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# Speciation of dimethylarsinic acid and monomethylarsonic acid by gas chromatography–mass spectrometry

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**Abstract**

A GC–MS method has been developed to determine two methylated arsenic species in human urine samples. This GC–MS method was compared with high-performance liquid chromatography–hydride generation–atomic fluorescence spectrometry. The yield of derivatisation for dimethylarsinic acid (DMA) and monomethylarsonic acid (MMA) using thioglycol methylate was measured. The detection limits for DMA and MMA using this GC–MS method are 0.95 and 0.8 ng/cm$^3$, respectively. © 1999 Published by Elsevier Science B.V. All rights reserved.

*Keywords:* Speciation; Dimethylarsinic acid; Monomethylarsonic acid; Organoarsenic compounds; Thioglycol methylate

## 1. Introduction

Speciation analysis has been one of the most progressing technique of modern instrumental element analysis in the last decade [1–4]. The aim of this technique is to determine the different chemical forms of a given element. Generally, for the separation of different species a high-performance separation technique and an element-selective atomic spectrometric detector are combined. The separation technique is in most cases liquid chromatography [5–9], since the atomic spectrometric detectors [10–14] were originally planned for determinations from liquid media, therefore, coupling is easy in this case. HPLC separations require element-selective detectors, because the selectivity of conventional liquid chromatographic detectors is insufficient for the determination of separated metal species. For example, for the determination of arsenic species HPLC—classical UV detection, which is not an element-selective detection method [4] is used for the actual analysis. Hyphenated systems are useful, but also have disadvantages: (i) they are remarkably expensive and complicated systems; (ii) their operation requires skilled (in atomic spectrometry for detection and in chromatography for separation); (iii) the atomic spectrometers software is either not able to accept transient signals, or they are not able to do it for 20–30 min, which is needed for the HPLC separations; (iv) although the commercially available atomic spectrometers are able to record time-intensity curves needed by chromatography, they are not able to treat and evaluate them as chromatograms; (v) data cannot be transferred from most of the spectrometric software to chromatographic software; and (vi) the efficiency of conventional nebulising sample introducing systems is often insufficient for/

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in speciation analyses, therefore, the use of different high-performance nebulisers, nebuliser-desolvation systems is necessary (ultrasonic nebuliser, USN; high-pressure nebuliser, HPN; microconcentric nebuliser, MCN; thermospray sample introduction system, TS).

The detection limit calculated for the sample to be measured in separation analyses is expected to be exceedingly high. This is explained by the following: (i) the already low metal content divides into further fractions during determination; and (ii) the sample introducing capacity of chromatographic systems varies between 1 and 200 $mm^3$ on average, therefore, transient signals never reach the intensity that is gained by the continuous introduction of the same sample solution.

For the above mentioned reasons, determinations from homogeneous systems where chromatographic separations have specific detectors constitute an important branch in analytical chemistry. GC–MS is a widely spread representative of this branch. It makes the determination of volatile metal species possible with high sensitivity and selectivity.

In the liver of humans and mammals there is a methylating mechanism for the detoxification of inorganic arsenic. As a result of this detoxifying process, the major part of the detectable amount of arsenic in the body—in blood and urine—is in methylated form. According to the literature, most is dimethylated [2]. Therefore, it is inevitable to determine the exact quantity of the different methylated species as well as the total amount of arsenic in body fluids [15]. This set of data represents the tolerance state of the body by telling us if the methylated amount is equal to the total concentration. If the methylated arsenic concentration is significantly lower than the total input, it means there is a large concentration of highly toxic, unmethylated species in the body.

There is a wide selection of methods for the determination of methylated arsenic species. The most characteristic systems are the ones using ion-pair or ion-exchange chromatography for separation. The most widely used detection methods are inductively coupled plasma atomic emission spectrometry (ICP-AES), inductively coupled plasma mass spectrometry (ICP-MS), hydride generation–atomic absorption spectrometry (HG–AAS) and atomic fluorescence spectrometry (AFS). The most sensitive and selective, and therefore, the most adequate systems are those applying ICP-MS or AFS for detection, although these techniques are also loaded with the above listed problems of hyphenation.

Methylated arsenic species cannot be determined with GC because their boiling point is too high for this analysis. By derivatisation these species can be transformed into volatile compounds. The most widespread compound for the volatilisation of the methylated arsenic species is thioglycol methylate (TGM) [16–19].

The aim of our work was the development of a method for the determination of methylated arsenic species from human urine. Besides the performance of the method, the selectivity and accuracy of the technique were investigated with the help of an independent arsenic speciation system—HPLC–HG–AFS.

## 2. Experimental

### *2.1. Instrumentation*

#### *2.1.1. GC–MS*

A HP5890 gas chromatograph (Hewlett-Packard, Waldbronn, Germany) was linked by a direct capillary interface to a VG TRIO2 mass spectrometer (VG Altrincham, UK). The gas chromatograph was temperature programmed and was operated in full scan (SCAN) mode or selected ion recording (SIR) mode, both in electron impact (EI) ionization. The concentration of analytes in the samples were determined by external standards method. The working parameters of the GC–MS system are summarized in Table 1.

#### *2.1.2. HPLC–HG–AFS*

A Shimadzu Model LC-7A HPLC pump was attached to a sample introduction valve (sixport Rheodyne system, LMIM, Hungary). A 250-$mm^3$ sample loop was used for sample introduction. The analytical column was a Bio Separation Technologies (BST) $C_{18}$ Rutin column (25 cm×4.6 mm I.D., 10-μm particle size). A four-channel peristaltic pump (Ismatec MS-CA 4, Switzerland), a 10-cm long mixing coil functioned as continuous hydride

Table 1
Working parameters of the GC–MS system

| Ion source | Electron collision |
|---|---|
| Energy of ionisation | 70 eV |
| GC column | HP-1 |
| GC–MS combination | Direct coupling |
| Injector | Split/splitless |
| Split ratio | 1:50 |
| $T_1$ | 110°C |
| Time program | 25°C/min |
| Final temperature | 230°C |
| Line temperature | 250°C |
| Injector temperature | 220°C |

generator. A laboratory-made liquid–gas separator was used in the system. The gas–liquid separator was further connected to an AFS system (PSA Excalibur, PS Analytical, Sevenoaks, UK) that utilises an arsenic boosted-discharge hollow cathode lamp (Superlamp, Photron, Australia) as excitation source. Measurements were carried out around the resonance wavelength of arsenic (193.7 nm) using a multi-reflectance filter having a spectral bandpass between 20 and 40 nm. Argon functioned as carrier gas and mixed with hydrogen it supported the diffusion flame. Constant gas flows were maintained by Cole–Palmer rotameters (Niles, IL, USA). Data collection and evaluation was totally automated by a Borwin Chromatographic Software (JMBS, Grenoble, France). All peaks were evaluated by their peak height.

### 2.2. Reagents

Monomethylarsonic acid (MMA) stock solution was prepared from Strychotonin solution (Chinoin, Budapest), dimethylarsinic acid (DMA) was obtained from Fluka. The 1000-mg/dm$^3$ stock solutions of MMA and DMA were diluted with deionised water before use.

The didocyldimethylammonium bromide (DDAB)

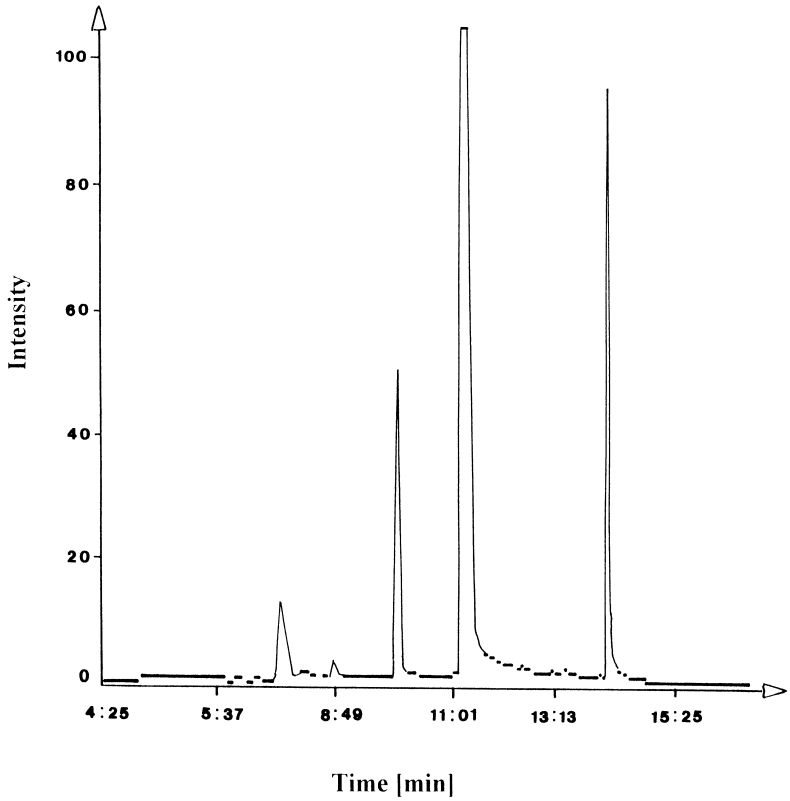


Fig. 1. SCAN chromatogram of DMA ($t_R$=7.75 min), MMA ($t_R$=13.92 min) and TGM ($t_R$=11.02 min).

(Fluka) solution (0.01 mol/dm$^3$) was prepared by adding 0.5% (v/v) methanol and 0.1% (v/v) of 0.01 mol/dm$^3$ DDAB solution to the $NaHPO_4$ buffer solution for the HPLC eluent. The pH was set to 6.0 by the addition of $NaH_2PO_4$ solution containing the same amount of phosphate, methanol and DDAB as the eluent. TGM and the organic solvents were obtained from Fluka.

*2.2.1. Chemicals for hydride generation*

Sodium tetrahydroborate solution (2%, m/v) was prepared daily by dissolving $NaBH_4$ powder (Aldrich) in 0.5% (m/v) NaOH solution, HCl was obtained from Reanal (Budapest, Hungary).

*2.3. Sample extraction procedure for GC–MS analysis*

The urine sample (1 cm$^3$) was placed in a 10-cm$^3$ glass tube, 100 mm$^3$ of hydrochloric acid and 50 mm$^3$ of TGM were added to it and then the tube was closed with a glass cork. After 2 min of intensive mixing (using a Vortex mixer) 1 cm$^3$ of hexane was added, which was followed by another 2 min of mixing [16–19]. Finally, after the separation of the phases, the upper, organic phase was used for the measurements.

## 3. Results and discussion

The SCAN chromatogram obtained under the circumstances provided by set parameters is presented on Fig. 1. The derivatisation reaction is seen in Fig. 2. From the figure it can be seen that MMA was substituted by two, while DMA was substituted with only one thioglycol side-chain. As a result the difference between the polarity of the two derived molecules, separation is a relatively simple task.

The determination of the efficiency of the derivatisation reaction was the first step in the development of this method. In this step an extraction test was carried out from aqueous solutions of given concentrations of MMA or DMA. The derivatisation reaction was tested and the concentration of the remaining arsenic species of interest was measured in the aqueous phase after the extraction with hexane. The measurement was actually the determination of total arsenic content from the aqueous phase and was carried out on a HG–AFS system. The results of the efficiency measurements of the derivatisation reaction was in case of MMA 88%, in case of DMA 95%. From the results and their relatively low R.S.D. value (5–8%) it can be concluded that the derivatisation reaction is characterised by high efficiency, because the arsenic content of the measured solutions had significantly decreased.

On the basis of the high efficiency values, the method seems to be suitable for the measurement of real samples, however, the efficiency of chemical reaction can be different at the samples matrix even from sample to sample. The need of certified reference materials (CRMs) in case of speciation analysis of biological samples is absolutely necessary, because without CRMs the efficiency of method development cannot be tested. The method described

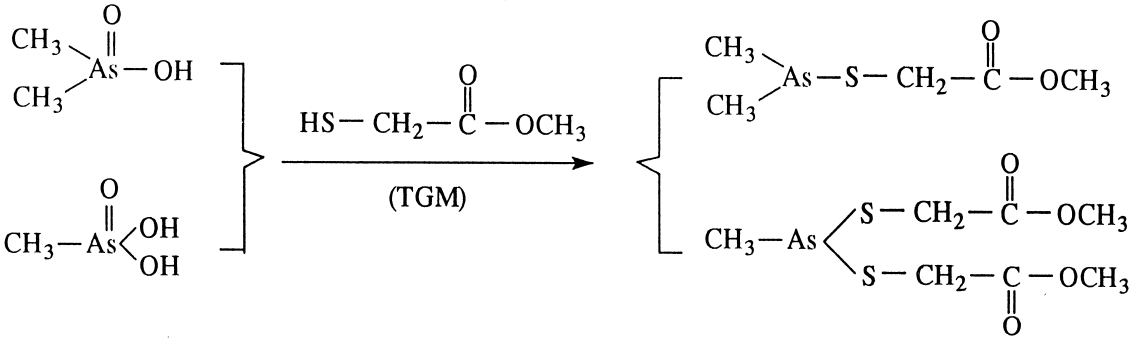


Fig. 2. The derivatisation reaction of DMA and MMA using TGM.

above is fast and it does not dilute the samples. This latter value is very advantageous, since the samples contain arsenic in small concentration in most cases.

In the next step the spectra of the two arsenic species were evaluated. Fig. 3a,b presents the (EI) spectra of DMA and MMA, where the base peak is the same value, $m/z=195$, and the molecular ions are $m/z=210$ and $m/z=300$, respectively, with an abundance of about 20%. Based on the EI spectra the following characteristic ions were selected for the SIR quantitation of DMA and MMA from urine. (Table 2).

Fig. 1 shows the mass chromatogram of the spiked urine sample at $m/z=195$. Table 2 shows the retention time of DMA and MMA. The peaks represent 500 ng/cm$^3$ DMA and MMA, where the signal-to-noise ratio is better then 1:10. The total separation time is less than 15 min.

During our work two real urine samples and their spiked varieties—with 500 ng/cm$^3$ of MMA and 500 ng/cm$^3$ of DMA—were measured on a GC–MS system. The selectivity and the accuracy of the method were controlled by an independent HPLC–HG–AFS system by measuring the original and the spiked samples. The efficiency of the hydride generation of arsenic species in our system was investigated earlier [20]. The operation parameters are summarised in Table 3, the gradient elution was linear between 2 and 4 min. The samples were directly injected into the system after a 1:5 dilution and measured in five replicates (the R.S.D. was in the range of 5–8%). The results were compared with results gained on the GC–MS system. In Table 4 it can be observed that the HPLC–HG–AFS system is not suitable for arsenic determination from unspiked, diluted urine samples, because the peaks are close to the detection limit. In the case of the spiked samples, although the added quantity is one and half orders of magnitude higher than the original amount, the recovery is still acceptable. In conclusion it can be stated that these two independent methods provide practically the same results. The main advantage of the GC–MS derivatisation system compared to the HPLC–HG–AFS system is the better detection limit. The detection limit of HPLC–HG–AFS system has already been published in a table together with a list of detection limits reported in the previous literature [21]. In the case of the GC–MS system the sample is not diluted and, in addition, after the extraction with hexane, the partial evaporation of the solvent causes the concentration of the analyte, which improves the detection limit. The detection limits for the two arsenic species are shown in Table 5. It can be concluded, that only the very expensive, sophisticated systems, e.g., HPLC–ICP-AES and HPLC–ICP-MS system possess better detection limits. On the other hand, these systems cannot tolerate undiluted body fluids. This means that the detection limits of these systems decrease proportionally with dilution. Another drawback of the HPLC separation systems is that the anion-exchange columns cannot tolerate the high organic content of body fluids.

## 4. Conclusion

The developed GC–MS method is suitable for the determination of methyl-arsenic species from urine samples. After controlling the quantitative and qualitative results obtained by GC–MS with the results of an independent method (HPLC–HG–AFS), it can be stated that the methods are comparable. The derivatisation process is quick, gives reproducible results and cheap, because of its low material requirement. The separation process is also relatively quick—it takes 15–17 min. The complete GC–MS system is homogeneous—it is not a ‘laboratory-made’ hyphenated system. It is not loaded with the problems of hyphenation, namely the conversion of physical states. The required selectivity for speciation analyses in this case is ensured by mass spectrometry.

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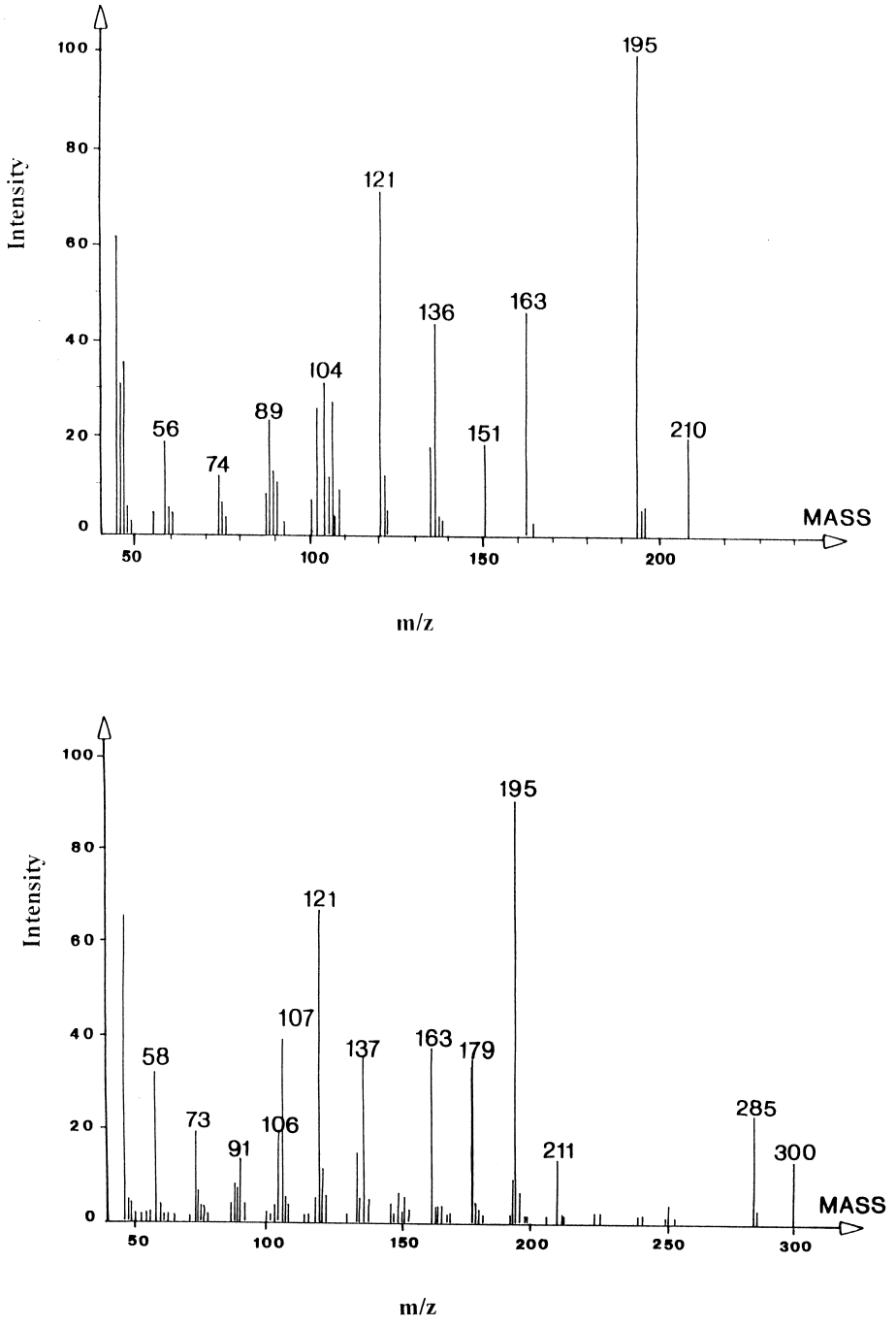


Fig. 3. The MS spectra of DMA (a) and MMA (b).

Table 2
SIR ions, retention times of TGM arsenic derivates

| Arsenic species | Target ion ($m/z$) | Qualifier ion ($m/z$) | Retention time (min) |
|---|---|---|---|
| DMA | 195 | 210 (M+) | 7.75 |
| MMA | 195 | 107 and 179 | 13.92 |

Table 3
Instrumental parameters of the HPLC–HG–AFS system

| HPLC system | |
|---|---|
| Column | BST $C_{18}$ Rutin column (25 cm×4.6 mm I.D., 10-μm particle size) |
| Column temperature | 24°C |
| Sample loop size | 250 $mm^3$ |
| Mobile phase | |
| 0–2 min | DDAB+methanol (no phosphate) |
| 2–4 min | 0⇒50 mmol/$dm^3$ $Na_2HPO_4$–$NaH_2PO_4$ buffer |
| 4–8 min | 50 mmol/$dm^3$ $Na_2HPO_4$–$NaH_2PO_4$ buffer<br>$+1\times10^{-5}$ mol/$dm^3$ DDAB<br>+0.5% (v/v) methanol<br>(pH 6.0) |
| Pump flow-rate | 2 $cm^3$/min |
| Hydride generation | |
| (a) | 1.5 *M* HCl |
| (b) | 2% (m/v) $NaBH_4$ |
| Pump flow-rate | 1.7 $cm^3$/min |
| Atomic fluorescence detector | |
| Primary current | 27.5 mA |
| Boost current | 35 mA |
| Detection wavelength range 190–210 nm | |
| Gas flow-rates | |
| Argon | 390 $cm^3$/min |
| Hydrogen | 70 $cm^3$/min |

Table 4
Comparison of the HPL–HG–AFS and GC–MS results for the DMA and MMA determination in urine and spiked urine samples

| | Measured values (ng/$cm^3$) | | | |
|---|---|---|---|---|
| | HPLC–HG–AFS | | GC–MS | |
| | MMA | DMA | MMA | DMA |
| Urine sample 1 | n.d. | n.d. | 8 | 6 |
| Spiked samle 1[a] | 510 | 502 | 513 | 505 |
| Urine sample 2 | n.d. | n.d. | 12 | 10 |
| Spiked samle 2[b] | 518 | 507 | 517 | 508 |

[a]Urine sample 1+500 ng/$cm^3$ MMA and 500 ng/$cm^3$ DMA.
[b]Urine sample 2+500 ng/$cm^3$ MMA and 500 ng/$cm^3$ DMA.

Table 5
Detection and quantification limits of the GC–MS system

| Arsenic species | Detection limit (ng/$cm^3$) | Quantification limit (ng/$cm^3$) | R.S.D. (%) ($n$=9) |
|---|---|---|---|
| MMA | 0.8 | 3.46 | 3.18 |
| DMA | 0.95 | 4.19 | 2.75 |

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