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# Determination of Organoarsenic Species in Blood Plasma by HPLC–ICP MS

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**A study of arsenic speciation in blood plasma of patients undergoing renal dialysis has been performed using HPLC coupled with ICP MS. It was found that the only detectable arsenic species present in the plasma was arsenobetaine. The limit of detection using an injection volume of 175 $\mu$l was found to be 0.25 ng of arsenic as arsenobetaine. Spiking experiments demonstrated recoveries of approximately 100%. In the absence of certified reference materials or an alternative technique, we believe this was the best way to demonstrate that the method was reliable and accurate. Arsenobetaine concentrations in pre-dialysis plasma were similar to those for the healthy volunteers, although after dialysis the concentrations were significantly reduced. It is thus concluded that, except for a few patients, dialysis removed the arsenobetaine efficiently (hence preventing an accumulation of arsenic) and that no biotransformations were occurring. The exceptions to this conclusion were in a few patients whose arsenobetaine levels increased marginally after dialysis, but this was attributed to the levels both pre- and post-dialysis being very close to the detection limit.** 

**Keywords: arsenic speciation; blood plasma; HPLC–ICP MS; renal dialysis**

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## INTRODUCTION

Recently, arsenic speciation has become of considerable interest because of the improved understanding of its speciation and how it is concentrated in marine products,[1–3] excreted in urine[3–6] and present in drinking water.[7] Some foods, particularly fish but also meats where fishmeal has been used in the diet, contain significant levels of arsenic, primarily in the non-toxic form arsenobetaine. The other arsenic species, such as inorganic arsenic, monomethylarsonic acid (MMAA) and dimethylarsinic acid (DMAA), are more toxic.[3] Arsenobetaine is easily absorbed and rapidly excreted in the urine of healthy humans within 6–24 h after ingestion. In an editorial,[8] Vahter posed the question of what types of arsenic may be present in urine, what techniques are available to determine them and whether we could come to any conclusions about exposure and the health risks. Some of these questions will be addressed in this paper.

There are a number of methods available for measuring arsenic in biological samples. Unfortunately, most of these techniques cannot measure arsenobetaine with any accuracy, because it does not form a hydride and it gives a different response from inorganic arsenic in electrothermal atomic absorption spectrometry. Other workers have measured arsenobetaine in urine by refluxing with alkali to produce trimethylarsine.[9] Such treatment is unacceptable when other species are to be determined because, under such harsh conditions, changes in speciation are inevitable. The analytical speciation of arsenic has usually been achieved by using high-performance liquid chromatography (HPLC) coupled to either inductively coupled plasma–atomic emission spectrometry (ICP AES) or inductively coupled plasma–mass spectrometry (ICP MS). Coupling with ICP MS may be hindered by the presence of chloride ions that form the isobaric interference $^{40}Ar^{35}Cl^{+}$ at $m/z$ 75. A variety of HPLC methods have been used which use

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reversed-phase, anion- and cation-exchange resins. In a recently developed procedure, an anion-exchange procedure was used to separate the arsenic species prior to determination by ICP MS.[10] In this paper, the interference from chloride ions is removed by using an anion-exchange column that retains the chloride. An alternative method of overcoming the interference is the addition of a molecular gas such as nitrogen[3] or ethene[11] to the nebulizer gas flow.

In three recent studies[12–14] from the same group in Belgium, the concentrations of different arsenic species in the blood serum of uraemic patients were determined. These authors indicated that the main species present was arsenobetaine,[12] but that small quantities of DMA also occurred. In two of these papers[12,13] the levels of arsenic species were all below or very close to the limits of detection. In the other paper,[14] Zhang *et al.* concluded that inorganic arsenic became bound to proteins and hypothesized that this may be important for detoxification. No selective removal of different arsenic species in the serum of haemodialysis patients was observed.

It is important to be able to differentiate the arsenic species as there is a concern that mildly toxic forms such as dimethylarsinic acid may be converted by biological processes to more toxic forms. Hence, a possible accumulation of organoarsenic species in the blood of patients with impaired kidney function would be undesirable. The aim of this study was to investigate whether there is an accumulation of any organoarsenic species in the blood plasma of patients with end-stage renal disease undergoing haemodialysis.

## EXPERIMENTAL

### Reagents and standards

High-purity water was obtained by reverse osmosis (Milli-RO4; Millipore, Harrow, UK) followed by adsorption, deionization and ultrafiltration (Milli-Q system; Millipore). A stock mobile-phase solution was prepare by dissolving 8.7125 g of potassium sulphate (Puriss; Fluka, Gillingham, Dorset, UK) in water to 500 ml and adjusting to pH 10 by the addition of ammonia (Aristar; Merck, Poole, Dorset, UK). The low-concentration mobile phase was then prepared by diluting 0.5 ml of the 0.1 M stock to 500 ml and then again adjusting to pH 10 with the ammonia. Standards (1000 $\mu$g ml$^{-1}$) of the arsenic species arsenobetaine, dimethylarsinic acid, monomethylarsonic acid and arsenate were obtained from Dr M. E. Foulkes, University of Plymouth, and diluted accordingly before analysis.

### Instrumentation

All analyses were performed using an HPLC pump (Waters Associates, MA, USA) coupled via a high-capacity, strong anion-exchange resin (Benson BA-X10; Benson Co., Reno, NV, USA) to an ICP MS instrument (PlasmaQuad II; Fisons Instruments, Winsford, Cheshire, UK). The resin was packed into two 150 mm $\times$ 4.6 mm columns arranged in series, with a guard column (50 mm $\times$ 4.6 mm) to protect the analytical columns from coagulated proteins or suspended solids. The system has been used previously and full details may be found in Ref. 9.

### Sample preparation

Blood (approximately 2 ml) was collected by venipuncture both before and after a 4 h haemodialysis session into lithium heparin tubes. The plasma was removed by Pasteur pipette after centrifugation (2000 rpm for 5 min) and stored at $-20$ °C before analysis. On thawing, it was filtered through a 0.2 $\mu$m pore-size filter (13 mm; Alltech Associates, Lanc., UK) to remove coagulated protein and other suspended solids and the filtrate was collected in clean vials of 2 ml capacity.

### Procedure

Aliquots (175 $\mu$l) of the samples were injected into the system via a sample loop on a six-port valve (Rheodyne 7125; Anachem, UK). The HPLC operating parameters were as follows. The stationary phase, in two 150 mm $\times$ 4.6 mm columns, was high-capacity strong anion-exchange resin (Benson BA-X10), the mobile phase was ammoniacal potassium sulphate (pH 10, 0.1 mM-stepped to 0.1 M) and the flow rate was 1.2 ml min$^{-1}$. The ICP MS parameters were: coolant gas flow 15 l min$^{-1}$, auxiliary gas flow 1.0 l min$^{-1}$, nebulizer gas flow 0.85 l min.$^{-1}$; the forward power was 1.5 kW and a single-pass spray chamber was used, in conjunction with an Ebdon high-solids nebulizer. The detection mode was single-ion monitoring ($m/z$ 75) with a dwell time of 163840 $\mu$s and 2095 channels. The peaks obtained in the chromatograms were quantified using peak area measurements obtained from the software of the ICP MS instrument.

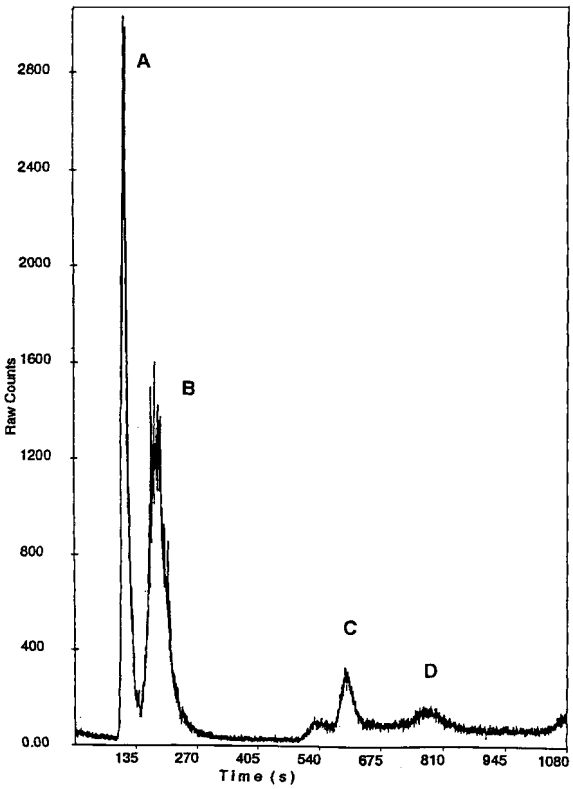


**Figure 1** A chromatogram of the standards.

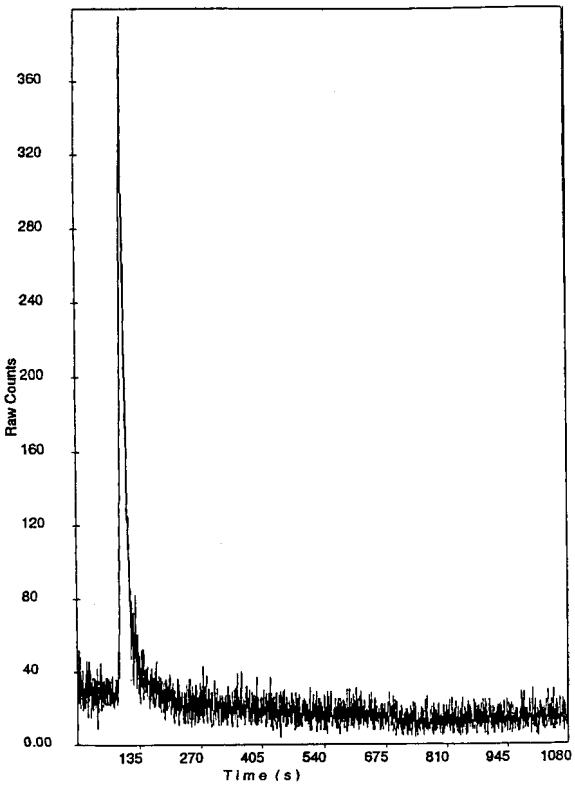


**Figure 2** A chromatogram of a plasma sample.

The limit of detection and precision of the technique were estimated by repeat analysis ($n = 7$) of a region where there were no peaks. The concentration equivalent to three times the standard deviation ($3_{\sigma_{n-1}}$) of the results was taken to be the limit of detection.

A mixed standard ($300\ \mathrm{ng\ ml^{-1}}$) of arsenobetaine, dimethylarsinic acid, monomethylarsonic acid and arsenate was prepared in water and covered to prevent photolysis. Unfortunately, there are no certified reference blood materials that may be used to validate the speciation method. As a truly independent technique is also unavailable, the method had to be validated using 'spike' recovery experiments. Samples of blood were taken from healthy volunteers and split into two sub-samples, of which one was treated in the normal manner, and the other was spiked with arsenobetaine and dimethylarsinic acid to a concentration of $300\ \mathrm{ng\ ml^{-1}}$ of each species, left overnight and then treated as described.

## RESULTS AND DISCUSSION

A typical chromatogram of the standards and of a plasma sample are shown in Figs 1 and 2 respectively. Peak A is arsenobetaine, peak B is dimethylarsinic acid, peak C is monomethylarsonic acid and peak D is the arsenate. From Fig. 1 it is clear that the four main species were resolved and hence would not interfere with each other. It can be seen that although the retention time of arsenobetaine was similar for both the standard and the plasma sample, the peak shape was slightly different. This was because of the relative different ionic strengths of the sample and the standard; therefore peak area measurements were preferred for accurate quantification.

The results of the spiking experiments indicated that recoveries of $96 \pm 3\%$ for arsenobetaine and $94 \pm 5\%$ for dimethylarsinic acid were obtained. The limit of detection ($3_{\sigma_{n-1}}$) for seven replicate analyses based on an injection volume of 175 µl

**Table 1** Summary of patient details and the effect of haemodialysis (HD) on plasma arsenobetaine concentration in 31 patients on regular haemodialysis

| | | | Arsenobetaine concentration (ng As $ml^{-1}$) | |
|---|---|---|---|---|
| | Duration of HD (months) | Haematocrit PCV (%) | Pre-dialysis | Post-dialysis |
| Mean | 9.7 | 27.1 | 57.3 | 14.5 |
| $\pm 1\sigma$ | ±30 | ±6.0 | ±78.8 | ±22.4 |
| ±S em | ±5.5 | ±1.1 | ±14.0 | ±5.5 |
| Range | 4–119 | 18.0–43.6 | <2.5–298 | <2.5–56 |
| Median | 17 | 25.9 | 42 | <2.5 |

was estimated to be 0.27 ng of arsenic as arsenobetaine (1.54 ng $ml^{-1}$).

Arsenobetaine appeared in most of the predialysis samples and in some of the postdialysis samples, but dimethylarsinic acid was not detected in any of the plasma samples analysed. Similarly, the monomethylarsonic acid and the arsenate were at levels below the limits of detection for plasma samples both pre- and post-dialysis. The amount of arsenic present would have been dependent on what the patient had eaten in the previous 12 h, especially if this had included fish or some other meat (e.g. bacon or chicken) from an animal or bird that had previously been fed on fishmeal. Arsenobetaine was not detected in the blood of some patients either pre- or post-dialysis. Unfortunately, no precise dietary records were available.

The results are summarized in Table 1, from which it can be seen that dialysis was quite efficient at removing arsenobetaine from blood and afterwards levels were generally similar to those in healthy individuals (mean concentration $\pm 1\sigma = 39.7 \pm 53$). For many patients, the arsenobetaine concentration dropped by 35–50 ng $ml^{-1}$ to below the limit of detection post-dialysis. The mean concentration of the arsenobetaine pre- and postdialysis was 57.3 ng $ml^{-1}$ and 14.5 ng $ml^{-1}$ respectively. It can be seen from Table 1 that not all of the patients had high levels of arsenobetaine. The concentration of arsenobetaine ranged from below the limit of detection (<2.5 ng $ml^{-1}$) to exceptionally high levels (298 ng $ml^{-1}$). Presumably the patients who had extremely low levels had not recently ingested an arsenic-containing foodstuff such as seafood, whereas the few patients who had high levels had done so. In a few cases, there was evidence of incomplete removal of arsenic, where the concentration before dialysis was as high as 170–200 ng $ml^{-1}$, but the level after dialysis had decreased to 40–60 ng $ml^{-1}$. The majority of patients, however, showed complete removal of the arsenic species; in one exceptional case, the concentration dropped from 298 ng $ml^{-1}$ to below the limit of detection. There were a few (three out of 31) patients whose blood samples yielded anomalous results, where the apparent arsenobetaine level increased marginally after haemodialysis. There is no ready explanation for this, although it must be stated that the concentrations involved were very close to the detection limit and the arsenobetaine concentrations pre- and post-haemodialysis were very similar. The significance of the increase must therefore be questioned. Also, the small sample volume obtained precluded the analysis of more than one replicate.

Comparison with results found in a previous study[14] indicated that the concentrations of arsenic species found were similar, and that they were very low. The exception to this was arsenobetaine, which, for a few patients, was approximately 10-fold higher in this study. This may be attributable to the use of different samples (i.e. plasma in this study instead of serum), or more probably, to differences in the diets of patients. In our study, no dietary constraints were imposed on patients.

Although a group of healthy control individuals did not have their blood analysed, it must be assumed that arsenic species were present, especially for those who had recently consumed a fish-based meal, because some species, e.g. arsenobetaine, pass into the urine unaffected within a period of 12–24 h.[3]

## CONCLUSIONS

Using high-performance liquid chromatography coupled with inductively coupled plasma–mass spectrometry, organoarsenic species have been determined successfully in blood plasma of patients undergoing renal dialysis. In the absence of

 

certified reference materials or a suitable alternative technique, the experimental results were validated using spiking experiments. Recovery was found to be close to 100%. Haemodialysis was efficient at removing the arsenobetaine, the only species detected in the plasma, and there was no evidence of an accumulation of this or other arsenic species in the plasma of patients on regular haemodialysis, indicating that biotransformation into more toxic species was not occurring.

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