

Determination of Organo-Arsenic Species in Seaweed Extracts Using HPLC Coupled with X Series ICP-MS

Key Words

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

Introduction

Arsenic is a ubiquitous component of our environment and is ranked as the twentieth most abundant element in the Earth's crust. Natural sources of arsenic in the environment include volcanic emissions to the atmosphere, release of hot volcanic spring waters, weathering of rocks enriched with arsenopyrites and associated soil runoff processes. More significant sources of environmental arsenic may result from anthropogenic activities such as mining, smelting, combustion of fossil fuels and the use of arsenic compounds such as disodium methylarsenate (DSMA), monosodium methylarsenate (MSMA), mono methylarsonic acid (MMAA), dimethylarsinic acid (DMAA), arsenic acid and phenylarsonates in agricultural pesticides, herbicides and animal feed additives. The majority of naturally derived arsenic in fresh and saline waters is present as inorganic arsenate As(V), although this inorganic arsenate can be alkylated and adenosylated readily by micro-organisms and marine algae to form organo-arsenic species such as arsenosugars and arsenobetaine. These species are readily taken up by marine animals through direct water contact and ingestion of food. Open ocean water may typically contain arsenic concentrations of the order of a few ng mL^{-1} due to the influence of natural and anthropogenic sources. However, marine animals and commercially available seafood products may exhibit exceptionally high arsenic concentrations in the range of $1\text{-}100 \mu\text{g g}^{-1}$ due to the influence of bioaccumulation processes.

The World Health Organisation (WHO) recognises arsenic as a potentially toxic element and specifies a guideline maximum drinking water value of 10 ng mL^{-1} . However, to date there is considerable interest in determining the chemical speciation of arsenic in environmental and biological samples, over and above total arsenic concentrations, to enable an assessment of the environmental mobility, bioavailability, potential for arsenic toxicity to biota and to aid elucidation of the arsenic biogeochemical cycle. Inorganic arsenic compounds are generally considered to exhibit greater toxicity than organic arsenic compounds and the toxicity of inorganic As(III) is several times greater than that of inorganic As(V). Some suggested modes of arsenic toxicity include reactivity with sulfhydryl groups of vital cellular enzymes and interference with phosphorylation processes. Symptoms of human toxicity following

ingestion of inorganic arsenic compounds may include damage to hepatic and renal functions and severe gastrointestinal irritation. The toxicity of organic arsenic compounds is reported to be variable depending upon the specific functionality. However, some of the most toxic organic species include methyl derivatives of arsenic such as MMAA and DMAA (including their sodium salts) and phenyl derivatives of arsenic acid such as phenylarsonate. MMAA and DMAA species are both readily absorbed by animals and humans although milder symptoms of toxicity may be observed in comparison to the above described inorganic arsenicals. Phenylarsonates are generally absorbed and excreted without any metabolic change, although prolonged exposure to high levels (i.e. in animal feeds) may result in damage to the sensory and peripheral nervous system. Arsenobetaine and arsenocholine species are observed primarily in marine animals such as fish and shellfish and these species exhibit non-toxic characteristics in comparison to the above described organo-arsenic species.

Techniques such as hydride generation coupled with atomic spectrometric detection have been used extensively in previous years to speciate arsenic in environmental and biological matrices although a number of drawbacks have been highlighted with regard to the use of these methodologies. For example, the hydride generation process is less efficient for DMAA species in comparison to inorganic arsenite species and lengthy sample preparation techniques may be required to decompose species such as arsenobetaine, arsenocholine and arsenosugar species before they can be converted to their volatile hydride species for analysis. High Performance Liquid Chromatography-Inductively Coupled Plasma Mass Spectrometry (HPLC-ICP-MS) techniques are becoming increasingly used in the field of speciation analysis and these techniques can offer some significant advantages over hydride generation techniques for the analysis of arsenic speciation. HPLC methodologies facilitate rapid on-line species separations with a high degree of species specificity. Low limits of detection are readily achievable for separated elemental species through the use of sensitive, element specific ICP-MS detection.

This application note describes the use of a high-speed separation method using nano-bore chromatography in conjunction with instrumentation from Thermo Electron Corporation to speciate four arsenic compounds

(arsenosugar I, arsenosugar II, arsenosugar III and arsenosugar IV) in a sample of kelp seaweed extract. The HPLC-ICP-MS methodology is developed to demonstrate the potential for rapid separation and quantification of arsenic species in biological extracts and to highlight the suitability of the instrumentation for high throughput, automated arsenic speciation analysis.

Instrument Configuration

The chromatographic mobile phase was pressurised using a Finnigan SpectraSYSTEM™ SCM1000 Solvent Degasser and was pumped through the analytical column to the ICP-MS using a Finnigan SpectraSYSTEM P4000 Quaternary Gradient Pump. Liquid samples are automatically injected into the flow of mobile phase using a Finnigan SpectraSYSTEM AS3500 autosampler and 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 Specification Note SN_E0634. A schematic diagram of this coupled instrumentation is presented in Figure 1.

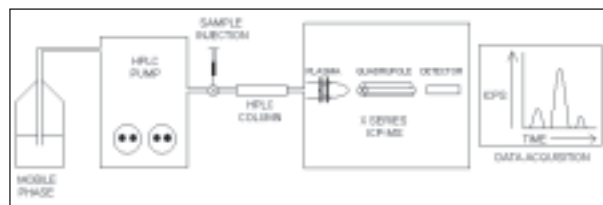


Figure 1: Schematic diagram of the HPLC-ICP-MS instrumentation

Analytical Conditions for HPLC

Table 1 presents analytical conditions for the HPLC.

Mobile Phase Composition	5 mM tetrabutylammonium hydroxide adjusted to pH 6.0 with malonic acid
Mobile Phase Flow Rate	0.7 ml min ⁻¹
Column Dimensions	narrow-bore stainless steel 150 x 2.1 mm id
Stationary Phase Composition	Discovery C18, Supelco, Bellefonte, PA, USA
Column Temperature	Ambient
Sample Injection Volume	1.0 µL

Table 1: HPLC conditions

Analytical Conditions for ICP-MS

The ICP-MS detector was configured with the Thermo Electron Xi 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 standard quartz concentric nebuliser, Peltier cooled impact bead spray chamber and quartz torch with an injector tube diameter of 1.5 mm, fitted with the PlasmaScreen *Plus* enhanced sensitivity screened torch option. The spray chamber was drained via the integral peristaltic pump. Table 2 presents operating conditions for the ICP-MS instrumentation.

Nebuliser Gas Flow	0.87 L min ⁻¹
Auxiliary Gas Flow	0.75 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) 20-100 ms
Timeslice Duration	246 ms
Channels per AMU	1
Run Duration	150 s

Table 2: ICP-MS operating conditions

Preparation and Analysis of Calibrant and Sample Solutions

Calibrant solutions were prepared daily for arsenosugar I, arsenosugar II, arsenosugar III and arsenosugar IV by making appropriate dilutions from a mixed stock solution of dimethylarsinoylriboside derivative standards in deionised water. These arsenosugars were previously isolated from biological sources, purified and characterised. The chemical structures for these species are shown in Figure 2. Dilutions were prepared to facilitate arsenic calibrations up to 50 ng mL⁻¹, 240 ng mL⁻¹, 200 ng mL⁻¹ and 44 ng mL⁻¹ from arsenosugar I, arsenosugar II, arsenosugar III and arsenosugar IV species respectively.

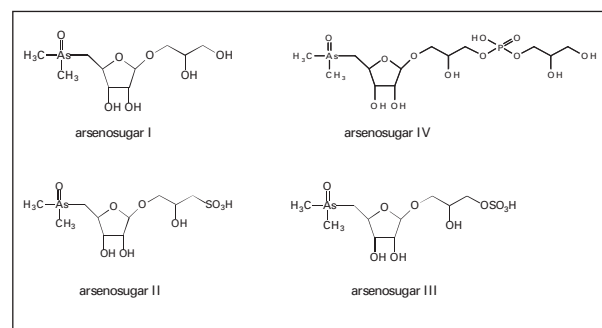


Figure 2: Chemical structures for arsenosugar species

Kelp extract samples were prepared by extracting 0.2 g of commercially available kelp powder in 5 ml of methanol:water (50:50 v/v) in an ultrasonic bath for 20 minutes. The extract was evaporated until almost completely dry, re-dissolved in 10 ml deionised water and filtered through a disposable 0.45 µm filter. Calibrant and extracted kelp sample solutions were diluted further as required in deionised water and were then injected into the flow of mobile phase for analysis without further preparation. All calibrant and sample solutions were analysed in triplicate unless specified otherwise.

Results and Discussion

The X Series ICP-MS PlasmaLab software suite enables fully automated speciation analysis 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 3 shows an example of the chromatography for a calibration standard containing arsenosugar I, arsenosugar II, arsenosugar III and arsenosugar IV and Figure 4 shows an example chromatogram for the unknown kelp extract sample.

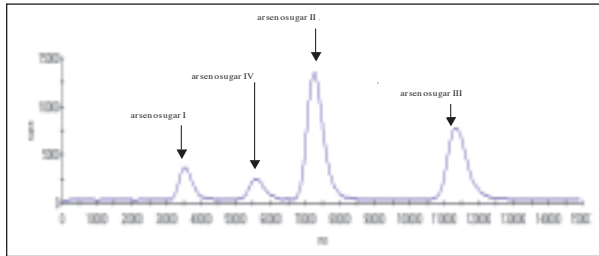


Figure 3: Calibration standard chromatograph for arsenosugar I, arsenosugar II, arsenosugar III and arsenosugar IV species.

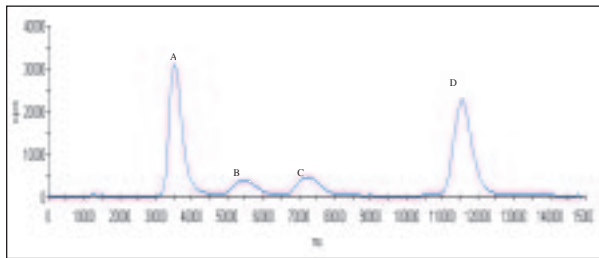


Figure 4: Chromatogram for kelp extract sample

The four arsenic species are baseline resolved in less than 150 secs (2.5 mins) when using this high-speed HPLC separation methodology and retention times for arsenosugar I, arsenosugar II, arsenosugar III and arsenosugar IV are 35 secs, 72 secs, 114 secs and 55 secs in the calibration standard matrix (Figure 3). Minimal time delays of approximately 5 secs are also achieved between consecutive sample analyses due to the optimised use of bi-directional communications between PlasmaLab and the HPLC autosampler accessory and incorporation of intelligent 'inject-hold' trigger commands in the PlasmaLab Accessory Control (ACL) script. This intelligent productivity enhancing feature compliments the rapid chromatographic separation methodology to enable high sample throughput analysis of up to 23 samples per hour. Four chromatographic peaks are observed in the kelp extract sample (Figure 4) and retention times for these peaks correlate closely with those observed in the calibration standards (Figure 3). Chromatographic peaks in the kelp extract sample are labelled A, B, C and D and these peaks are identified as arsenosugar I, arsenosugar IV, arsenosugar II and arsenosugar III respectively.

Chromatographic peak integration parameters are assigned in the PlasmaLab transient TRA data to generate fully quantitative calibration curves for each arsenic species and Figures 5-8 show the PlasmaLab calibrations for As in the arsenosugar I, arsenosugar II, arsenosugar III and arsenosugar IV species. Fully quantitative analytical data for the extracted kelp sample is shown in Table 3.

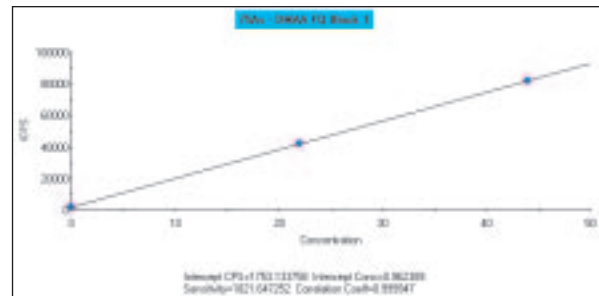


Figure 5: Calibration curve for arsenosugar I

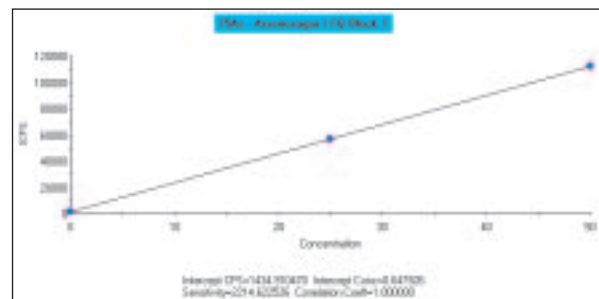


Figure 6: Calibration curve for arsenosugar II

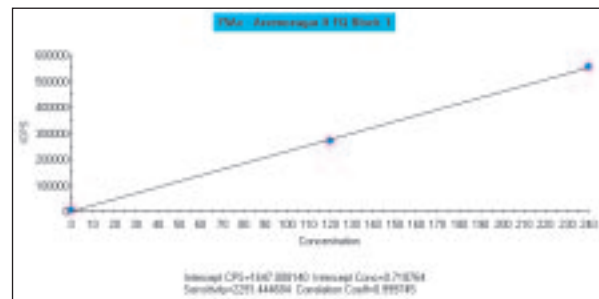


Figure 7: Calibration curve for arsenosugar III

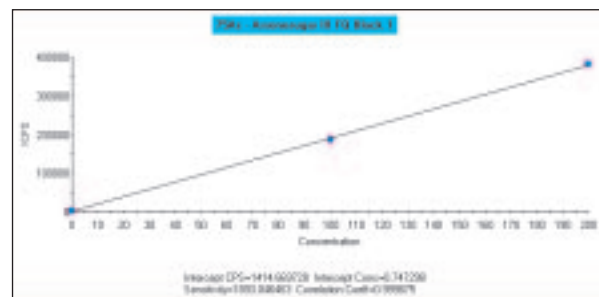


Figure 8: Calibration curve for arsenosugar IV

Parameter	arsenosugar I	arsenosugar II	arsenosugar III	arsenosugar IV
Measured As Extract				
Conc. (ng mL ⁻¹)*	113	24.7	159	23.0
%RSD	0.9	0.5	0.9	0.8
Calculated As Species				
Conc. (ng mL ⁻¹)	494	129	865	148
Actual Species Conc. (µg g ⁻¹ dry kelp)	24.7	6.45	43.3	7.4

*Dilution corrected concentration derived from peak area integration

Table 3: Arsenic speciation data for the kelp extract sample

The HPLC-ICP-MS methodology is shown to produce calibration curves with excellent linearity (r^2 correlation coefficients ≥ 0.999) for the measured species. The kelp extract sample is found to contain concentrations of arsenosugar I, arsenosugar II, arsenosugar III and arsenosugar IV at 24.7, 6.45, 43.3 and 7.4 µg g⁻¹ respectively. Excellent analytical precision is observed from triplicate analyses of the kelp extract sample and % RSD values are calculated as 0.9%, 0.5%, 0.9% and 0.8% for the arsenosugar I, arsenosugar II, arsenosugar III and arsenosugar IV species respectively.

Method Detection Limits

Peak area integrations are performed in the PlasmaLab software for arsenosugar I, arsenosugar II, arsenosugar III and arsenosugar IV species following 10 repeat injections of the lowest calibration standard and method detection limits derived from the 3σ model are shown in Table 4.

SPECIES	DETECTION LIMIT	
	As/ng mL ⁻¹	SPECIES/ng mL ⁻¹
arsenosugar I	0.3	1.3
arsenosugar II	0.4	2.1
arsenosugar III	0.4	2.2
arsenosugar IV	0.3	0.6

Table 4: Detection limit data for As species

Conclusions

The X Series ICP-MS is easily coupled to HPLC accessories to enable sensitive elemental speciation analysis in liquid samples and the Thermo Electron HPLC-ICP-MS speciation package offers a complete instrument solution to the analytical technique. A rapid nano-bore HPLC separation method is utilised 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. This methodology facilitates high sample throughput capability with minimum cost per sample and detection limits are achieved in the pg mL⁻¹ to low ng mL⁻¹ range for the selected organo-arsenic species.

Acknowledgements

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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 SpectraSYSTEM™ HPLC Option:	
SpectraSYSTEM SCM1000 Solvent Degasser	(1600623)
SpectraSYSTEM P4000 Quaternary Gradient Pump	(1600624)
SpectraSYSTEM AS3500 Autosampler	(1600625)
Finnigan™ Surveyor HPLC Option:	
Finnigan Surveyor HPLC Pump	(1600621)
Finnigan Surveyor HPLC Autosampler	(1600622)

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