements. The results were compared with the certified values for accuracy, precision, and reproducibility of the method. The certified arsenic content of BCR-CRM 397 (Hair) was 0.31±0.02 mg/kg, while the measured value was 0.30±0.01 mg/kg, with the successful recovery and relative standard deviation (RSD) as 96.77% and 3.97%, respectively. Similarly, the certified arsenic content of Seronorm™ Trace Elements Whole Blood L-2 was 13.20±1.3 µg/L, while the measured value was 12.87±0.77 µg/L, with a satisfactory recovery and RSD as 97.50% and 5.98%, respectively. The analytical results of the certified reference materials are summarized in Table IV.

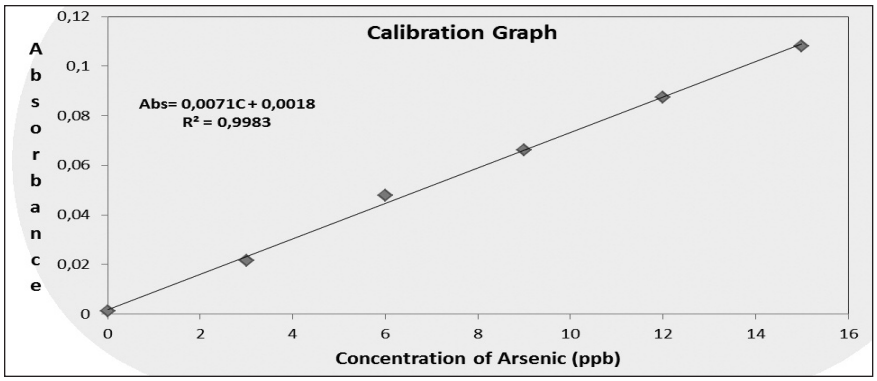

Fig. 3. (Yüksel et al.) Calibration graph of arsenic, performed by graphite furnace atomic absorption spectrometry (GFAAS), equipped with Zeeman-effect background correction.

TABLE IV
Analysis of Certified Reference Materials (CRMs)

CRMs	Number of Analyses (n)	Certified Value	Measured Value	Recovery	RSD
BCR-CRM 397 (Hair)	10	0.31±0.02 mg/kg	0.30±0.01 mg/kg	96.77 (%)	3.97 (%)
Seronorm™ Trace Elements Whole Blood L-2 (Blood)	10	13.20±1.30 µg/L	12.87±0.77 µg/L	97.50 (%)	5.98 (%)

Limit of Detection and Quantification

The limit of detection (LOD) and lowest limit of quantification (LOQ) were determined based on the standard deviation of the response and the slope of the calibration curve, according to ICH guidelines (23, 24) (LOD=3.3σ/S, LOQ=10σ/S, where σ is the standard deviation of the response and S is the slope of the calibration curve). The LOD and LOQ values were calculated for arsenic in the blood samples and found as 0.37 µg/L and 1.1 µg/L, respectively.

RESULTS AND DISCUSSION

Epidemiological studies have provided compelling evidence that inorganic arsenic is carcinogenic to humans. Chronic ingestion of arsenic increases the risk of developing skin, lung, urinary bladder, and liver cancer (25).

The assessed arsenic levels of the biological samples from the metal workers ranged between 3.83 and 52.44 µg/L in blood; 1.26 and 27.54 µg/L in urine; 0.06 and 7.90 mg/kg As in hair. The mean arsenic levels in the blood, urine, and hair samples of the silver metal workers were found as 21.25±12.47 µg/L, 6.43±4.99 µg/L, 1.81±1.79 mg/kg As, respectively, while the acceptable arsenic levels in human biological samples (blood, urine, hair, and nail) were below: 1 µg/L, 100 µg/L, 1 mg/kg, and 1 mg/kg, respectively, and are listed in Table V (8).

According to the results obtained from this toxicological arsenic analysis, 43 of 95 individu-

TABLE V
Normal Arsenic Levels in Human Biological Samples (8)

Blood	Urine	Hair	Nail
<1 µg/L	<100 µg/L	≤1 mg/kg	≤1 mg/kg

als have hair-arsenic concentrations above the safe limits. As for the blood-arsenic and urine-arsenic levels, all individuals have above normal levels of blood-arsenic, but are at safe limits for urine-arsenic levels (21). The evaluated arsenic levels in the biological samples of the metal workers are listed in Table VI.

TABLE VI
Descriptive Statistics of Arsenic Levels in Biological Samples of Metal Workers

N=95	Age (Years)	Body Mass Index	Exposure Time (Year)	Blood Arsenic Level (ppb)	Urine Arsenic Level (ppb)	Hair Arsenic Level (ppm)
Mean	33.22	25.45	3.49	21.25	6.43	1.81
Standard Deviation	8.08	4.22	2.09	12.47	4.99	1.79
Minimum	18	17.34	1.0	3.83	1.26	0.06
Maximum	61	37.50	10	52.44	27.54	7.90

CONCLUSION

A graphite furnace atomic absorption spectrometry (GFAAS) method, using Zeeman background correction, was developed for the determination of arsenic in human blood, urine, and hair. Using the Zeeman-effect for background correction, a strong magnetic field is turned on and off in rapid sequence. Total absorbance (element-specific and non-specific background absorption) is measured with the magnetic field in OFF-position and the background absorption with the magnetic field in ON-position The difference of the two values gives the corrected element-specific absorption. The advantages of the Zeeman-effect technique are as follows:

- Measurement of total and back ground absorption on the same wavelength
- Correction of rapid and structured background
- No special lamp required
- Correction over the entire wavelength range
- Better signal-to-noise ratio

The method developed for arsenic determination in human biological samples is relatively simple, rapid, sensitive, and offers very good precision and accuracy. The method is low-cost since it does not require large amounts of reagents. In addition, the method proposed is quite competitive in relation to other analytical approaches used for toxicological purposes.

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REFERENCES

1. IARC (International Agency for Research on Cancer): Some drinking water disinfectants and contaminants, including arsenic, IARC Monographs on the evaluation of carcinogenic risks to humans, 84 (2004)
2. J.A. Centeno, F.G. Mullick, L. Martinez, N.P. Page, H. Gibb, D. Longfellow, C. Thompson, and E.R. Ladich, Environ. Health Perspect. 110 (5), 883 (2002).
3. World Health Organization (WHO), Diagnosis and treatment of chronic arsenic poisoning. In United Nations synthesis report in drinking water, Geneva, Switzerland, 4, 1 (2000).
4. K. Bailey, Y. Xia, W.O. Ward, G. Knapp, J. MO, J.L. Mumford, R.D. Owen, and S.F. Thai, Toxicol. Pathol. 37(7), 849 (2009).
5. S.M. Cohen, L.L. Arnold,.M. Eldan, A.S. Lewis and B. D. Beck, Crit. Rev. Toxicol. 36, 99 (2006)
6. M.F. Hughes, B.D. Beck, Y. Chen, A.S. Lewis and D.J. Thomas, Toxicol. Sci. 123(2), 305–332 (2011).
7. J. Liu, Y. Lu, Q. Wu, R.A. Goyer and M.P. Waalkes, J. Pharmacol. Exp. Ther. 326(2), 363 (2008).
8. Agency for Toxic Substances and Disease Registry (ATSDR), Toxicological profile for arsenic, Atlanta, GA: US Public Health Service (2007).
9. R.G. Abrefah, D.K. Adotey, E. Mensimah, E. Ampomah-Amoako, R.B.M. Sogbadji and N.S. Opata, Environm. Research, Engineering and Management 2(56), 43 (2011).
10. World Health Organisation (WHO), Environmental Health Criteria (18), Arsenic, Geneva, Switzerland (1981).
11. National Research Council (NRC), Arsenic in drinking water, Washington, DC, USA, National Academy Press (1999).
12. H. Sela, Z. Karpas, M. Zoriy, C. Pickhardt, and J.S. Becker, Int. J. Mass Spectrom, (261), 199 (2007).
13. R.D. Koons and C.A. Peters., J. Anal. Toxicol. 18, 36 (1994).
14. M.F. Hughes, Environ. Health Perspect. 114(11), 1790 (2006).
15. K. Gellein., S. Lierhagen., P.S. Brevik, M. Teigen, P. Kaur, T. Singh, T.P. Falten, and T. Syversen, Biol. Trace. Elem. Res. 123, 250 (2008).

16. D.A. Bass, D. Hickok, D. Quig, and K. Urek, Altern. Med. Rev. 6(5), 472 (2001).

17. A. Unkiewicz-Winiarczyk, A. Bagniuk, K. Gromysz-Kałkowska, and E. Szubartowska, Biol. Trace Elem. Res. 128, 152 (2009).

18. R. Wietecha, P. Kos´cielniak, T. Lech, and T. Kielar, Microchim. Acta 149, 137 (2005).

19. W. Goessler and D. Kuehnelt, Environm. Chem. of Arsenic, Marcel Dekker, New York, USA, 27 (2002).

20. Fundemantals, Instrumentation and Techniques of Atomic Absorption Spectrometry, Analytik Jena AG, Jena, Germany, [http://www.analytik-jena.de], (Access Date: December 20, 2014).

21. B. Yüksel, Z. Kayaalti, V.A., Türksoy, E. Tutkun, and T. Söylemezoglu, Toxicol. Lett., Supplement, 221, 251 (2013).

22. B. Yüksel, G. Mergen, and T. Söylemezoglu, At. Spectrosc. 31(1), 1 (2010).

23. International Conference on Harmonization (ICH) of Technical Requirements for the Registration of Pharmaceuticals for Human Use, Validation of Analytical Procedures: Methodology (ICB-Q2B) (1996).

24. G. Mergen, T. Söylemezoglu, I. Ilhan, and Y.B. Dogan, LC GC Eur. 22(4), 180 (2009).

25. H. Wanibuchi, E.I. Salim, A. Kinoshita, J. Shen, M. Wei, K. Morimura, K. Yoshida, K. Kuroda, G. Endo, and S. Fukushima, Toxicol. Appl. Pharmacol. 198, 366 (2004).

26. B.Yüksel, Ph.D Thesis, Ankara University, Ankara, Turkey (2013).