

Determination of Arsenic Species in Urine Using HPLC Coupled with X Series ICP-MS

Key Words

- Arsenic
- HPLC
- ICP-MS
- Speciation
- Transient TRA

Introduction

Arsenic is a ubiquitous element which enters the environment through both natural and anthropogenic sources. Natural sources of arsenic can result from volcanic activity, weathering of rocks rich in arsenopyrite and soil run-off processes. However, anthropogenic sources may also include mining, smelting, burning of fossil fuels and the use of arsenic compounds in fertilizers and animal feeds.

A variety of organic and inorganic arsenic species have currently been identified in environmental and biological samples and the element is known to exhibit oxidation states of 0, +3 and +5. The importance of arsenic speciation is being increasingly recognized by the scientific community because intrinsic arsenic species are known to exhibit differences in mobility, bioavailability and toxicity.

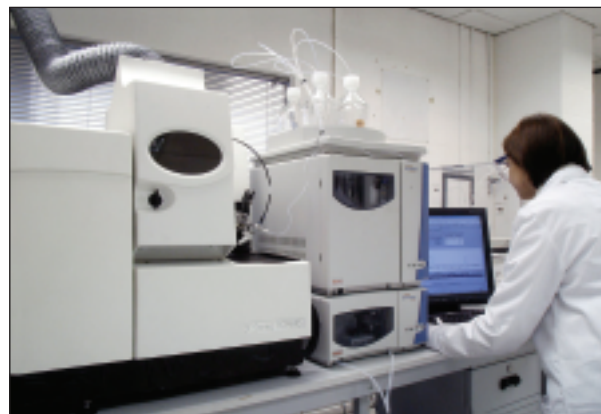
Major sources of human exposure to arsenic result through the consumption of selected foods and drinking water. For example, marine organisms are known to bioaccumulate arsenic up to 200 mg kg⁻¹ from food sources and surrounding waters. These arsenic concentrations are bio-magnified as they are transferred through the food chain and high concentrations of arsenic can be found in selected seafoods which are eaten as part of the human diet. Seafoods are suggested to provide the major source of arsenic in the human diet, despite the fact that only small quantities of seafood are consumed relative to other food groups.

The metabolism of accumulated arsenic in seafood and macroalgae is known to yield arsenobetaine (AB) and arsenosugars (AS) as the predominant species. The AB species is known to be excreted in the urine and remains unchanged from its point of formation. However, arsenosugars are suggested to decompose to dimethylarsinic acid (DMA) species prior to excretion in the urine and bio-methylation pathways for inorganic arsenicals have also been confirmed in humans following the identification of monomethylarsonic acid (MMA) and DMA metabolites in the urine. AB species found in seafoods are known to exhibit relatively non-toxic characteristics and methylated species such as MMA and DMA exhibit intermediate human toxicity. The inorganic arsenic species such as arsenite (As³⁺) and arsenate (As⁵⁺) generally exhibit greater toxicity than organo-arsenic species (e.g. As³⁺ is a known carcinogen) and these inorganic species are found more commonly in waters. Notably high concentrations of arsenic (up to 2000 mg L⁻¹)

have been found in drinking waters in developing countries such as Argentina, Bangladesh and India and significant concerns have been raised with regards to the potential for human toxicity from the inorganic As species resident in these waters.

The most popular techniques used for arsenic speciation applications include HPLC-HG-AAS or HPLC-ICP-MS. However, HPLC-ICP-MS is often the preferred analytical technique for these applications due to the simplicity of the coupling between the HPLC and ICP-MS, potential for on-line separations with high species specificity and the capability for optimum limits of detection without the necessity for use of complex hydride generation mechanisms.

This application note describes the use of an HPLC-ICP-MS instrument package from Thermo Electron Corporation to enable quantitative analysis of arsenic speciation in human urine samples. Urine samples were collected from three individuals with different dietary habits to enable comparison of the associated arsenic speciation. The associated methodology is evaluated in the context of method detection limits and spike recoveries for As species in each of the urine sample matrices.



Instrument Configuration

The chromatographic mobile phase was degassed and pumped through the analytical column to the ICP-MS using a Finnigan™ Surveyor Quaternary Gradient Pump. Liquid samples were automatically injected into the flow of mobile phase using a Finnigan Surveyor autosampler and the species of interest eluted using a gradient elution profile. An X Series ICP-MS detector was used to quantify the elemental species constituents. Use of the HPLC-ICP-MS coupling pack (P/N 4600485) and X Series PlasmaLab software facilitated failsafe automated analysis of the HPLC-ICP-MS instrumentation using bi-directional communications. Further technical information on the coupling of HPLC to X Series ICP-MS is provided in Product Note SN_E0634. A schematic diagram of this coupled instrumentation is presented in Figure 1.

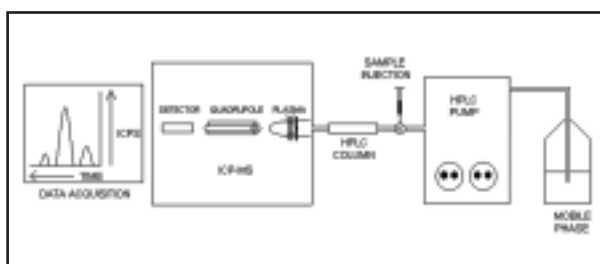


Figure 1: Schematic diagram of the HPLC-ICP-MS instrumentation

Analytical Conditions for HPLC

Mobile Phase Composition	Phase A: 10 mM ammonium carbonate at pH 8.9 Phase B: 20 mM ammonium carbonate at pH 8.9
Gradient Elution Profile	100% A: 0-5.0 minutes 100% A to 100% B: 5.0-6.0 minutes 100% B: 6.0-22.0 minutes 100% B to 100% A: 22.0-23.0 minutes
Mobile Phase Flow Rate	1.0 ml min ⁻¹
Column Dimensions	300 x 4.6 mm id
Stationary Phase Composition	Hamilton PRP X100
Column Temperature	Ambient
Sample Injection Volume	20 µL

Table 1 presents analytical conditions for the HPLC.

Analytical Conditions for ICP-MS

The ICP-MS detector was configured with the unique Thermo Xt interface to promote enhanced matrix tolerance and reduced formation of polyatomic interferences. Liquid sample was pumped to the ICP-MS by the HPLC pump and was introduced to the plasma through a Burgener AriMist nebulizer, Peltier cooled impact bead spray chamber and quartz torch with an injector tube diameter of 1.5 mm, fitted with the PlasmaScreen Plus screened torch option. The spray chamber was drained via the integral peristaltic pump. Table 2 presents operating conditions for the ICP-MS instrumentation.

Nebulizer Gas Flow	0.78 L min ⁻¹
Auxilliary Gas Flow	0.85 L min ⁻¹
Cool Gas Flow	13.0 L min ⁻¹
Forward Plasma Power	1400 W
Data Acquisition Mode	transient Time Resolved Acquisition (TRA)
Monitored Masses	⁷⁵ As, ⁷⁷ ArCl, ⁸² Se, ⁸³ Kr
Dwell Times	(mass specific) 50-150 ms
Timeslice Duration	308 ms
Channels per AMU	1
Run Duration	1800 s

Table 2: ICP-MS operating conditions

Preparation and Analysis of Calibrant and Sample Solutions

Calibrant solutions were prepared daily for AB, As³⁺, MMA, DMA and As⁵⁺ by making appropriate dilutions from a solution of mixed species (1000 ng mL⁻¹) prepared in deionised water. Dilutions were prepared to facilitate arsenic calibrations in the 0-10 ng mL⁻¹ range for each species.

Three human urine samples were collected for analysis using the above described HPLC-ICP-MS methodology. Sample A was collected from an individual who had eaten a seafood meal consisting of Salmon and Prawns within 24 hours of producing the sample. Sample B was collected from an individual who had eaten no fish products within the last 7 days and sample C was collected from a non-fish eater. The urine samples were diluted 1:10 using deionized water and were then injected into the flow of mobile phase for analysis without further preparation. An aliquot of each 1:10 diluted urine sample was also spiked with 10 ng mL⁻¹ of each arsenic species and analysed directly to verify the performance of the HPLC-ICP-MS methodology.

Results and Discussion

The X Series ICP-MS PlasmaLab software suite enables fully automated speciation analyses with flexible data acquisition and integration parameters. The transient TRA data is plotted automatically within the PlasmaLab software to enable the integration of chromatographic peaks and quantification of species in unknown samples. Figure 2 shows an example of the chromatography for a calibration standard containing 1 ng mL⁻¹ of AB, As³⁺, MMA, DMA and As⁵⁺.

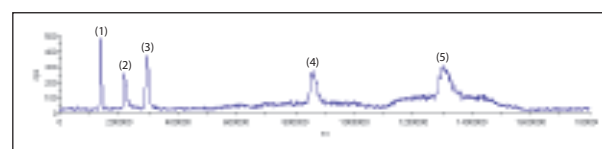


Figure 2: Example calibration standard chromatography (1)AB (2)As³⁺ (3)DMA (4)MMA (5)As⁵⁺

The five arsenic species are baseline resolved in approximately 1350 seconds using the above described methodology and retention times for AB, As³⁺, DMA, MMA and As⁵⁺ are 135, 218, 296, 862 and 1308 seconds respectively. Minimal time delays are achieved between consecutive samples due to the optimized use of bi-directional communications between PlasmaLab and the HPLC autosampler and incorporation of intelligent inject-hold and timed trigger events in the PlasmaLab Accessory Control (ACX) script. This intelligent productivity enhancing feature enables failsafe automated analysis with enhanced sample throughput capability.

Chromatographs derived from the analysis of the three 1:10 diluted urine samples (A, B and C) are shown below in Figures 3, 4 and 5 respectively and example chromatography for sample B spiked with AB, As³⁺, DMA, MMA and As⁵⁺ (10 ng mL⁻¹ as As) is also shown in Figure 6. Chromatographic peaks in each sample are labelled in accordance with peaks observed in the calibration standard chromatography.

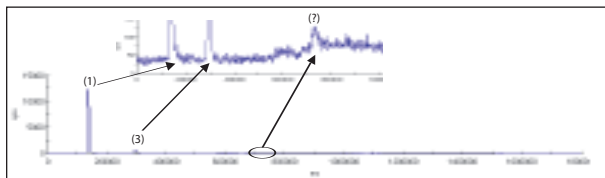


Figure 3: Chromatogram for urine sample A (1:10 diluted)

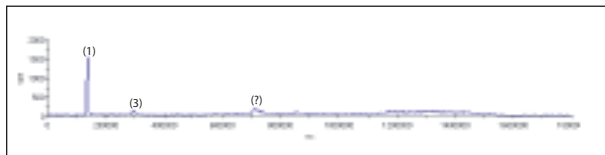


Figure 4: Chromatogram for urine sample B (1:10 diluted)

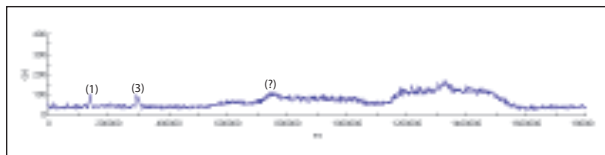


Figure 5: Chromatogram for urine sample C (1:10 diluted)

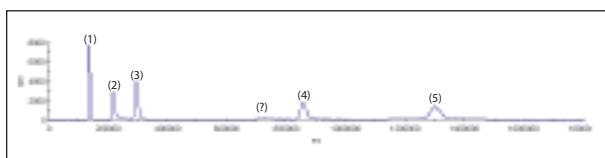


Figure 6: Chromatogram for urine sample B (1:10 diluted) spiked with 10 ng mL⁻¹ AB, As³⁺, DMA, MMA and As⁵⁺ species

Urine samples A, B and C each exhibit peaks which correspond to AB (1) and DMA (3) although the largest peaks were observed for these species in sample A. Retention times for AB, As³⁺, DMA, MMA and As⁵⁺ were found to be identical in both the calibration standard and the urine matrices (e.g. Figures 2 and 6 respectively).

However, an unidentified arsenic species (?) was also observed in each sample and exhibits a retention time of 713 seconds.

Chromatographic peak integration parameters were assigned in the PlasmaLab transient TRA data to enable fully quantitative analysis for AB, As³⁺, DMA, MMA and As⁵⁺ species and the resultant fully quantitative calibrations for these species are below shown in Figures 7-11. The uncharacterized As peak (?) was integrated in each sample and semi-quantitative analysis (Compound Independent Calibration) was then performed in the PlasmaLab software using the mean As sensitivity derived from the fully quantitative calibrations.

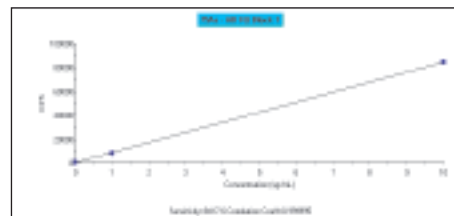


Figure 7: Calibration curve for arsenosugar AB

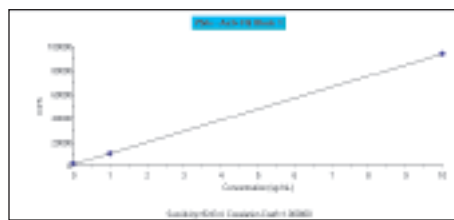


Figure 8: Calibration curve for arsenosugar As³⁺

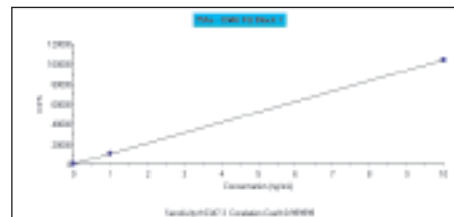


Figure 9: Calibration curve for DMA

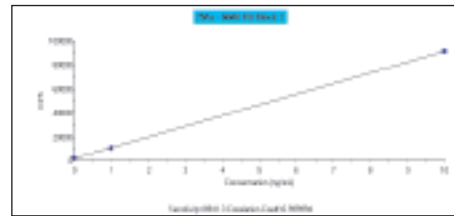


Figure 10: Calibration curve for MMA

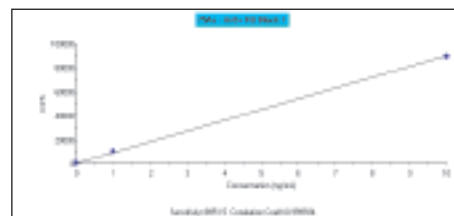


Figure 11: Calibration curve for As⁵⁺

Table 3 presents concentration data (prior to dilution correction) for samples A, B and C both with and without the spike of 10 ng mL⁻¹ AB, As³⁺, DMA, MMA and As⁵⁺ (as As) and Table 4 presents spike recoveries for each sample. The use of mixed unit sets in PlasmaLab allows species concentrations to be reported independently of the measured As concentration and Table 5 presents dilution corrected concentrations for the arsenic species in samples A, B and C.

MEASURED CONCENTRATIONS (NG ML ⁻¹ AS AS)						
Sample	AB	As ³⁺	DMA	MMA	As ⁵⁺	?*
Sample A	25.0	ND	0.940	ND	ND	0.101
Sample A + 10 ng mL ⁻¹ spike	35.9	10.8	11.0	10.2	9.86	NS
Sample B	3.08	ND	0.474	0.420	ND	0.123
Sample B + 10 ng mL ⁻¹ spike	13.3	10.1	10.8	10.1	10.0	NS
Sample C	0.0550	ND	0.209	0.129	ND	0.288
Sample C + 10 ng mL ⁻¹ spike	10.5	10.6	11.0	10.8	9.87	NS

Table 3: Uncorrected concentration data for samples A, B and C

ND: Not Detected NS: Not Spiked

*semi-quantitative data derived from compound independent calibration

SPIKE RECOVERIES (%)					
Sample	AB	As ³⁺	DMA	MMA	As ⁵⁺
Sample A	109	108	100	102	98.6
Sample B	102	101	104	96.3	100
Sample C	104	106	108	107	98.7

Table 4: Spike recovery data for samples A, B and C

DILUTION CORRECTED SPECIES CONCENTRATIONS (NG ML ⁻¹)					
Sample	AB	As ³⁺	DMA	MMA	As ⁵⁺
Sample A	593	ND	17.3	ND	ND
Sample B	73	ND	8.72	7.84	ND
Sample C	1.31	ND	3.85	2.41	ND

Table 5: Species concentration data for samples A, B and C

The HPLC-ICP-MS methodology is shown to produce calibration curves with excellent linearity (r^2 correlation coefficients ≥ 0.9999) for each of the measured species and the PlasmaLab Semi-Quantitative analysis tools provide an integral mechanism for quantification of uncharacterized chromatographic peaks using Compound Independent Calibration.

Highest concentrations of the AB and DMA species (250 and 9.4 ng mL⁻¹ respectively) were measured in the urine sample collected from the individual who consumed a meal of Prawns and Salmon within 24 hours of producing the sample. However, lowest concentrations of the AB and DMA species (0.550 and 2.09 ng mL⁻¹ respectively) were observed in the urine sample collected from the non-fish eating individual. The urine sample collected from the individual who had eaten no fish within the last 7 days was found to contain AB and DMA concentrations that were higher than those in sample C but lower than those in sample A (30.8 and 4.74 ng mL⁻¹ respectively). Interestingly, concentrations of MMA were determined in both samples B and C (4.20 and 1.29 ng mL⁻¹ respectively) although MMA was not determined

in sample A. An uncharacterized arsenic species (?) was quantified in each of the supplied urine samples (1.01, 1.23 and 2.88 ng mL⁻¹ for samples A, B and C respectively). However, traces of the toxic inorganic As³⁺ and As⁵⁺ species were not identified in any of the supplied samples.

The HPLC-ICP-MS methodology was shown to enable excellent spike recoveries for AB, As³⁺, DMA, MMA and As⁵⁺ in each urine matrix (96.3-109%) demonstrating the suitability of the analytical methodology. Retention times for the quantified As species were stable throughout the analysis and were not affected by the sample matrix.

Method Detection Limits

Peak area integrations were performed in the PlasmaLab software for AB, As³⁺, DMA, MMA and As⁵⁺ species following 5 repeat injections of the blank and the lowest calibration standard and method detection limits derived from the 3σ model are shown in Table 6.

AS SPECIES	3 SIGMA DL (NG ML ⁻¹)	ABSOLUTE DL (PG)
AB	0.0300	0.601
As ³⁺	0.105	2.09
DMA	0.106	2.11
MMA	0.0436	0.872
As ⁵⁺	0.0739	1.48

Table 6: Method detection limits for As species

Conclusions

The X Series ICP-MS is easily coupled to HPLC accessories to enable highly sensitive elemental speciation analyses in biomedical samples and the Thermo HPLC-ICP-MS speciation package offers a complete instrument solution to the analytical technique. An optimized HPLC method is utilized in conjunction with the intelligent productivity enhancing features of the X Series PlasmaLab software and external trigger card to enable fully automated quantitative arsenic speciation analysis. PlasmaLab Semi-Quantitative analysis tools are also available to quantify uncharacterized species using the Compound Independent Calibration technique.

Parts List

HPLC-ICP-MS Coupling Pack(4600485)

- PEEK Tubing (0.25 mm i.d.) (2.0 m)
- PEEK Tee-Piece (1/16)
- Ezyfit Connector
- Advantech PCI Trigger Card
- 7/02 Electrical Wire (2.0 m)
- 37 Pin D-Type Male Connector

Finnigan Surveyor HPLC Option:

- Finnigan Surveyor HPLC Pump (1600621)
- Finnigan Surveyor HPLC Autosampler (1600622)

Spectra System™ HPLC Option:

- Spectra System SCM1000 Solvent Degasser(1600623)
- Spectra System P4000 Quaternary Gradient Pump (1600624)
- Spectra System AS3500 Autosampler(1600625)

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