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Arsenic and arsenic compounds – Determination of arsenic species (As(III), As(V), monomethylarsonic acid, dimethylarsinic acid and arsenobetaine) in urine by HPLC-ICP-MS

Biomonitoring Method – Translation of the German version from 2018

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Keywords: arsenic; arsenic species; arsenic acid; arsenous acid; monomethylarsonic acid; dimethylarsinic acid; arsenobetaine; biomonitoring; urine; HPLC-ICP-MS

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Arsenic and inorganic arsenic compounds – Determination of arsenic species (As(III), As(V), monomethylarsonic acid, dimethylarsinic acid and arsenobetaine) in urine by HPLC-ICP-MS

Biomonitoring Methods

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Abstract

The working group "Analyses in Biological Materials" of the Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area verified the presented biomonitoring method.

The analytical method described hereinafter permits the simple and specific determination of the arsenic species arsenic (III) (As^{3+}), arsenic (V) (As^{5+}), monomethylarsonic acid (MMA), dimethylarsinic acid (DMA) and arsenobetaine in urine. The limits of quantitation allow the quantification of the above-mentioned arsenic species in both occupational and environmental medicine.

To this end, the arsenic species are separated by anion-exchange high-performance liquid chromatography (anion-exchange HPLC) and detected by inductively coupled plasma mass spectrometry (ICP-MS). Sample preparation is done by diluting the urine with water. Calibration is performed using either single or mixed standards of the different arsenic species or using the standard addition method.

The method was extensively validated and the reliability data were confirmed by an independent laboratory, which has established and cross-checked the whole procedure.

Keywords

arsenic; arsenic species; arsenic acid; arsenous acid; monomethylarsonic acid; dimethylarsinic acid; arsenobetaine; urine; biomonitoring; Analyses in Biological Materials; HPLC; ICP; mass spectrometry

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Arsenic and arsenic compounds – Determination of arsenic species (As(III), As(V), monomethylarsonic acid, dimethylarsinic acid and arsenobetaine) in urine by HPLC-ICP-MS

Matrix:	Urine
Hazardous substances:	Arsenic and arsenic compounds
Analytical principle:	High-performance liquid chromatography with inductively coupled plasma mass spectrometry (HPLC-ICP-MS)
Completed in:	May 2000

Overview of the parameters that can be determined with this method and the corresponding hazardous substances:

Hazardous substance	CAS	Parameter	CAS
Arsenic and arsenic compounds	7440-38-2 (arsenic)	Arsenic (III) (arsenous acid)	36465-76-6
		Arsenic (V) (arsenic acid)	7778-39-4
		Monomethylarsonic acid	124-58-3
		Dimethylarsinic acid	75-60-5
		Arsenobetaine	64436-13-1

Summary

The analytical method described hereinafter permits the simple and specific determination of the arsenic species arsenic(III) (As^{3+}), arsenic(V) (As^{5+}), monomethylarsonic acid (MMA), dimethylarsinic acid (DMA) and arsenobetaine in urine. The limits of quantitation allow the quantification of the above-mentioned arsenic species in both occupational and environmental medicine.

To this end, the arsenic species are separated by anion-exchange high-performance liquid chromatography (anion-exchange HPLC) and detected by inductively coupled plasma mass spectrometry (ICP-MS). The samples are prepared by diluting the urine with water. Calibration is performed using either single or mixed standards of the different arsenic species or using the standard addition method.

Reliability data of the method
As(III)

Within-day precision:	Standard deviation (rel.)	s_w = 6.3%
	Prognostic range	u = 15.4%
	at a spiked concentration of 5.0 µg As(III) per litre urine and where n = 7 determinations	
Day-to-day precision:	Standard deviation (rel.)	s_w = 21.8% or 27.0%
	Prognostic range	u = 45.3% or 56.2%
	at a spiked concentration of 0.2 µg or 0.4 µg As(III) per litre urine and where n = 22 determinations	
Accuracy:	Recovery rate (rel.)	r = 119%
	at a nominal concentration of 5.0 µg As(III) per litre urine and where n = 7 determinations	
Detection limit:	0.03 µg As per litre urine	
Quantitation limit:	0.09 µg As per litre urine	

As(V)

Within-day precision:	Standard deviation (rel.)	s_w = 2.1%
	Prognostic range	u = 5.1%
	at a spiked concentration of 5.0 µg As(V) per litre urine and where n = 7 determinations	
Day-to-day precision:	Standard deviation (rel.)	s_w = 17.8% or 5.1%
	Prognostic range	u = 37.0% or 10.6%
	at a spiked concentration of 0.3 µg or 5.6 µg As(V) per litre urine and where n = 22 determinations	
Accuracy:	Recovery rate (rel.)	r = 102%
	at a nominal concentration of 5.0 µg As(V) per litre urine and where n = 7 determinations	
Detection limit:	0.05 µg As per litre urine	
Quantitation limit:	0.15 µg As per litre urine	

MMA

Within-day precision:	Standard deviation (rel.)	s_w = 2.0%
	Prognostic range	u = 4.9%
	at a spiked concentration of 5.0 µg As as MMA per litre urine and where n = 7 determinations	
Day-to-day precision:	Standard deviation (rel.)	s_w = 8.9% or 4.6%
	Prognostic range	u = 18.5% or 9.6%
	at a spiked concentration of 0.7 µg or 2.8 µg As as MMA per litre urine and where n = 22 determinations	

Accuracy:	Recovery rate (rel.)	r = 112%
	at a nominal concentration of 5.0 µg As as MMA per litre urine and where n = 7 determinations	
Detection limit:	0.04 µg As as MMA per litre urine	
Quantitation limit:	0.12 µg As as MMA per litre urine	

DMA

Within-day precision:	Standard deviation (rel.)	s_w = 3.5%
	Prognostic range	u = 8.6%
	at a spiked concentration of 5.0 µg As as DMA per litre urine and where n = 7 determinations	
Day-to-day precision:	Standard deviation (rel.)	s_w = 5.1% or 2.8%
	Prognostic range	u = 10.6% or 5.8%
	at a spiked concentration of 2.2 µg or 19.6 µg As as DMA per litre urine and where n = 22 determinations	
Accuracy:	Recovery rate (rel.)	r = 114%
	at a nominal concentration of 5.0 µg As as DMA per litre urine and where n = 7 determinations	
Detection limit:	0.02 µg As as DMA per litre urine	
Quantitation limit:	0.06 µg As as DMA per litre urine	

Arsenobetaine

Within-day precision:	Standard deviation (rel.)	s_w = 1.8%
	Prognostic range	u = 4.4%
	at a spiked concentration of 5.0 µg As as arsenobetaine per litre urine and where n = 7 determinations	
Day-to-day precision:	Standard deviation (rel.)	s_w = 4.9% or 4.7%
	Prognostic range	u = 10.2% or 9.8%
	at a spiked concentration of 2.4 µg or 19.8 µg As as arsenobetaine per litre urine and where n = 22 determinations	
Accuracy:	Recovery rate (rel.)	r = 106%
	at a nominal concentration of 5.0 µg As as arsenobetaine per litre urine and where n = 7 determinations	
Detection limit:	0.03 µg As as arsenobetaine per litre urine	
Quantitation limit:	0.09 µg As as arsenobetaine per litre urine	

General information on arsenic and its compounds. Arsenic (As, relative atomic mass 74.92, atomic number 33) is an element that is ubiquitous in the environment in low concentrations and that has the properties of both metals and nonmetals. Arsenic occurs naturally in the earth's crust mainly in the form of arsenides (e.g. arsenopyrite FeAsS and cobaltite CoAsS), sulphides (e.g. realgar As_4S_4 and orpiment As_2S_3) as well as oxides (arsenic trioxide As_2O_3). It can occur in the oxidation states -III, 0, +III and +V [IARC 2012].

Today, arsenic is primarily used in semiconductor manufacturing, as an alloying component, as a refining and decolourising agent in the glass industry as well as in arsenical pigments. In these areas of application, occupational exposure to arsenic is possible. Another source of exposure is the extraction of arsenic and its by-metals from arsenic-containing ores, as arsenic often occurs in copper, iron or nickel ores. By smelting these arsenic-containing ores and burning fossil fuels, arsenic and its compounds are also released into the environment [IARC 2012].

Arsenic concentrations in soil are strongly influenced by geological or local conditions. In some countries, such as Bangladesh, China, India, Chile or the U.S., very high arsenic concentrations have been found in the soil. Arsenic also enters groundwater and drinking water from both natural and anthropogenic sources. In Germany, the limit value for arsenic in drinking and mineral water is 10 µg/L [TrinkwV 2001], which is normally not exceeded [UBA 2003]. For geological reasons, there are much higher arsenic concentrations in drinking water of up to 1,000 µg/L in some areas of the world [UBA 2003]. High concentrations of arsenic can especially be found in seafood and sea fish, mainly in the form of organic arsenic compounds such as arsenobetaine, arsenocholine and arsenosugars [IARC 2012; UBA 2003].

Orally administered inorganic and organic arsenic is quickly and readily absorbed by humans. By contrast, inhalation and dermal absorption is significantly lower [IARC 2012; UBA 2003]. Inhalation exposure to inorganic arsenic occurs mainly at the workplace. In air, arsenic and its inorganic compounds are particle-bound and respiratory absorption therefore takes place in a two-phase process [Drexler 2003, translated]. Absorbed inorganic arsenic compounds are primarily methylated in the human liver to MMA and DMA and excreted in the urine [UBA 2003; Ochsmann 2011]. Renal main metabolites of inorganic arsenic compounds are As^{3+}, As^{5+}, MMA and DMA [UBA 2003; Ochsmann 2011]. By contrast, organic arsenic compounds are mainly renally eliminated in unchanged form. It should be noted that marine animals contain DMA, which is also excreted unchanged upon ingestion and may thus erroneously point to exposure to inorganic arsenic.

Arsenic is eliminated from the human body in three phases, with most of the substance being eliminated at a half-life of between one day and a few days [UBA 2003; Drexler 2003, translated].

Figure 1 shows the arsenic species that can be detected with this method.

Arsenic and its inorganic compounds have been classified by the Commission as Category 1 carcinogens and Category 3A germ cell mutagens [DFG 2017]. For more detailed information on the toxicological evaluation of arsenic and its compounds, please refer to the respective MAK Value Documentations [Hartwig 2015, translated; Hartwig 2014, translated]. For inorganic arsenic and its methylated metabolites in urine, a biological guidance value (BLW) of 50 µg/L arsenic has been established. For the sum of As(III), As(V), MMA and DMA, there are exposure equivalents for

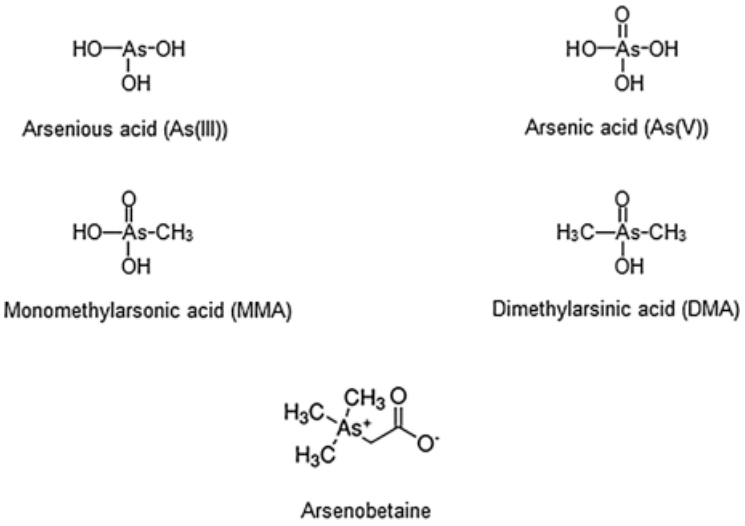

Figure 1 Arsenic species that can be detected with this method.

carcinogenic substances (EKA values) available; besides, biological reference values (BAR) are available for the individual arsenic species, namely 0.5 µg/L for As^{3+} and As^{5+}, respectively, as well as 2 µg/L for MMA and 10 µg/L for DMA [DFG 2017].

Table 1 shows selected study data on arsenic species concentrations in the urine of the general population.

Table 1 Selected study data on the concentrations of analysed arsenic species in the urine of the general population.

Study	Collective	No. of samples n	Concentration of arsenic species in urine, median (95th percentile) [µg As/L]				
			As^{3+}	As^{5+}	MMA	DMA	Arseno-betaine
Foà et al. 1984	General population	184	1.9 (10[a])		1.9 (9[a])	2.1 (10[a])	–
Kalman et al. 1990	General population	696	1.3		1.6	6.4	–
Navas-Acien et al. 2009	General population	60	0.6 (1.9[b])		1.1 (3.3[b])	4.9 (15[b])	<0.5 (4.3[b])
Rivera-Nunez et al. 2011	General population, drinking water[1]	387	0.3 (1.0)	0.3 (0.8)	1.0 (3.4)	5.6 (16)	13.6 (72)
Heitland and Köster 2008	General population	82	0.2 (0.5)	0.2 (0.5)	0.5 (1.6)	4.0 (9.1)	5.9 (23)

[1] Use of drinking water with elevated arsenic levels, [a] Max. value, [b] 90th percentile

Contents

1 General principles

The analytical method described hereinafter permits the simple and specific determination of the arsenic species arsenic(III) (As^{3+}), arsenic(V) (As^{5+}), monomethylarsonic acid (MMA), dimethylarsinic acid (DMA) and arsenobetaine in urine. The limits of quantitation allow the quantification of the above-mentioned arsenic species in both occupational and environmental medicine.

To this end, the arsenic species are separated by anion-exchange high-performance liquid chromatography (anion-exchange HPLC) and detected by inductively coupled plasma mass spectrometry (ICP-MS). The samples are prepared by diluting the urine with water. Calibration is performed using either single or mixed standards of the different arsenic species or using the standard addition method.

2 Equipment, chemicals and solutions

2.1 Equipment

- HPLC: Two-channel gradient pump (Beckman System Gold 127NM); 6-port valve (Rheodyne) with 100 µL sample loop; all parts coming into contact with the sample are made from PEEK
- Analytical column: Thermo-Dionex AS14 (250 x 4 mm) with pre-column AG14 (40 x 4 mm) (e.g. Thermo Scientific/Dionex, Idstein)
- ICP mass spectrometer: Quadrupol ICP-MS (ELAN 5000 from Perkin Elmer) coupled to the HPLC by a Meinhard nebuliser and cyclone spray chamber
- Piston-stroke pipettes, with variably adjustable volume between 1–10 µL, 10–100 µL or 100–1000 µL with suitable pipette tips (e.g. Eppendorf)
- 250 mL urine containers with screw caps (e.g. Sarstedt No. 77.577)
- Various volumetric flasks and beakers made of glass (e.g. Schott)
- 0.45 µm cellulose filter (e.g. VWR)
- 10 mL plastic vials (PE) for the autosampler (e.g. Sarstedt)
- Ultrasonic bath (e.g. VWR)

2.2 Chemicals

- As(III) standard solution in nitric acid, TraceCERT® 1000 mg/L As (e.g. Sigma-Aldrich No. 39436)
- As(V) standard solution in water, TraceCERT® 1000 mg/L As (e.g. Sigma-Aldrich No. 76686)
- Monomethylarsonic acid (MMA), 99.5% (e.g. Chem Service No. N-12922)
- Dimethylarsinic acid (DMA), 99% (e.g. Aldrich No. 20835)
- Arsenobetaine, 95% (e.g. Sigma-Aldrich No. 11093)
- Tetramethylammonium hydroxide (25% aqueous solution) (e.g. Merck No. 814748)
- Ammonium carbonate, 99.999% (e.g. Sigma-Aldrich No. 379999)
- Sodium hydroxide, pellets (e.g. Merck No. 106498)
- Nitric acid, 1 M (e.g. Merck No. 109966)
- Ultrapure, deionised water (e.g. Milli-Q)
- Argon 5.0 (e.g. Air Liquide)
- Argon/hydrogen (8% hydrogen in argon) (e.g. Air Liquide)

2.3 Solutions

- **Sodium hydroxide solution (1 M)**
  Exactly 4.0 g sodium hydroxide are weighed into a 100 mL volumetric flask and dissolved in ultrapure water. The flask is then filled with ultrapure water.

- **Mobile phase A**
  0.8 mL of the 25% tetramethylammonium hydroxide solution are placed into a 1000 mL volumetric flask. The flask is then filled to the mark with ultrapure water.

- **Mobile phase B**
  Exactly 790 mg ammonium carbonate are weighed into a 1000 mL beaker and dissolved in approximately 900 mL ultrapure water. The pH value is adjusted to pH 9 with 1 M sodium hydroxide solution and the solution is transferred to a 1000 mL volumetric flask. The flask is then filled to the mark with ultrapure water.

Each eluent (mobile phase) must be freshly prepared for analysis, filtered prior to use (0.45 µm cellulose filter) and degassed in an ultrasonic bath for five minutes.

2.4 Calibration standards

- **Stock solution MMA, DMA and arsenobetaine (100 mg/L each)**
  10 mg MMA, 10 mg DMA and 10 mg arsenobetaine are weighed into a 100 mL volumetric flask and dissolved in ultrapure water. The flask is then filled with ultrapure water.

If stored in the dark in a refrigerator at +4°C, the stock solution is stable for at least 12 months.

- **Working solution (1 mg/L)**
  100 µL each of the purchased As(III) and As(V) stock solutions as well as 1000 µL of the MMA, DMA and arsenobetaine stock solution are pipetted into a 100 mL volumetric flask. The flask is filled to the mark with ultrapure water and the solution is thoroughly mixed. This solution contains – in relation to the respective arsenic species – 1 mg/L As.

It is stored in the refrigerator at a maximum of +4°C and must be freshly prepared every week.

- **Calibration standards**
  Calibration standards are prepared in the concentration range up to 50 µg/L by diluting the working solution according to the scheme given in Table 2.

The calibration standards must be freshly prepared every day.

A matrix-adapted calibration is performed by standard addition of the analytes from the working solution to a human urine sample with a low arsenic background level. Six calibration standards are prepared by gradually adding a defined volume of the working solution and ultrapure water to this urine so that the urine is diluted at a ratio of 1:5. This standard addition calibration is then converted into an external calibration.

Table 2 Pipetting scheme for the preparation of calibration standards to determine arsenic species in urine.

Calibration standard	Volume of the working solution	Volume of ultrapure water	Final volume	Concentration of the calibration standard
	[mL]	[mL]	[mL]	[µg/L]
0	0	100	100	0
1	0.1	99.9	100	1.0
2	0.2	99.8	100	2.0
3	0.5	99.5	100	5.0
4	1.0	99.0	100	10
5	2.0	98.0	100	20
6	5.0	95.0	100	50

3 Specimen collection and sample preparation

3.1 Specimen collection

As with all trace element analyses, the reagents and materials used must be of the highest purity. Any contamination must also be avoided during sampling. Thus, the polyethylene containers used for sampling must be cleaned with 1 M nitric acid prior to use. To this end, 1 M nitric acid is filled into the containers and allowed to stand for at least 2 h. The containers are then thoroughly rinsed with ultrapure water and left to air dry. For determination in the range of the detection limit, the cleaning effect can be further enhanced by heating the nitric acid.

For the determination of the arsenic species, the use of 24-h urine is recommended. If the analytical determination cannot be carried out immediately, the urine can be stored in the refrigerator at +4°C for about one week. For longer-term storage (weeks or months), storage at –20°C is recommended.

3.2 Sample preparation

The urine samples are brought to room temperature and mixed thoroughly. Afterwards, an aliquot of 1 mL of the urine sample is diluted with 4 mL ultrapure water and filtered (0.45 µm cellulose filter).

4 Operational parameters

Analysis is performed using HPLC with an anion exchange column coupled with ICP-MS detection.

4.1 High performance liquid chromatography

Analytical column:	Material: Dionex IonPac AS14
Length:	250 mm
Inner diameter:	4.0 mm
Particle size:	9 µm
Separation principle:	Anion exchange
Pre-column:	Material: Dionex IonPac AG14
Mobile phase:	**Eluent A:** Tetramethylammonium hydroxide solution **Eluent B:** Ammonium carbonate solution, pH 9
Flow rate:	1.5 mL/min
Injection volume:	100 µL

The gradient program is given in Table 3.

Table 3 Gradient program for the determination of arsenic species in urine.

Time [min]	Eluent A [%]	Eluent B [%]
0	100	0
0.5	100	0
0.6	0	100
8.0	0	100
8.1	100	0
15.0	100	0

The HPLC is directly connected to the Meinhard nebulizer with a cyclone spray chamber of ICP-MS by short Teflon tubing.

4.2 Inductively coupled plasma mass spectrometry

The settings described below are intended as a rough guide only. These parameters must be optimised individually for each system. Additional setting and parameter optimisation may be required when using spectrometers from other manufacturers.

Plasma power:	1200 W
Plasma gas:	15 L/min argon
Auxiliary gas:	0.6 L/min argon
Nebulizer gas:	0.75 L/min argon + 0.175 mL/min hydrogen
Nebulizer:	Meinhard
Nebulizer chamber:	Cyclone type
Analysed mass traces:	75, 77

5 Analytical determination

100 µL of each sample prepared as described in Section 3 are injected into the HPLC-ICP-MS system. It is recommended to determine each sample in duplicate. Identification of the arsenic species is based on the retention times compiled in Table 4. The retention times given are intended to be a rough guide only. Users of the method must ensure proper separation performance of the analytical column used influencing the resulting retention behaviour of the analytes. A reagent blank value (deioinised water instead of the urine sample) is included in each analytical run.

Figure 2 (in the Appendix) shows an example of a chromatogram of a urine sample spiked with standard solutions.

Table 4 Retention times of the arsenic species analysed.

Species	Retention time [min]
Arsenobetaine	1.4
DMA	3.2
As(III)	4.0
MMA	6.1
As(V)	7.8

6 Calibration

The calibration solutions prepared as described in Section 2 are processed in the same way as the samples and analysed using HPLC-ICP-MS (cf. Section 4). The calibration graph is obtained by plotting the concentration of the calibration solution against the peak area of the ^{75}As mass peak. Under the described conditions, the calibration graphs are linear for all analytes in a range between the detection limit and 100 µg As/L. Calibration should be performed anew for each analytical run. Figure 3 (in the Appendix) shows examples of calibration graphs of the analytes.

7 Calculation of the analytical results

To calculate the analyte concentration in a sample, the peak area of the ^{75}As mass peak of the analytes determined in the analysed urine sample is inserted into the corresponding calibration graph. The arsenic concentration is obtained in µg per litre urine. The dilution of the urine sample during preparation is accounted for by multiplication by a factor of 5. Any reagent blank values, which may be present, are accounted for by subtraction.

8 Standardisation and quality control

Quality control of the analytical results is carried out as stipulated in the guidelines of the Bundesärztekammer (German Medical Association) and in a general chapter of the MAK-Collection for Occupational Health and Safety Part IV: Biomonitoring Methods [Bader et al. 2010, translated; Bundesärztekammer 2014]. To check precision, at least one quality control sample with known and constant analyte concentrations is analysed within each analytical run. As material for quality control is not commercially available, it must be prepared in the laboratory. To this end, pooled urine from individuals not occupationally exposed to arsenic is spiked with a defined amount of the arsenic species and aliquoted. When stored at –20°C, aliquots of these solutions can be used for up to one year for quality control. The nominal value and the tolerance ranges of the quality control material are determined in a pre-analytical period [Bader et al. 2010, translated]. The measured values of the control sample analysed within each analytical run should each lie within the determined tolerance ranges.

9 Evaluation of the method

The reliability of the method was proved by comprehensive validation and by implementation and validation of the procedure in a second, independent laboratory.

9.1 Precision

To determine within-day precision, urine samples were spiked with 5 µg/L each of the individual arsenic species, processed several times in parallel and then analysed. The obtained within-day precision data are given in Table 5.

Day-to-day precision was determined during external verification of the method. Therefore, spiked urine samples were processed on different days and analysed. The results are summarised in Table 6 and 7.

Table 5 Within-day precision for the determination of arsenic species in urine (n = 7).

Analyte	Concentration [µg As/L]	Standard deviation (rel.) [%]	Prognostic range [%]
As(III)	5.0	6.3	15.4
As(V)	5.0	2.1	5.1
MMA	5.0	2.0	4.9
DMA	5.0	3.5	8.6
Arsenobetaine	5.0	1.8	4.4

Table 6 Day-to-day precision for the determination of arsenic species in urine (Q_{low}, n = 22).

Analyte	Mean value [µg As/L]	Standard deviation (rel.) [%]	Prognostic range [%]
As(III)	0.2	21.8	45.3
As(V)	0.3	17.8	37.0
MMA	0.7	8.9	18.5
DMA	2.2	5.1	10.6
Arsenobetaine	2.4	4.9	10.2

Table 7 Day-to-day precision for the determination of arsenic species in urine (Q_{high}, n = 22).

Analyte	Mean value [µg As/L]	Standard deviation (rel.) [%]	Prognostic range [%]
As(III)	0.4	27.0	56.2
As(V)	5.6	5.1	10.6
MMA	2.8	4.6	9.6
DMA	19.6	2.8	5.8
Arsenobetaine	19.8	4.7	9.8

9.2 Accuracy

To determine the relative recovery rate, urine was spiked with 5 µg As/L each of the individual arsenic species, processed several times in parallel and then analysed. The relative recovery was calculated on the basis of the determined concentrations in spiked urine by subtracting any background levels of the analytes in unspiked urine. The results are summarised in Table 8.

Besides, four individual urine samples were analysed, in which the total arsenic concentration (after UV digestion and SF-ICP-MS) was determined in addition to the arsenic species. Overall, there was a good consistency between the sum of the arsenic species and the total arsenic concentration in urine. The results are presented in Table 9.

The accuracy of the method was also validated by repeated successful participation in the German external quality assessment scheme (G-EQUAS) by the external verifier of the method. The results are shown in Table 10.

9.3 Limits of detection and limits of quantitation

Under the specified analytical conditions, the detection limits in urine were determined using the three-fold standard deviation of the background signal. The quantitation limit was determined analogously on the basis of the nine-fold standard deviation of the background signal. The results are presented in Table 11.

Table 8 Relative recovery rates for the determination of arsenic species in urine (n = 7).

Analyte	Concentration [µg As/L]	Mean relative recovery [%]
As(III)	5.0	119
As(V)	5.0	102
MMA	5.0	112
DMA	5.0	114
Arsenobetaine	5.0	106

Table 9 Comparison of the sum of the arsenic species with the total arsenic concentration in four individual urine samples.

Analyte	Urine 1	Urine 2	Urine 3	Urine 4
As(III) [µg/L]	< LOD	< LOD	< LOD	< LOD
As(V) [µg/L]	2.5	3.1	3.6	4.0
MMA [µg As/L]	6.5	6.1	< LOD	0.7
DMA [µg As/L]	36.1	22.0	1.7	3.5
Arsenobetaine [µg As/L]	19.5	87.5	4.2	5.0
unknown [µg As/L]	~3.0	~3.7	–	–
Sum of species [µg/L]	**67.6**	**122.4**	**9.5**	**13.2**
Total As concentration [µg/L]	**71 ± 3**	**125 ± 5**	**10 ± 2**	**10 ± 3**

9.4 Sources of error

Due to the spectral interference of $^{40}Ar^{35}Cl^+$, both $^{75}As^+$ and $^{77}As^+$ are measured in urine. In the low concentration range, the broad $^{40}Ar^{35}Cl^+$ peak between DMA and As(III) may interfere with the quantitative determination of As(III) at concentrations < 1 µg/L As(III). The native sodium chloride concentration in urine, which is usually around 1%, also has an interfering effect on the determination of DMA and As(III). As interference is negligible at sodium chloride concentrations of < 0.2%, the urine samples must be diluted.

When verifying the method, a minor As(V) signal interference was noted, which may be caused by an accumulation on the analytical column. Nevertheless, good reproducibility was achieved. For real samples, however, this is a possible source of error as the intensity of the As(V) interference peak depends on the regeneration time. Therefore, it must be ensured that the samples are always injected with a constant delay after the previous measurement, which, however, is usually the case with large analytical runs.

Due to the very early elution of arsenobetaine, this signal may be overlapped by unknown arsenic species, which also elute with the dead volume.

Table 10 Results of participation in the interlaboratory test G-EQUAS (German external quality assessment scheme) to determine the accuracy of the method.

G-EQUAS	Analyte concentration [µg As/L]				
	As(III)	**As(V)**	**MMA**	**DMA**	**Arsenobetaine**
57/2016 8A					
Actual	<LOD	0.74	0.77	2.39	5.45
Nominal	0.1	0.80	0.64	2.74	5.12
Accuracy [%]	–	**92.5**	**120.3**	**87.2**	**106.4**
57/2016 8B					
Actual	0.75	0.72	0.88	1.79	23.3
Nominal	0.64	0.68	0.66	2.04	24.2
Accuracy [%]	**117.2**	**105.9**	**133.3**	**87.7**	**96.2**
58/2016 8A					
Actual	0.70	0.60	1.94	7.17	48.6
Nominal	0.68	0.68	1.44	7.95	51.1
Accuracy [%]	**102.9**	**88.2**	**134.7**	**90.2**	**95.1**
58/2016 8B					
Actual	0.87	1.18	2.90	18.3	35.6
Nominal	0.82	1.25	2.19	20.5	36.9
Accuracy [%]	**106.1**	**94.4**	**132.4**	**89.0**	**96.5**
59/2017 8A					
Actual	0.36	0.30	0.77	2.17	2.59
Nominal	0.35	0.46	1.02	2.37	2.89
Accuracy [%]	**102.9**	**65.2**	**75.5**	**91.6**	**89.6**
59/2017 8B					
Actual	1.37	1.47	1.28	6.82	29.0
Nominal	1.62	1.60	1.65	7.22	31.6
Accuracy [%]	**84.6**	**91.9**	**77.6**	**94.5**	**91.8**
60/2017 8A					
Actual	0.24	0.39	0.65	4.94	23.9
Nominal	0.30	0.46	0.73	5.14	28.7
Accuracy [%]	**80.0**	**84.8**	**89.0**	**96.1**	**83.3**
60/2017 8B					
Actual	1.18	0.77	2.96	16.0	58.1
Nominal	1.41	0.97	3.10	16.8	65.7
Accuracy [%]	**83.7**	**79.4**	**95.5**	**95.2**	**88.4**

Table 11 Detection limits and quantitation limits of the arsenic species analysed.

Analyte	Detection limit [µg As/L]	Quantitation limit [µg As/L]
As(III)	0.03	0.09
As(V)	0.05	0.15
MMA	0.04	0.12
DMA	0.02	0.06
Arsenobetaine	0.03	0.09

10 Discussion of the method

Methods for the determination of toxicologically relevant arsenic [Schaller et al. 1991, translated] and of arsenic species [Begerow et al. 2000, translated] have already been published in this collection. In the past, the hydride AAS technique was frequently used to determine the toxicologically relevant arsenic concentration. This technique allows the determination of the arsenic species (As(III), As(V), MMA and DMA) that form the gaseous hydride, while other organic arsenic compounds (arsenobetaine, arsenocholine) cannot be detected by this method. However, in practice difficulties with calibration are often encountered when using modern flow injection methods for hydride AAS, as not all toxicologically relevant arsenic species can be determined with the same sensitivity.

This method permits the simultaneous and reliable determination of the five arsenic species As(III), As(V), MMA, DMA and arsenobetaine, which mainly occur in urine. The method is sufficiently sensitive to both cover the concentration range relevant to occupational medicine and enable determination of the environmental background exposure. In the low-dose range of the individual arsenic species, various interferences may lead to overlapping (see Section 9.4). Therefore, at concentrations of the individual species below 5 µg/L, it is recommended to use the standard addition method for quantification. For the concentration range relevant to occupational medicine, however, external calibration using the multi-species standard is generally sufficient. In addition to the device used here, a number of newer and more powerful ICP-MS systems are available today. To avoid interferences, these are fitted with collision cells or dynamic reaction cells.

For method verification, the procedure described above was slightly modified. Chromatographic separation was performed not using anion exchange HPLC but reverse-phase chromatography (column used: Hamilton PRP-X100 (250 x 431 mm, 10 µm) with Hamilton PRP-X100 pre-column). A gradient of aqueous ammonium dihydrogen phosphate buffers was used as the mobile phase. Validation of the method yielded comparable reliability data.

Instruments used:

ICP mass spectrometer ELAN 5000 (Perkin Elmer).

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12 Appendix

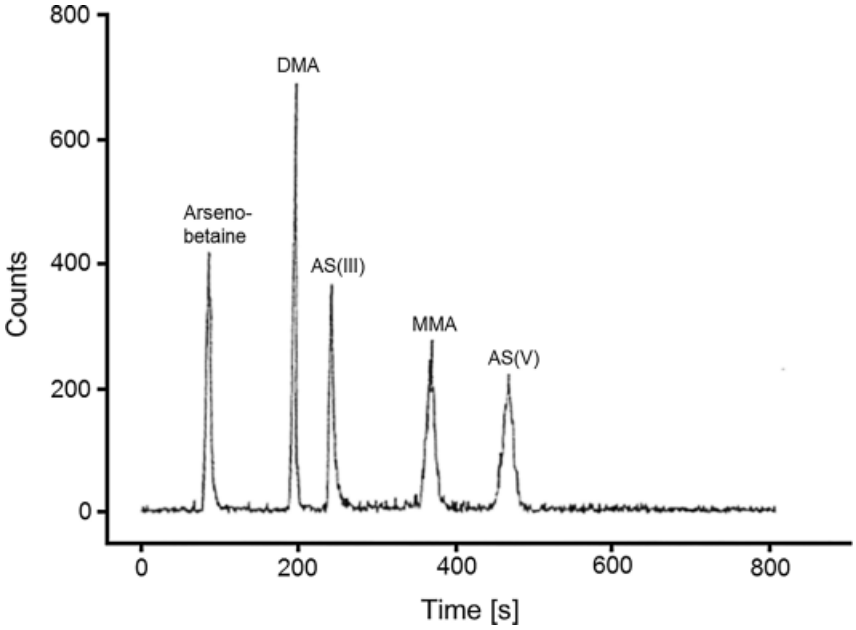

Figure 2 Chromatogram of a urine sample spiked with standard solutions.

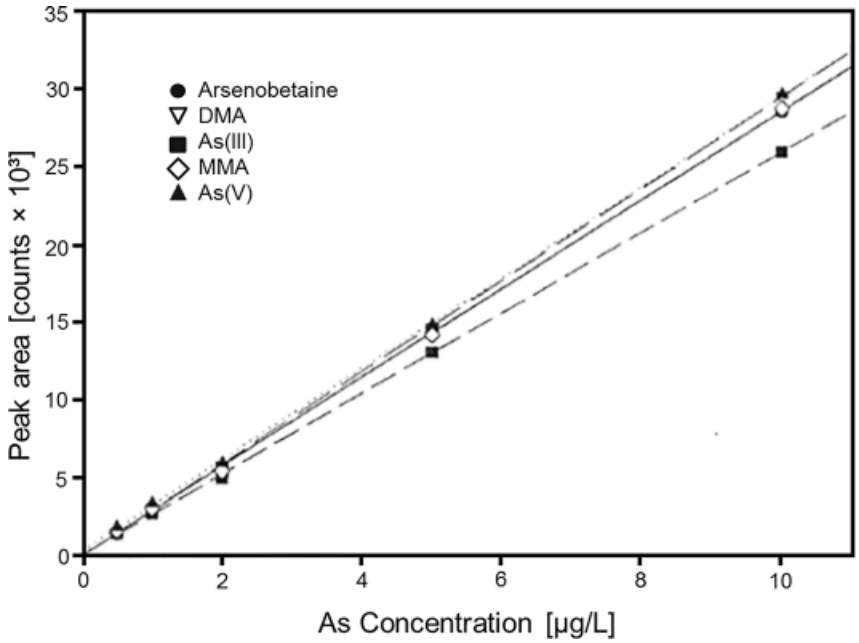

Figure 3 Examples of calibration curves for the arsenic species that can be determined with this method.