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Speciation of dimethylarsinic acid and monomethylarsonic acid by solid-phase microextraction–gas chromatography–ion trap mass spectrometry

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Abstract

A solid-phase microextraction (SPME) method has been developed to determine two methylated arsenic species in human urine samples by GC–MS. The direct extraction of the methyl arsenic compounds by SPME after thioglycol methylate derivatization was studied. Direct extraction with SPME was suitable for the determination of trace levels of dimethylarsinic acid (DMA) and monomethylarsonic acid (MMA) in urine samples. Four different commercial SPME fibers were tested for the extraction of methyl arsenic compounds, and the best results were obtained using the polydimethylsiloxane coating. The extraction and desorption time profiles of DMA and MMA were determined. The detection limits for DMA and MMA using the SPME–GC–MS method were 0.12 and 0.29 ng/ml, respectively. The method is linear in the 1 to 200 ng/ml range.

Keywords: Dimethylarsinic acid; Monomethylarsonic acid; Arsenic compounds; Organoarsenic compounds; Thioglycol methylate

1. Introduction

Speciation analysis has been one of the fastest progressing techniques of modern instrumental element analysis in the last decade [1–4]. This technique aims at determining the different chemical forms of a given element. Generally, for the separation of different species, a high-performance separation technique (e.g. HPLC, capillary GC) and an element selective atomic spectroscopic detector are coupled. In the liver of humans and mammals, there is a methylation 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 the form of dimethylarsinic acid (DMA) and monomethylarsonic acid (MMA). According to the literature the majority of arsenic is in the form of DMA. Therefore, it is necessary to determine the exact quantity of the differently-methylated species as well as the total amount of arsenic in body fluids [5].

In the case of arsenic speciation the separation technique is typically liquid chromatography, while the detection method is atomic spectroscopy. The typical choices for the separation of anionic arsenic species [MMA, DMA, As(III), As(V)] are ion-exchange chromatography [6,7] and ion-pair chromatography [7–10]. An interesting new approach was described by Taniguchi et al. [11] employing ion-exclusion chromatography for the separation of As(V), As(III) and MMA. The advantage of this separation method is that it does not employ buffers;

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rather it uses only diluted acids as the mobile phase. The use of high purity acids decreased the background signal level and improved the detection limit. However, the method could only be used to separate three of the four important anionic arsenic species. A characteristic effort in speciation analysis is to simplify complicated analytical systems. Yalcin and Le [12] described a low-pressure liquid separation system employing only a solid-phase extraction (SPE) cartridge for the separation of arsenic species. A popular method for the separation of arsenic species is capillary electrophoresis (CE) [13,14]. The clear advantage of CE, coupled with atomic spectroscopic detection, is the very low liquid flow-rate, which offers the possibility of using different high efficiency micro-sample-introduction systems.

The detection methods used in the speciation systems are typically atomic fluorescence spectrometry (AFS) [8–10], atomic absorption spectrometry (AAS) [15] and inductively coupled plasma–mass spectrometry (ICP–MS) [7,11]. Typical problems related to this type of hyphenation (liquid phase separation + element selective detection) were described in our earlier work [16].

To avoid the technical problems related to the above described hyphenated systems we applied a GC–MS method for the determination of methyl DMA and MMA. Only a few methods have been described in the literature for arsenic speciation by GC. One of these, the thioglycol methylate derivatization method [16–20], is based on the ''affinity'' of arsenic compounds to thiol groups. A schematic diagram of this reaction is shown in Fig. 1. In our recent paper the fragmentation pattern of thioglyco-derivative DMA and MMA was fully discussed [21]. All of the earlier applications based on these reactions applied past-derivatization, liquid–liquid extractions to transfer the derivatized arsenic species to the organic phase.

Solid-phase microextraction (SPME) was successfully applied for the headspace- and direct-extraction of several organic and organometallic compounds (e.g. benzene [22], caffeine [23], volatile organic compounds in water [24], pesticides [25], phenols [26,27], organolead [28], organotin [29], organomercury [29,30]). The general advantages of SPME are well known: solvent-free, fast, integrated sampling-extraction-sample introduction system.

The objective of our work was to develop a SPME method for the speciation of DMA and MMA by GC–MS after thioglycol methylate (TGM) derivatization.

2. Experimental

2.1. Instrumentation

Ion trap mass spectrometric experiments were performed using a Varian Star 3400 gas chromatograph coupled to a Varian Saturn 4D ion trap MS (Varian, CA, USA), controlled by a computer with dedicated software. The quantification of samples was performed using external calibration and standard addition methods. A VWR Dylastir magnetic stirrer (VWR Scientific of Canada) and PTFE-coated stir bars were used for stirring the samples.

2.2. Reagents

MMA was obtained from Supelco, DMA was obtained from Fluka. The 1000 mg/l stock solutions

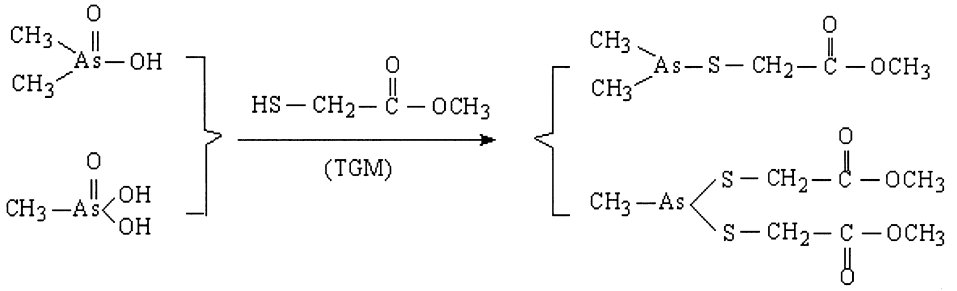

Fig. 1. Derivatization reaction of DMA and MMA using TGM.

of MMA and DMA were diluted with de-ionized water before use. Thioglycol methylate was obtained from Fluka.

2.3. Sample extraction procedure

A 1 ml urine sample (10× diluted with water) was placed in a 2 ml glass vial which was sealed with a septum, 10 μl of hydrochloric acid and 50 μl of TGM were added to it and then the tube was closed and intensively stirred for 2 min (1000 rpm). To thermally insulate the sample from the stirring plate (to avoid heating), a PTFE silicon septum was placed between the glass vial and the plate. SPME fiber was exposed in the solution for a predetermined sampling time, then the fiber was withdrawn into the needle and transferred to the desorption unit (i.e. GC injector). The glass vials were used only once and were disposed after each analysis. Because the applied desorption methods also serve as fiber clean up, no further cleaning was employed.

3. Results and discussion

The parameters of the developed derivatization, extraction and separation/detection system are shown in Table 1. A chromatogram obtained under these conditions is presented in Fig. 2.

The derivatization reaction is shown in Fig. 1. 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 of the difference between the polarity of the two derivatized molecules, separation is a relatively simple task.

4. Optimization of SPME extraction

4.1. Determination of the desorption time

Desorption time profiles for the two DMA and MMA compounds were determined. Desorption from the fiber was complete after 40 s. The chromatogram and the mass spectra of these compounds were compared at different injector temperatures (from 100 to 260°C). No decomposition of DMA and MMA was observed. Therefore, 250°C injector temperature was employed to obtain fast desorption of the compounds.

4.2. Extraction time study

The determination of the extraction time profile of

Table 1
Parameters of SPME–GC–MS method

Derivatization	
Solution	1 ml sample + 10 μl concentrated HCl + 50 μl TGM
Reaction time	2 min
Extraction	
SPME Fibre	PDMS 100 μm
Extraction time	40 min
GC	
Ion source	Electron collision
Energy of ionization	70 eV
GC column	Supelco SPB-5 15 m×0.25 mm, 0.25 μm
GC–MS combination	Direct coupling
Injector	250°C
Initial temperature	110°C
Time program	20°C/min
Final temperature	230°C
Transfer line temperature	240°C
Injector temperature	250°C

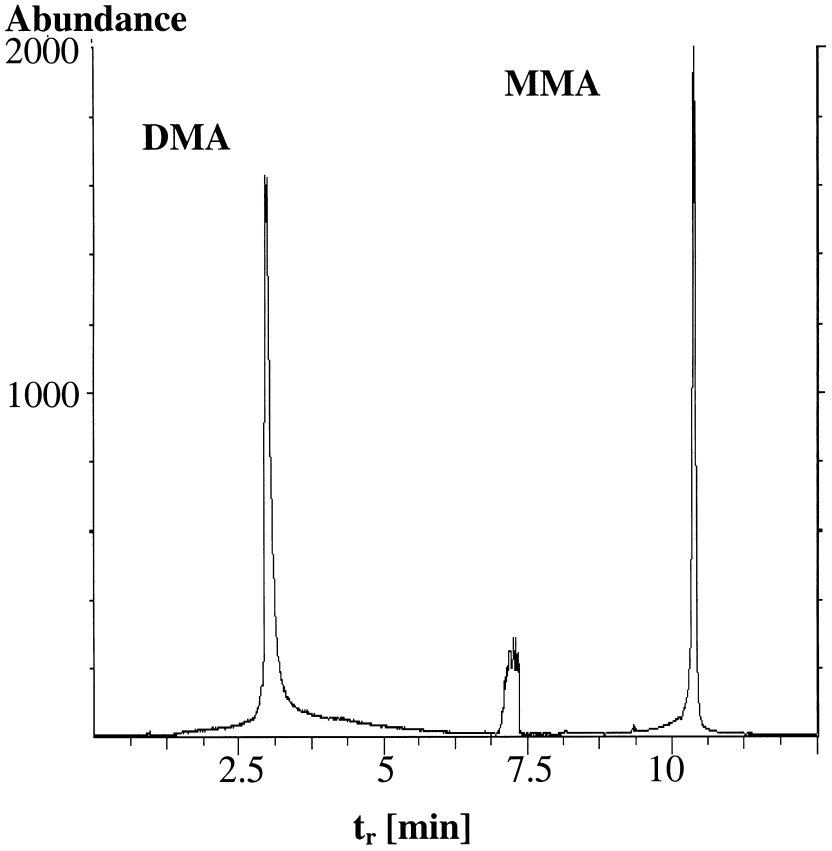

Fig. 2. Chromatogram of DMA (t_R=3.12 min), MMA (t_R=10.43 min). The observed ion was m/z 195 (according to our earlier work [16,21]).

the measured compounds is a crucial part of the equilibrium extraction methods. Fig. 3 shows the results obtained for DMA and MMA. After 1 h, both compounds reach equilibrium between the aqueous phase and the extracting polymer. For sample analysis, 40 min extraction time was used.

Fig. 4 shows the results of the comparison of the different commercial SPME coatings for the extraction of DMA and MMA. As can seen for both compounds, the best results were obtained using the polydimethylsiloxane (PDMS)-coated fiber.

5. Characteristic of the method

Table 2 shows the retention time, the detection limit (3σ value), quantification limit (9σ value) and the relative standard deviation (RSD) of the developed SPME–GC–MS method. The detection limits (low ppb range) are comparable to those obtained with the more complicated and expensive HPLC–ICP-MS systems.

The linear response range for the two compounds was three orders of magnitude (1–2000 ng/ml). Fig. 5 shows the calibration curve of MMA and DMA, where the sensitivity of the method can be observed. The SPME–GC–MS method was applied on real urine samples. Table 3 shows the results of the determination of MMA and DMA in a urine sample. External calibration and standard addition calibration methods were applied for quantification. The standard addition method provided about 10% lower concentration for both compounds and better reproducibility. In complex matrixes, the standard addition

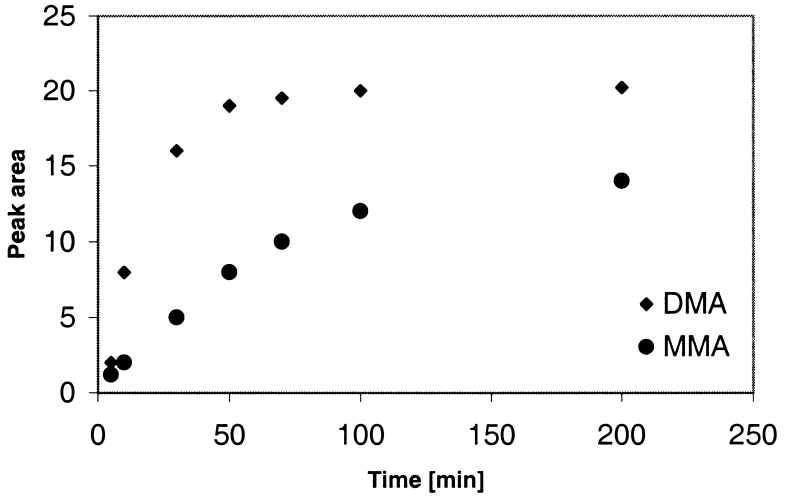

Fig. 3. Absorption time profile of DMA and MMA.

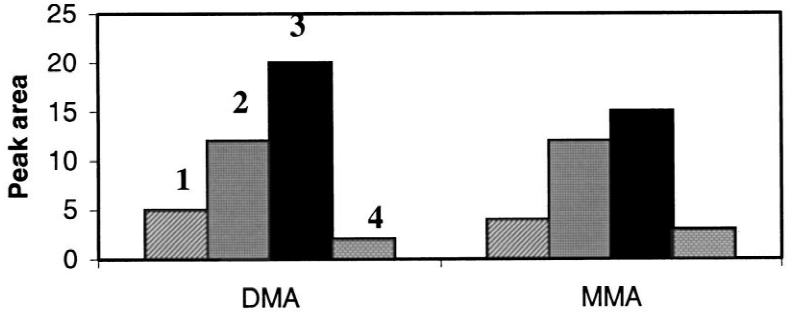

Fig. 4. Comparison of commercial SPME coatings for DMA and MMA extraction. 1=Polyacrylate; 2=PDMS–divinylbenzene; DMS; 3=PDMS; 4=Carboxen.

method is recommended for quantification. The effect of time-lapse between extraction and analysis was studied. In the studied ''storage'' interval (from 5 min to 2 h), the fluctuation in the analytical data was within the standard deviation of the method. (During the storage period the SPME needle was covered by a PTFE septum.)

Table 2
Retention time, detection limits, quantification limits and RSDs, for DMA and MMA determination

Arsenic species	t_R (min)	DL[a]	QL[b]	RSD[c] (%)
MMA	10.43	0.29	1.16	9.2
DMA	3.12	0.12	0.66	7.2

[a] Detection limit (ng/ml).
[b] Quantification limit (ng/ml).
[c] %, $n=9$, measured using a 10 ng/ml standard solution.

6. Limitations of the method

The above described analytical system provides information only about the mono- and dimethylarsenic compounds. To obtain information about the total concentration of arsenic, the conventional analytical methods can be applied.

7. Conclusion

The SPME–GC–MS method is suitable for the

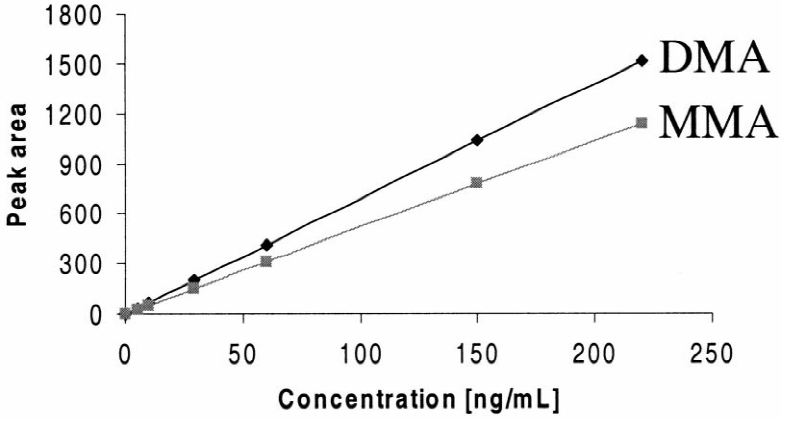

Fig. 5. Calibration curves of MMA ($y=51.7x+2.3$) and DMA ($y=68.9x+3.1$).

determination of methyl arsenic species from urine samples The detection limit achieved with this system is favorably compared with other, more complicated hyphenated systems used for arsenic speciation. In comparison with the liquid–liquid extraction methods, SPME has been shown to be an environmentally friendly, valid alternative.

The derivatization process is relatively fast, reproducible and inexpensive. The GC separation is fast – it takes less than 12 min. In this case, the required selectivity for speciation analyses is ensured by mass spectrometric detection. The long-term stability of analyte on the fiber offers the possibility of performing the extraction on-site – in this case in the doctor's office and sending the fiber to the laboratory for analysis.

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Table 3
Determination of DMA and MMA in urine by external calibration and standard addition

Arsenic species	Sample concentration[a] (ng/ml)	Sample concentration[b] (ng/ml)
MMA	4.12±0.6	3.85±0.23
DMA	7.26±0.45	6.49±0.28

[a] Results obtained by external calibration.
[b] Results obtained by standard addition.

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