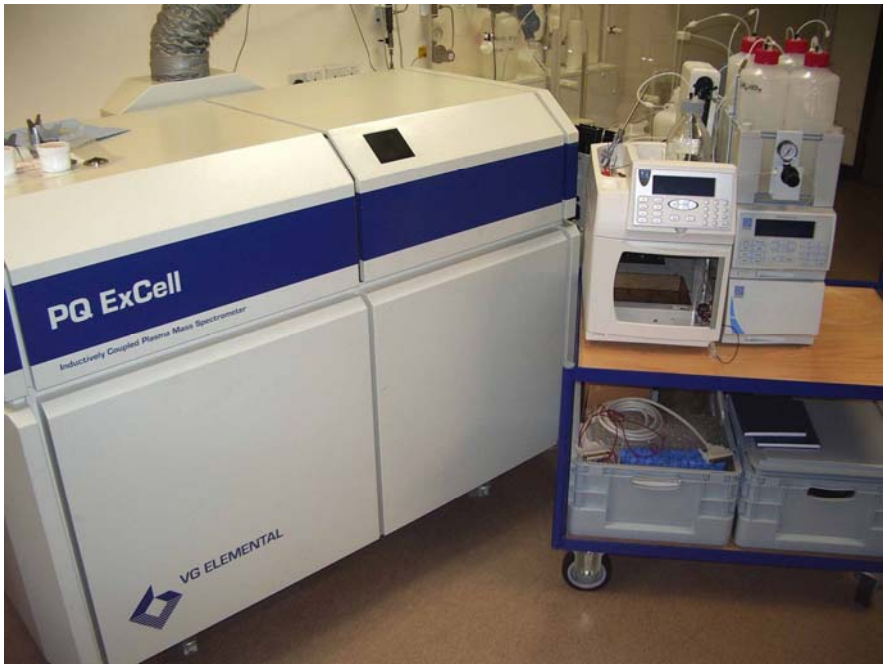
Measurement of Arsenic Compounds in Water by HPLC-ICP-MS

Laboratory Operations

Open Report OR/07/021



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Measurement of Arsenic Compounds in Water by HPLC-ICP-MS

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Summary

This report provides a brief introduction to the application of high-pressure liquid chromatography coupled to inductively coupled plasma mass spectrometry (HPLC-ICP-MS) to determine the concentration of various arsenic species in water samples. It describes the validation of this arsenic speciation method developed under the Laboratory Maintenance and Development Capability Programme to provide data on arsenic speciation in a range of challenging sample matrices to support BGS science projects and university collaborative research projects.

The validation was carried out in two stages. Firstly, the chromatographic conditions for separating five arsenic species and coupling of the HPLC to the ICP-MS instrument for subsequent measurement of arsenic at mass 75 were optimised, together with establishing the stability of standards and reagents. Once the methodology had been optimised, validation data were obtained based on the method of Cheeseman and Wilson (Cheeseman & Wilson, 1989).

The measurement of arsenobetaine (AB) and dimethylarsinous acid (DMA) by the proposed methodology provided good performance data with respect to certified values for the certified reference material NIES CRM 18 human urine. Performance data obtained for spiked low and high TDS solutions was generally within acceptance criteria outlined in the validation plan (Appendix 1) for all of the arsenic species.

1 Introduction

The abundance of evidence for the toxicological effects of elevated concentrations of arsenic in drinking water and the improvements in the ability to measure arsenic quantitatively, led to the reduction in the recommended and regulatory limits of many national and international authorities. The World Health Organisation (WHO) guideline value for arsenic in drinking water was reduced in 1993 from 50 to 10 $\mu g\ L^{-1}$ (WHO, 1993). The EC maximum contaminant level (MCL) in drinking water has been reduced to 10 $\mu g\ L^{-1}$. The Japanese limit for drinking water is also 10 $\mu g\ L^{-1}$, whilst the Canadian MCL is 25 $\mu g\ L^{-1}$ (Smedley and Kinniburgh, 2002). In 2001, the US EPA limit was reduced from 50 to 10 $\mu g\ L^{-1}$, to be enforced by 2006 (EPA, 2004).

Knowledge of the speciation of arsenic in natural water is important since its toxicity, mobility and bioavailability is dependent upon its chemical form. Arsenate [$As^{(V)}$] is the major arsenic species present in surface water, whilst arsenite [$As^{(III)}$], monomethylarsononous acid (MA), dimethylarsinous acid (DMA) and arsenobetaine (AB) have been frequently detected at lower concentrations in surface and ground waters. Overall, approximately 20 to 30 arsenic compounds have been identified to date, many of which are the result of metabolic processes, plant uptake and microbial conversion. This report focuses on the five most common arsenic species found in surface waters, since all of these can be measured using one chromatographic set-up, rather than two or three chromatographic approaches that would be required to provide a complete range of arsenic compounds, many of which may be present at levels below detection in any case. The severe health implications of high arsenic intake reported in the West Bengal, Bangladesh, Taiwan and Inner Mongolia (Le, 2004) were mainly attributed to the high levels of inorganic arsenic in well water. Owing to the differences in toxicity between arsenic species, a knowledge of the speciation of arsenic is essential in improving the understanding of health implications and design processes to reduce the levels of arsenic present in drinking water.

The quantification of trace element species is a difficult task since they are often present at low concentrations relative to the detection limits of most analytical instrumentation. A number of methods have been employed and reviewed in the scientific literature, including spectroscopy, chromatography and electrochemical methods (Jain, 2000; Franscesconi, 2000; Szpunar, 1999 and 2000). ICP-MS is often a favoured method for the determination of arsenic because of its sensitivity and ability to couple to HPLC for the separation and measurement of arsenic species at low concentrations (Feldmann, 1999; Gong, 2002; Hamon, 2004; Polya, 2003; Sathrugnan, 2004; Xie, 2002).

This report provides an overview of recent efforts at BGS to set up and validate the operation of HPLC-ICP-MS instrumentation for in-house measurements of the most common arsenic species present in environmental water samples. The objective of this validation process was to demonstrate the robustness of the method and obtain figures of merit for its routine operation.

2. Experimental

The arsenic species arsenobetaine (AB), monomethylarsonic acid (MA), dimethylarsinous acid (DMA), As^{3+} and As^{5+} were determined by coupling a Dionex HPLC unit to a ThermoElemental ICP-MS instrument. The two instruments were coupled so that communication between them enabled automated injection and analysis for up to 50 separate solutions. This approach vastly improved sample throughput compared to manual operation (between 10 and 40 samples per day), reduced staff time required to perform the measurements and enhanced the reproducibility of sample injections. This development in itself was a huge achievement considering the lack of technical knowledge from the instrument manufacturers on how to achieve full automation of the coupled instruments.

Following the injection of a 100 µl sample solution onto the HPLC column, chromatographic separation was achieved through anion exchange and a modification of the chromatographic conditions described by Martinez-Bravo *et al*. (2001). The ICP-MS instrument was used as a sensitive detector operated in continuous scan mode for monitoring the arsenic (^{75}As) signal in the eluant from the HPLC unit. The continuous signal resulted in the production of a chromatogram in the ICP-MS software, illustrating the elution and separation of the arsenic species over time (Figure 1). Owing to the deficiencies of the Plasmalab software in determining peak areas accurately, raw data (as time slices) were transferred to a specialist chromatography package for the accurate determination of peak areas, with subsequent calculation of sample concentrations from known standards in Microsoft Excel, i.e. calibration graphs. Performance checks were included throughout each analytical run by the inclusion of a calibration standard at 10 µg L^{-1}, to monitor any change in sensitivity, and a quality control solution (NIES CRM-18 Human Urine at 5-fold dilution, only certified for AB and DMA (Nakazato *et al*., 2000; Sloth *et al*. 2004; Yoshinaga *et al*., 2000 and 2004) after every 10 samples.

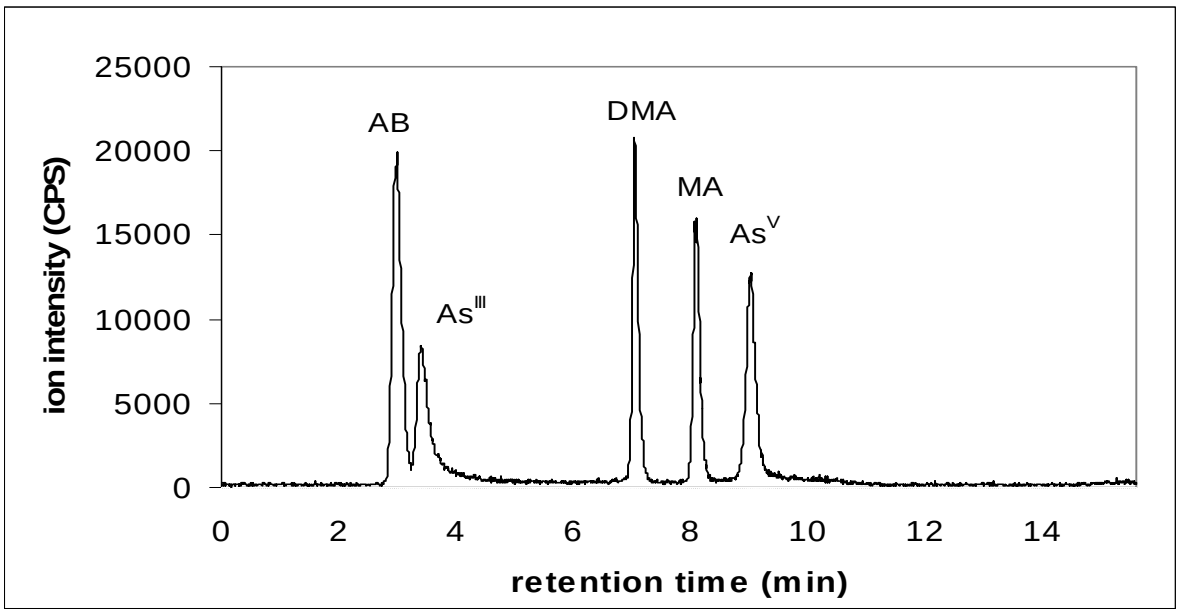

Figure 1. Separation of five arsenic species of interest by instrumental conditions outlined.

2.1 REAGENTS AND EQUIPMENT

Freeze Dried NIES CRM-18 Human Urine, National Institute for Environmental Studies, Ibaraki, Japan (Manufacturer), LGC, Teddington, UK (Supplier).

Arsenobetaine (BCR-626) standard solution, 1000 mg L^{-1}, LGC, Teddington, UK.

Arsenate (As^{5+}), Fisher Scientific Ltd, Loughborough, UK. (1000 mg L^{-1})

Arsenite (As^{3+}), Fisher Scientific Ltd, Loughborough, UK. (1000 mg L^{-1})

Monomethylarsonic acid (MA), Cacodylic Acid, Sigma-Aldrich Company Ltd, Dorset, UK. (solid)

Dimethylarsonous acid (DMA), Disodium-methyl arsenate, Greyhound Chromatography, Birkenhead, UK. (solid)

Ammonium nitrate, Sigma-Aldrich Company Ltd, Dorset, UK.

Fe, Mn, Ca, PO_4, SO_4, Na and Cl single element standards, Sigma-Aldrich Company Ltd, Dorset, UK. (1000 mg L^{-1})

Deionised water <18MΩcm^{-1}, Millipore Element A10, Millipore UK, Watford, UK.

Table 1. Summary of instrument parameters for coupled HPLC-ICP-MS

ThermoElemental PQ ExCell ICP-MS, ThermoElemental UK	
Mass monitored	As –75
Monitoring mode	TRA – continuous scan
ICP-MS software	Plasmalab 1.06.007
Manipulative software	PeakFit V4.02
Dionex Ion Chromatograph, Dionex UK Ltd, Camberley	
Injection volume	100 µl
Mobile phase	Gradient: 4 mM / 60 mM ammonium nitrate, pH 8.7
Column (anion exchange)	Hamilton PRP X-100 250 mm x 4.6 mm, 10 µm
Flow rate	1 ml/min

2.2 STABILITY OF STANDARDS AND REAGENTS

Owing to the inherent instability of some arsenic species, in particular the inorganic species which are subject to redox changes, the stock standard solutions (1000 mg L^{-1}) purchased directly from commercial suppliers, the diluted intermediate standards at 1 mg L^{-1} prepared on a weekly basis and the calibration standards prepared on a daily basis were monitored for their stability in deionised water over a period of 6 months. In addition, the stability of NIES CRM-18 Human Urine was monitored. Initially, the NIES CRM-18 human urine was supplied in a freeze-dried form, requiring reconstitution in deionised water, as per the accompanying instructions. The manufacturer assumed (NIES) that the arsenic species in this solution should be stable when stored in the dark at 4ºC for at least one month based on measurements of AB over two years and one year for DMA (Yoshinaga *et al*., 2000). The supplier also quoted the findings of Feldmann *et al*. (1999) in that As^{3+}, As^{5+}, MA, DMA and AB concentrations in urine samples were stable for up to 2 months when stored at 4 to 20ºC. In practice, the reference

material was supplied in two bottles and reconstituted separately as required. The solution was found to be stable for up to 8 weeks, by which time it was generally fully consumed.

Stock standard solutions ranging from 10 to 50 mg L^{-1}, commercially available as solutions or diluted from solid in deionised water, were found to be stable after 12 months of storage in the dark at 4ºC. Intermediate standard solutions at 1 mg L^{-1} were found to be stable for at least 3 months, beyond which their stability was questionable. The As^{3+} intermediate standard solution was the exception and was found to be unstable when diluted in deionised water. The intermediate standard for As^{3+} was prepared in 2% HCl, the same medium as the stock solution, which prevented transformation to As^{5+}. Daily standards for As^{3+} still required dilution in deionised water to avoid chromatographic changes in the retention time. All stock and intermediate standard solutions were only stable for the periods stated if stored in the dark at 4ºC. Intermediate standard solutions could not be removed from the fridge for long periods of time without deterioration.

The NH_4NO_3 mobile phase was prepared as a stock solution at 1 M concentration. This solution was found to be unstable after 2 to 4 weeks, resulting in changes to the chromatographic separations. Consequently, the stock solution was kept for a maximum of 2 weeks and subsequently stored in the dark at 4ºC. Eluents of 4 and 60 mM NH_4NO_3 (pH 8.7) were prepared for each analytical run from the stock solution.

2.3 STABILITY OF ARSENIC SPECIES IN SAMPLE SOLUTIONS

The preservation of samples is the most problematical step in the determination of arsenic species. Events such as changes in the oxidation state, changes induced by microbial activity or losses by volatilisation or adsorption should be avoided. Aqueous samples intended for total arsenic measurements are not subject to losses during storage when filtered and acidified on collection. Gomez-Ariza *et al*. (2000) reviewed methods for preserving the integrity of arsenic species, including freezing, cooling, acidification, sterilisation, de-aeration, addition of ascorbic acid and storage in the dark. However, there is no general agreement on these procedures and reports often conflict. For example, Hall *et al*. (1999) suggested preservation with weak HCl, which McCleskey *et al*. (2004) questioned strongly. Huang and Ilgen (2004) recommended EDTA, although the present authors have experienced problems with poor chromatographic performance resulting from the use of EDTA. Preservation by adsorption onto ion exchange cartridges was applied to the inorganic species by Ficklin (1983) and Miller *et al*. (2000), and successfully extended to include DMA and MA by O'Reilly (2005). The issue of preserving the integrity of samples with respect to arsenic speciation is clearly critical, but still requires further research outside of the scope of this report. For now, the most practical approach is to store sample solutions in the dark at 4ºC, following filtration (0.45µm) and no addition of a preservative.

2.4 TEST SAMPLES

Water samples were collected from Devon Great Consols on the Tavistock Estate in Southwest England. The site was a former arsenic mine and, through previous BGS surveys (BGS, 2005), the local surface water was known to contain high total arsenic concentrations (50 to 10,000 µg L^{-1}). The samples were generally acid mine drainage waters with pHs around pH 4, well below those of most natural waters. They also contained other elements, such as Fe at >500 mg L^{-1}, that can influence the distribution of arsenic species, via redox changes or precipitation of arsenic from solution (Daus *et al*., 2002; Bednar *et al*., 2005). Total arsenic concentrations in these test samples were found to be between 20 and 500 µg L^{-1}. Arsenic was generally present as As^{5+}, which is in agreement with the findings of Williams (2001). Traces of DMA and As^{3+} (<5 µg L^{-1}) were also present in these samples, but often near to or below the limit of detection.

Owing to the instability of the mine drainage waters collected, synthetic solutions containing major components, *e.g*. Fe, Mn, Cl, Na, at similar concentrations to those found in the test samples were prepared to assess the influence that such samples may have on chromatographic separations of arsenic species (Appendix 1). Matrix components were selected on the basis that they were the main factors reported to influence arsenic speciation measurements. As suggested by Thompson *et al*. (2002), it was not practicable to account for all potential factors that may affect speciation measurements.

The validation of methodologies, required the testing of the analytical procedure for each type of sample matrix where it will be applied (Gonzalez, 2007). In this case the matrix was water, but with different pH and TDS content. Therefore, two synthetic solutions were prepared, one with a pH adjusted to 4.0 to simulate mine acid drainage waters and the second solution was adjusted to pH 7.5 to simulate a more common water type with near neutral pH. Water samples were also collected from a stream adjacent to the BGS Keyworth site, where water samples contained <5 $\mu g\ L^{-1}$ total arsenic (pH: 7, Fe <40 mgL^{-1}), with the arsenic present mainly as As^{5+} or As^{3+}. The Keyworth water samples were spiked as for the synthetic solutions prior to analysis. Two standard solutions containing 5 and 40 $\mu g\ L^{-1}$ of each arsenic species were also analysed with each analytical run. These standards represented measurements at 20 and 80% of the top calibration standard, as required by the Cheeseman and Wilson (Cheeseman & Wilson, 1989) validation procedure, to demonstrate the validity of the method over the whole calibration range. The accuracy and precision of the method were calculated from data obtained from a number of analytical runs, together with an assessment of systematic and random errors from consideration of the within and between run precision.

3 Methodology

The proposed plan of work to validate the measurement of arsenic species by HPLC-ICP-MS is given Appendix 1. Instructions on the operation of the HPLC-ICP-MS instrumentation and an example of the HPLC Method Run Sheet are contained in Appendices 2 and 3, respectively. Details of the preparation of standard solutions for each of the arsenic species is outlined in Appendix 4.

Initially the stability of standards and reagents was investigated to explore the shelf life of these solutions, with an intention of recommending expiry dates (section 2.2). The test solutions were characterised for their total elemental content by ICP-MS and ICP-AES, and arsenic species by HPLC-ICP-MS, prior to commencing the spike tests. This was necessary to ensure that the matrix components were as stated in the validation plan and to calculate the spike recoveries accurately.

Each validation run contained a calibration block containing multi-arsenic species standards (2, 10 and 50 $\mu g\ L^{-1}$) at the beginning and end of the run. Drift correction standards containing all five arsenic species at 10 $\mu g\ L^{-1}$ were inserted after every ten sample solutions, along with a quality control solution (NIES CRM-18). Low and high matrix solutions were analysed, followed by spiked tests for each of these solutions. In addition, standard solutions at 20 and 80% of the concentration of the top calibration standard (5 and 40 $\mu g\ L^{-1}$) were measured. All of these solutions were analysed in duplicate on each occasion. Nine analytical runs were performed by three operators, with shutdown in between, requiring separate tuning and set-up of the instrumentation.

The limits of detection for each arsenic species were calculated by extrapolation from the 2 $\mu g\ L^{-1}$ calibration standard and the baseline signal. This approach was taken because of the presence of random analyte peaks in the blank chromatograms. Concentrations of each analyte were measured in a total of 26 blank solutions over nine analytical runs, which is much greater than the minimum of 10 blanks suggested by Gonzalez *et al*. (2007). The limit of detection for each

analyte was calculated as three times the standard deviation (SD) of the blank measurements (3 x SD). The limits of detection determined in solution were: As^{3+}: 0.8 µg L^{-1}, As^{5+}: 1.5 µg L^{-1}, MA: 0.7 µg L^{-1}, DMA: 0.3 µg L^{-1}, AB: 1.3 µg L^{-1}.

4 Results and Discussion

4.1 NIES CRM-18 HUMAN URINE

As in other fields of measurement, arsenic speciation requires suitable reference materials to be available to verify accuracy and to meet quality assurance needs. The high degree of difficulty of this type of analysis is illustrated by the number of tests needed to certify arsenic species in reference materials, in that results must be obtained by three independent methods for certification as a reference material. NIES CRM-18 human urine was initially tested by six laboratories, using nine approaches (Shibata *et al*., 1989, Chatterjee *et al*., 1999, Hanoaka *et al*., 2001, Sakai *et al*., 2001) to chromatographic separation, with ICP-MS as the means of detection. NIES CRM-18 was only certified for AB and DMA, with reports of other peaks present in the scientific literature (Sloth, 2004). Table 2 summarises the certified values for AB and DMA in NIES CRM-18 and the concentrations measured during validation. In total, nine analytical runs were completed and 21 separate measurements of the reference material were made. The mean measured concentrations of 67 ± 7 µg L^{-1} AB and 36 ± 4 µg L^{-1} for DMA compared favourably to the certified concentrations of 69 ± 12 µg L^{-1} and 36 ± 9 µg L^{-1}, respectively, providing a degree of confidence in the data obtained overall.

Table 2: Results for NIES CRM-18 human urine (9 analytical runs, 21 separate measurements) in µg L^{-1}.

	AB	DMA
NIES Certified value	69	36
NIES Certified SD	12	9
Mean measured	**67**	**36**
Measured SD	7	4
Precision (%RSD)	11	11

4.2 PERCENTAGE BIAS ON HIGH AND LOW STANDARDS

Multi-species standards at 5 and 40 µg L^{-1} (20 and 80% of the top calibration standard) were analysed in duplicate within each analytical run. Tables 3 and 4 relate to performance data for the 5 and 40 µg L^{-1} standards, respectively. Table 3 shows that the mean measurements for AB, As^{3+}, DMA and MA were within the specified target value of ± 15% (Appendix 1), whereas the bias was up to 20% below the target value for As^{5+}. Mean measurements for all of the 40 µg L^{-1} multi-species solution were comfortably within ± 15% of the target value, although As^{5+} did follow a general negative trend for measurements. Initially, it was considered that the As^{5+} was undergoing transformation via redox changes to As^{3+}. However, As^{3+} remained consistently close to the target value. This may point to a requirement for wider tolerances at lower concentrations, such as ± 20% at < 10µg L^{-1} for As^{5+} measurements until the problem can be resolved.

Table 3. 5 µg L^{-1} standard measured in duplicate in each of nine analytical runs.

	AB	As^{3+}	DMA	MA	As^{5+}
Target value (µg L^{-1})	5.0	5.0	5.0	5.0	5.0
Mean measured (µg L^{-1})	**5.1**	**5.0**	**4.7**	**4.8**	**4.2**
Measured SD	1.0	0.3	0.3	1.0	1.0
Precision (%RSD)	10.0	7.0	6.5	11.7	12.1
Bias (%)	2.5	0.1	-5.1	-4.4	-16.7

Table 4: 40 µg L^{-1} standard measured in duplicate in each of nine analytical runs.

	AB	As^{3+}	DMA	MA	As^{5+}
Target value (µgL^{-1})	40	40	40	40	40
Mean measured (µgL^{-1})	**40.5**	**38.8**	**40.1**	**40.1**	**35.3**
Measured SD	3.2	3.3	2.9	4.2	2.7
Precision (%RSD)	7.7	8.4	7.2	10.4	7.7
Bias (%)	2.7	-2.9	0.2	0.3	-11.6

4.3 SPIKE RECOVERY FROM LOW AND HIGH TDS SOLUTIONS

Table 5 summarises spike recoveries for all five arsenic species in a low TDS water. Accuracies were generally very good (within ± 15%) for each of the species and precision was less than 10%.

Table 5. Keyworth (low TDS) streamwater spiked with 20 µg L^{-1} of each analyte and measured in duplicate in each of the nine analytical runs.

	AB	As^{3+}	DMA	MA	As^{5+}
Target value (µg L^{-1})	20	20	20	20	20
Measured mean (µg L^{-1})	**19.8**	**20.7**	**21.5**	**22.1**	**22.1**
Measured SD	1.4	1.3	1.7	1.3	2.2
Precision (%RSD)	7.3	6.3	7.8	6.1	10.2
Bias (%)	-1.0	3.4	7.4	10.6	10.4

Tables 6 and 7 summarise measurements of high TDS synthetic solutions prepared at pHs of 4 and 7.5, spiked with each arsenic species and analysed in duplicate in each analytical run. Accuracies for each arsenic species were within the ± 15% target for the high TDS solution at pH 4, although again As^{5+} provided a slightly lower response compared to the other arsenic species. The precision of the measurements for each of the arsenic species in this matrix were generally good at <10% (Table 6). Table 7 summarises spike data for the high TDS solution at pH 7.5, for which accuracies were less than ± 15% for all species with the exception of As^{5+}, which had a bias of 20%.

Table 6: pH 4 high TDS synthetic water, spiked with 20 µg L^{-1} of each analyte and measured in duplicate in each of the nine analytical runs.

	AB	As^{3+}	DMA	MA	As^{5+}
Target value (µg L^{-1})	20	20	20	20	20
Mean measured (µg L^{-1})	**20.9**	**19.3**	**20.6**	**19.9**	**17.5**
Measured SD	1.8	1.9	0.9	1.4	1.6
Precision (%RSD)	8.4	9.7	4.3	7.0	9.3
Bias (%)	4.4	-3.3	2.8	-0.3	-12.7

Table 7: pH 7.5 high TDS synthetic water, spiked with 20 µg L^{-1} of each analyte and measured in duplicate for each of the nine analytical runs.

	AB	As^{3+}	DMA	MA	As^{5+}
Target value (µg L^{-1})	20	20	20	20	20
Mean measured (µg L^{-1})	**20.9**	**20.0**	**20.7**	**20.3**	**15.7**
Measured SD	2.2	2.0	1.5	2.0	1.8
Precision (%RSD)	10.3	10.2	7.1	9.8	11.3
Bias (%)	4.4	0.1	3.4	1.7	-21.3

5 Conclusions

The measurement of AB and DMA by the proposed methodology provided comparable performance data with respect to the certified values for NIES CRM 18 human urine. Further tests to provide data for the other arsenic species would be useful when suitable certified reference materials become commercially available. There is a clear need to produce certified reference materials (CRMs) for a wider range of arsenic species to validate methods in general.

There is also a need for analytical procedures to use quality control solutions to evaluate the long-term reproducibility of methods, through the establishment of analytical quality control charts and for intercomparison studies. This is often achieved through the use of non-certified reference materials produced within the trace element speciation community, where producers are generally smaller and not as well advertised as producers of CRMs. Information on non-certified reference materials can be searched in the VIRM (Virtual Institute for Reference Materials) database at www.virm.net (Leermakers et al., 2006). The prohibitive cost of CRMs is also a potential barrier to validating a broad range of sample matrices for arsenic speciation and for continuing to monitor performance, using CRMs.

The data obtained for the spiked low and high TDS solutions was generally within the acceptance criteria outlined in the original validation plan for all of the arsenic species (Appendix 1). Accuracies for all of the arsenic species in each of the spiked matrices were within ± 15% of the target value, except for As^{5+} in the pH 7.5 high TDS water, which was 20% below the target value. The precision of measurements for all spiked matrices, high and low

standards, was within the target of ± 15% and often less than ± 10%. The performance data indicated that the acceptance criteria for each species within the validation plan were generally met, although a wider tolerance of ± 20% is required for measurements of As^{5+} across the concentration range up to 50 µg L^{-1}. The poorer quality of the As^{5+} measurements may be due to the chromatographic separation, with As^{5+} eluting last from the HPLC column and a slight tailing effect on the peak, which may lead into a slight bleeding of the As^{5+} signal along the baseline measurement. The apparent retention of As^{5+} is also evident in the blank measurements, where As^{5+}, although low, is clearly present unlike the other arsenic species. This phenomenon resulted in a slight loss of the As^{5+} signal to the baseline or background signal. The limit of detection for each analyte was calculated as three times the standard deviation (SD) of the blank measurements (3 x SD). The limits of detection determined in solution were: As^{3+}: 0.8 µg L^{-1}, As^{5+}: 1.5 µg L^{-1}, MA: 0.7 µg L^{-1}, DMA: 0.3 µg L^{-1}, AB: 1.3 µg L^{-1}.

In summary, apart from the bias on the As^{5+} measurements, the performance data for each of the arsenic species determined during the validation process met the acceptance criteria outlined in the validation plan. Further work beyond the scope of this exercise is also required to address the commonly recognised problem of stabilising sample solutions to preserve the integrity of the sample from the point of sample collection to the time of analysis.

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Appendices

Appendix 1

HPLC-ICP-MS Validation Plan: Measurement of arsenic species in water samples

1. Background

The purpose of the HPLC-ICP-MS validation plan is to describe the steps in validating the procedure and provide confidence in the robustness and reproducibility of the method.

2. Scope

The HPLC-ICP-MS instrumentation will be used to measure five arsenic species (As^{3+}, As^{5+}, AB, MA, DMA) in aqueous solutions containing high amounts of matrix elements such as Cl, Fe, Mn and Na that may interfere with the accurate determination of arsenic.

3. Test Solutions

Validation data will be acquired for three test solutions representing high and low concentrations of matrix elements encompassing the scope of the method.

(i) Keyworth stream water

(ii) Synthetic solutions containing high concentrations of matrix elements at pH 4.0 and pH 7.5:

Matrix	High Matrix Water (μg l^{-1})	High Matrix Water (μg l^{-1})
Fe	1000	1000
Mn	1000	1000
Ca	1000	1000
PO_4	500	500
SO_4	1000	1000
Na / Cl	1000 / 1500	1000 / 1500
pH	4.0	7.5

All test solutions will be prepared as a 500 ml bulk sample and analysed by ICP-MS to confirm their elemental composition, prior to the spiking experiments. Where the levels in the test solutions permit, the spikes will be added at 20 μg l^{-1} for each arsenic species.

4. Calibration standards

Commercial standard solutions will be used to prepare the calibration standards. The daily calibration standards for all arsenic species will be prepared at 50, 10 and 2 μg l^{-1}plus a blank, in deionised water. Measurements will be made in TRA mode and the calibrations should be linear.

5. Preliminary tests

Before commencing the validation tests, data from a number of analytical batches spread over a six month period will be evaluated for the following:

(i) stability of standards, including stock, intermediate and daily solutions;

(ii) accuracy of measurements for NIES CRM-18 human urine;

(iii) stability of NIES CRM-18 human urine solution in reconstituted form;

(iv) concentrations of matrix elements and arsenic species in Keyworth stream water and synthetic matrix waters.

6. Acceptance Criteria

Initial tests indicate that the following criteria should be achievable:

(i) percentage bias on high and low standards (40 and 5 $\mu g l^{-1}$) <10%;

(ii) precision of high and low standards, spiked and unspiked samples <15%;

(iii) The percentage recovery of all spikes should be between 85 and 115%.

7. Programme of Work

- Assess data compiled over a 6-month period relating to standard and CRM stability.
- Define levels of matrix components.
- Review HPLC-ICP-MS method form.
- Write a draft technical procedure.
- Review and refine methodology at all stages.
- Prepare synthetic high matrix solutions and collect Keyworth stream water (Jan 2007).
- Review validation plan.
- Analyse a set of solutions, consisting of a blank, sample, spiked sample, matrix solution, spiked matrix solution, high standard and low standard, twice in a sufficient number of separate runs to confirm the performance characteristics of the method.
- Compare the results with the acceptance criteria.
- Write internal report on methodology (March).

Dr Michael Watts

21st December 2006

Appendix 2

Technical Operating Procedure for the HPLC- ICP-MS

Mobile Phase- 1M Stock solution NH_4NO_3

Weigh out 4.02 g into a clean beaker

Measure out 500 ml of MQ water into a measuring cylinder.

Pour half of the MQ water into the beaker and swirl to allow the NH_4NO_3 to dissolve.

Empty contents of beaker into the designated Nalgene bottle.

Add remainder of MQ water to designated Nalgene bottle and shake thoroughly until all solid has dissolved.

Record a preparation date on the bottle (shelf life of solution is 2 weeks, based on observation of deteriorating chromatograms). Store in fridge at 4°C.

Mobile Phase- 4 mM and 60 mM

Partially fill designated 1 L volumetric flasks (found in grey box in HPLC trolley) with MQ water

4 mM: Add to designated vol. flask, via auto pipette 4 ml of 1 M Stock solution.

60 mM: Measure out in a measuring cylinder, 60 ml of 1 M and decant to designated vol. flask.

Make vol. flasks up to line with MQ water and shake thoroughly.

Decant into designated 1 L nalgene bottles, for pH adjustment

Mobile Phase- pH adjustment

To pH adjust; you need ammonia solution (this is kept in a sterilin in the grey box on the HPLC trolley, if it runs out there is some more under the fume cupboard in U029, just decant an appropriate amount for use).

4 mM: Usually you need about 90µl to achieve the desired 8.67 ± 0.1.

60 mM: Usually you need about 1000 µl to achieve the desired 8.67 ± 0.1. (These volumes vary slightly but it should not be too different from the above.)

Shake vigorously to encourage complete mixing, when desired pH is achieved, decant solutions to designated HPLC bottles.

*Repeat Mobile Phase procedure for 4 mM, as 2 L is usually needed)

Operation of the HPLC-ICP-MS

Turn on the ICP-MS PC and load up plasmalab software. The ICP-MS should be tuned for the ^{75}As signal and instrument parameters saved in the HPLC Trigger configuration. TRA mode should be selected in Acquisition Parameters to monitor the ^{75}As signal.

Switch on the HPLC pump and autosampler and attach the correct column*.

*Maintenance of HPLC column, guard columns and frits – the column should have 50% MeOH in it; this will need to be flushed out with either MQ or the mobile phase. All columns are stored on the HPLC trolley. Regularly check them for residue and blockages and note any observations in the instrument log book.

Attach the 4 mM mobile phase to the tube from port 2, the left slot labelled A, and open relevant helium valve. Repeat for the 60 mM mobile phase and attach the tube from port 3 to the right slot labelled B and open relevant helium valve.

Degassing – disconnect the helium line and use the tube provided to reconnect to the line, turn on the helium very low! Degas the 4 and 60 mM mobile phase by bubbling helium through it for about a minute each, then seal eluent containers. Helium pressure should be about 4 to 6 psi into the top of the eluent container.

Ensure that there is no air in the system; do this by opening the little black valve where the tube leaves the HPLC pump head to join the sample loader, and then press the prime button. Make sure all of the air bubbles are removed from both eluent lines. This will ensure stable pump pressure during the analytical run. A syringe can also be used to suck air out via the valve located on the HPLC pump head if the Prime function is not sufficient to remove all of the air bubbles in the eluent lines. Also try turning the little black valve where the tube leaves the HPLC to join the sample loader. This will cause the pressure to drop and force the air and some mobile phase down the waste tubing.

Maintenance of pump heads / pistons – on the pump there are two white plastic holes, put the two small syringes containing a few millilitres of MQ into these and push the MQ through each way a few times, before and after every analytical run. This prevents the precipitation of salts onto the glass piston rods and subsequent scratching and leading to unstable pump pressure.

Select mobile phase A (4 mM), now press the on button; this will start running the mobile phase through the system and the column. Run the 4 mM mobile phase through the machine for at least 20 minutes before any run. Also run other eluents to make sure air is removed. Repeat for mobile phase B, the pressure on the HPLC pump should read between 1500- 1700 psi (record in log book) depending on the column used.

Connect the HPLC to the ICP computer via the Advantech trigger board, restart the computer to ensure communication between ICP software and HPLC.

Make sure that the ICP-MS peristaltic pump speed is adjusted in Plasmalab to 50% (varies depending on flow rate, mobile phase and run time).

Once you have completed all MS related tasks such as tuning and stability tests, you are ready to couple the HPLC with PEEK tubing to the ICP-MS nebuliser and allow mobile phase (50% A and 50% B) to run through ICP-MS for at least 20-30 minutes to allow the plasma to stabilise and the sampler / skimmer cones to be coated with the mobile phase.

Set up of sample list on ICP-MS – set the scan time and make sure the scan finishes just before HPLC is ready to inject next sample to avoid communication errors between the Advantech trigger board and the AS-50 HPLC autosampler. The calibration blanks need to be labelled as blank in the column “type” and the standards need to labelled as fully Quant in the same column, all the rest of the blanks and samples should be left as unknown. In the sample list tab, in quantitative standards the expected concentration values need to be added in for example, for the 2 ppb put 2 in the box etc.

Set-up of experiment on HPLC - for the ammonium nitrate mobile phase, make sure the method is selected as method 1 in the right hand column. Method 1 has been set to run a gradient elution over a period of time (it should never be altered as it is the default devised method) but always check that timings are correct before each analytical run. For other mobile phases, timings can be altered to suit specific needs in other methods (e.g. method 2, 3 etc). Also check that the flow rate is correct and that the HPLC is running on 100% 4 mM mobile phase.

Set-up of experimental queue on the AS-50 autosampler – go to menu 4, which is "schedule". You can either edit a previous method or create a new one. Make sure the volume for uptake is correct. The next stage is fairly intuitive by following the on-screen instructions.

The number on the left of the columns is how many samples are being tested; make sure that this is the same as the number of samples in the ICP sample list. Use the HPLC-ICP-MS method run form to compare the sample lists in the AS-50 HPLC autosampler and ICP-MS Plasmalab software.

Ensure the sample list order matches the vial order in the AS-50 HPLC autosampler tray.

Check one last time that all settings are correct on both the HPLC and ICP-MS, once that is done, you are ready to run.

Press run on the auto sampler, and "queue" the experiment in the ICP-MS Plasmalab software. The HPLC AS-50 autosampler will begin flushing the injection needle, uptake the sample, load the sample loop and inject the sample onto the column. The AS-50 autosampler will then trigger the ICP-MS to commence scanning for ^{75}As for the set time period and the gradient pump to begin the gradient program.

Data handling – When the run is completed, all of the raw data (counts per second) are selected and imported to an excel spreadsheet for manipulation in PeakFit software.

Tips

Always wait for the first sample to start analysis, it is the only way to be sure that the communication and timing between the two instruments are synchchronized. If the run needs to be aborted, stop both the HPLC and ICP-MS sample runs and reset communications between the two instruments by restarting the ICP-MS computer.

Ensure you go to acquisition parameters and the calculated defined peaks, to check that there are expected chromatograms, if there are none it could mean, that the sample is not getting to the plasma in the ICP-MS. This is usually technical; you would need to investigate this in order to run an experiment.

Always check for leaks and blockages, especially the column connections.

Appendix 3

HPLC Method run sheet

Date…….

Run Name:…… Analyst:……

Sample Type……Sample Number:……

Calibration Details:

……
……

Comments/Other Information:

……
……
……

Sheet 1 of

SN	Sample Name	P	Type	Dilution Factor	Comments
1	MQ	1	Unknown		
2	Cal Blank 1	2	Blank		
3	2ppb All As -1	3	Fully Quant Standard		
4	10ppb All As -1	4	Fully Quant Standard		
5	50ppb All As –1	5	Fully Quant Standard		
6	Dummy	6	Unknown		
7	CRM18-1	7	Unknown		
8	BLK-1	8	Unknown		
9					
10					
11					
12					
13					
14					
15					
16					
17					
18	BLK-2		Unknown		
19	Cal Blank 2		Blank		
20	10ppb All As -2		Fully Quant Standard		
21	CRM18-2		Unknown		
22	BLK-3		Unknown		
23					

Run Name: ……

Sheet 2 of

SN	Sample No.	AS	Dilution	ED	AD	Comments
24						
25						
26						
27						
28						
29						
30						
31						
32						
33						
34	BLK-4		Unknown			
35	Cal Blank 3		Blank			
36	10ppb All As -3		Fully Quant Standard			
37	CRM18-3		Unknown			
38	BLK-5		Unknown			
39						
40						
41						
42						
43						
44						
45						
46						
47						
48						
49	BLK-6		Unknown			
50	CRM18-4		Blank			
51	Cal Blank 4		Fully Quant Standard			
52	2ppb All As –2		Fully Quant Standard			
53	10ppb All As –4		Fully Quant Standard			
54	50ppb All As –2		Fully Quant Standard			
55	Dummy		Unknown			

Appendix 4

Preparation of Arsenic speciation standards for HPLC-ICP-MS

MA (50 ppm)

Weigh out 0.0243 g.

Place into designated 125 ml nalgene bottle (rinse bottle 3x with MQ before beginning).

Using appropriate measuring cylinder decant 125 ml of MQ water into bottle. Place lid on bottle and shake thoroughly until solid fully dissolved.

Record preparation, expiration details on a label and place on bottle.

Place standard in fridge.

DMA (50 ppm)

Weigh out 0.0115 g.

Place into designated 125 ml nalgene bottle (rinse bottle 3 times with MQ before beginning).

Using appropriate measuring cylinder decant 125 ml of MQ water into bottle. Place lid on bottle and shake thoroughly until solid fully dissolved.

Record preparation, expiration details on a label and place on bottle.

Place standard in fridge.

As^{III} and As^{V} (10 ppm)

Using autopipette, pipette 100 µl of commercial (conc) stock solution into designated 30 ml nalgene bottle (rinse bottle 3 times with MQ before beginning).

Using micropipette, pipette 9.99 ml of MQ water into bottle**. (As ^{III} is made up in 2% HCl)**

Place lid on bottle and shake thoroughly.

Record preparation, expiration details on a label and place on bottle.

Place standard in fridge.

AB (10 ppm)

Using an autopipette, transfer 230 µl of AB commercial (conc) stock into designated 15 ml nalgene bottle (rinse bottle 3 times with MQ before beginning).

Using a micropipette, add 9.77 ml of MQ water.

Place lid on bottle and shake thoroughly.

Record preparation, expiration details on a label and place on bottle.

Place standard in fridge.

1 ppm Intermediate standards

For **MA & DMA** transfer 200 µl of standard and 9.8 ml MQ into an autosampler tube.

For **As ^{III}, As ^{V} & AB** transfer 1ml of standard and 9.0 ml MQ into an autosampler tube.

Place lid on tube and shake thoroughly

2 ppb

Pipette 20 µl from each of the 1 ppm for all 5 standards into an autosampler tube.

Then pipette 9.0 ml of MQ into the same tube.

Place the lid on the tube and shake thoroughly.

Decant solution to an HPLC autosampler vial (clearly labelled).

10 ppb

Pipette 1000 µl from each of the 1 ppm for all 5 standards into an autosampler tube.

Then pipette 5 ml of MQ into the same tube.

Place the lid on the tube and shake thoroughly.

Decant solution to an HPLC autosampler vial (clearly labelled).

50 ppb

Pipette 500 µl from the each of the 1 ppm for all 5 standards into an autosampler tube.

Then pipette 7.5 ml of MQ into the same tube.

Place the lid on the tube and shake thoroughly.

Decant solution to an HPLC autosampler vial (clearly labelled).

CRM-18 x 5 DILUTION

Pipette 1 ml of reconstituted freeze dried human urine reference material into auto sampler vial.

Pipette 4 ml of MQ into the same auto sampler vial.

Place lid on vial and shake thoroughly and label.