Application Note: 40741

Speciation of Arsenic in Fish Tissues using HPLC Coupled with XSERIES 2 ICP-MS

Dr Martin Nash, Thermo Fisher Scientific, Winsford, UK; Dr Shona McSheehy, Group of Biogeochemistry, Ultra-Trace and Isotopic Analysis, Laboratoire de Chimie-Analytique Bio-inorganique et Environnement, University of Pau, France.

Key Words

- Arsenic
- Fish
- HPLC-ICP-MS
- ICP-MS
- Speciation

Introduction

The accumulation and biomagnification of arsenic in marine flora and fauna is a phenomenon that has generated a great deal of interest in the nutrition and trade industries in recent years. The notorious association of arsenic with poisoning has led to many studies on the possible risks associated with human exposure. However, as it is the chemical form of arsenic that controls the toxicity of the element, it is information from speciation that generates the most appropriate data on which to evaluate toxicological risk. As a general rule, inorganic arsenic species are more toxic than organoarsenic species and trivalent species are more toxic than pentavalent species. The range of arsenic species encountered in the environment is diverse due to natural and anthropogenic releases and subsequent chemical and/or biological transformations.

Marine organisms are known to accumulate arsenic in the range of 1-100 mg kg-1 from their environment and food sources. The majority of arsenic is present as organoarsenic species, metabolised from inorganic arsenic present in seawater or accumulated from food sources such as algae or other fish. Arsenobetaine (AsB), the major species found in fish and shellfish, often contributes to 50-90 % of the total arsenic. Minor species, such as arsenocholine, trimethylarsonium proprionate, dimethyl arsinic acid (DMA) and tetramethylarsonium ion have been reported. In some shellfish, the group of species known as arsenosugars, which are predominantly found in macroalgae, have also been observed in small quantities. Carnivorous fish tend to have the highest arsenic concentrations due to the biomagnification process of AsB from their food source. Although herbivores and detritivores also contain AsB as the major species, they are also likely to contain inorganic arsenic, methylated arsenic and other organoarsenic species. However, the complete speciation of arsenic in fish is often hampered by the lack of commercially available arsenic-containing standards.

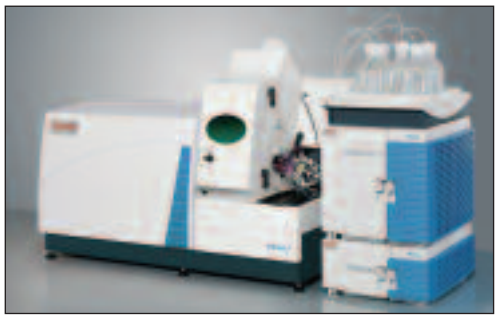
Most modern analytical techniques concerning the speciation of arsenic employ a liquid chromatographic (HPLC) separation coupled with inductively coupled plasma mass spectrometry (ICP-MS). The majority of arsenic species are aqueous soluble, thus HPLC mechanisms (anion exchange, cation exchange, ion-pair) are particularly suited for this application. The ICP-MS allows sensitive and specific detection of eluting arsenic species. Aqueous extraction is often sufficient for the quantitative extraction of arsenic species from fish muscle, whereas more fatty tissues such as shellfish or specific organs of fish may require solvent extraction. Extraction efficiencies are improved with such techniques as sonication or accelerated solvent extraction (ASE). Care must be taken by the analyst to ensure there is minimum degradation of species during the extraction and analytical protocol. Species degradation and sources of analytical error can be detected with the use of a certified reference material (CRM). The CRM should be representative of the sample being analyzed and will serve to validate the analytical methodology.

This application note describes the use of the HPLC-ICP-MS instrument package from Thermo Fisher Scientific for the determination of arsenic species in a range of commercially available fish tissues. Analytical recoveries were determined by spiking two independent extracts with a mixture of arsenic standards and method detection limits were established using the 3σ model. The method is validated using the CRM DORM-2, a dogfish muscle matrix.

Thermo SCIENTIFIC

Instrument configuration

The Thermo Scientific Surveyor™ HPLC pump and autosampler were coupled to the Thermo Scientific XSERIES 2 ICP-MS with the aid of an HPLC-ICP-MS Coupling Pack (P/N 4600485) and Surveyor LC Wiring Harness (P/N 4600487). The Thermo Scientific HPLC-ICP-MS coupling pack includes all the required components to establish electrical and analytical connections between the HPLC and ICP-MS instrumentation. The PlasmaLab software and External Trigger Card (P/N 4600261) enable automated HPLC accessory control using bi-directional communications and intelligent peak integration facilities.



Analytical Conditions for HPLC-ICP-MS

The Surveyor HPLC pump with autosampler was programmed from the XSERIES 2 ICP-MS PC using Atlas software to enable separation of the aqueous soluble arsenic species. The XSERIES 2 ICP-MS was performance tested, tuned and optimized as required for HPLC-ICP-MS analysis using the automated PlasmaLab Performance Test and Autotune facilities. The arsenic-containing species were separated using an anion-exchange HPLC column and eluted with an ammonium carbonate mobile phase at pH 8.9. HPLC parameters and analytical conditions for HPLC-ICP-MS are shown in Table 1.

Column	Polymeric Anion Exchange (250 x 4.6 mm, 10 µm)
Injection volume	20 µL
Flow rate	1 mL min^{-1}
Buffer A	10 mM ammonium carbonate, pH 8.9
Buffer B	20 mM ammonium carbonate, pH 8.9
Gradient	0 to 10 min, Buffer A, 10 to 11 min
0 to 10 min	100 % Buffer A
10 to 11 min	100 % Buffer A to 100 % Buffer B
11 to 20 min	100 % Buffer B
Forward Power	1350 W
Nebulizer Gas Flow	0.8 L min^{-1}
Auxilliary Gas Flow	0.8 L min^{-1}
Cool Gas Flow	13 L min^{-1}
Data Acquisition Mode	PlasmaLab Transient Time Resolved Analysis (TRA)
Isotopes and dwell times, ms	^{75}As (150 ms)
	^{77}Se, ^{82}Se, ^{83}Kr (50 ms)
Channels per AMU	1
Timeslice duration	306 ms
Transient acquisition time	1800 s
Spray chamber	Quartz impact bead
Nebulizer	Glass concentric
Cones	Xt

Table 1. HPLC-ICP-MS conditions

Preparation of Arsenic-Containing Standards

Stock solutions of 1000 µg g^{-1} of each arsenic standard (arsenite, arsenate, monomethyl arsonic acid, dimethyl arsenic acid and arsenobetaine) were prepared by dissolving the appropriate quantity of the commercially available salt in milli-Q water (18.2 MΩ). The stock solutions were diluted to produce daily working standards of 1 µg g^{-1}. The stock solutions were kept at 4 °C in the dark.

Preparation of CRM and Fish Samples

Sample preparation:

Commercially available fish samples (red tuna, canned tuna, fillet of trout, freshwater perch, ling, alaskan hake, and squid) were individually washed in 18.2 MΩ water, cut into small pieces with a scalpel and transferred to clean, dry vials. After freezing during 24 hours the fish were subjected to freeze-drying during 48 hours to remove all water from the tissues. The dried tissues were homogenized with a pestle and mortar and reduced to a powder.

Sample extraction:

Prior to sub-sampling, the powdered CRM DORM-2 was removed from storage at -20 °C, left to achieve ambient temperature for 1 hour and homogenized by manual agitation for 5 min. Triplicate sub-samples of 250 ± 10 mg of the CRM were weighed accurately into clean, dry extraction vessels. Approximately 250 mg portions of freeze-dried commercially available fish samples were also used for extraction. 10 mL of 18.2 MΩ milli-Q water was added to each vessel and the vessels were capped. The vessels were vortexed briefly before extraction and every 15 min during extraction to ensure dispersion of the sample. Extraction was performed in an ultrasonic bath at 50 °C over a period of 2 hours. After extraction, the samples were centrifuged for 20 m at 2000 rpm. The supernatant was decanted and appropriate dilutions of each sample were performed such that the peak areas of arsenic-containing species fell within the fully quantitative calibration curve. 3 reagent blanks were also prepared by the above procedure and used to determine the method limit of detection.

Results and Discussion

The HPLC methodology allowed the baseline separation of arsenite (As^{III}), AsB, DMA, monomethyl arsonic acid (MMA) and arsenate (As^{V}). The chromatographic data is displayed automatically in the XSERIES 2 PlasmaLab software package following analysis and an example of the chromatographic separation of arsenic-containing standards is shown in Figure 1. The PlasmaLab software enables flexible data acquisition and integration parameters, automatically applicable to a series of sample analyses in an experiment.

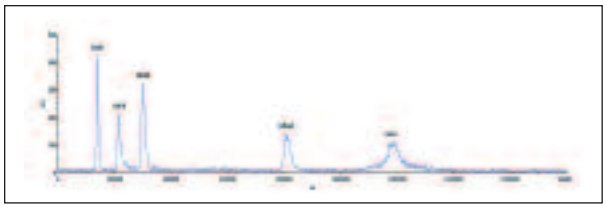


Figure 1. Calibration standard chromatography of five commercially available arsenic-containing standards (1 ng As mL^{-1} for each standard)

Quantification of arsenic-containing species was performed by external calibration. A blank and calibration standards of 1, 5, 10 and 25 ng g^{-1} of each of the five arsenic-containing standards were used to generate species specific calibration curves. The calibrations for AsB and DMA are shown for reference in Figure 2. The CRM DORM-2, certified for AsB was extracted and analyzed in triplicate to verify the HPLC-ICP-MS procedure. A method blank was prepared and analyzed to correct for any contamination.

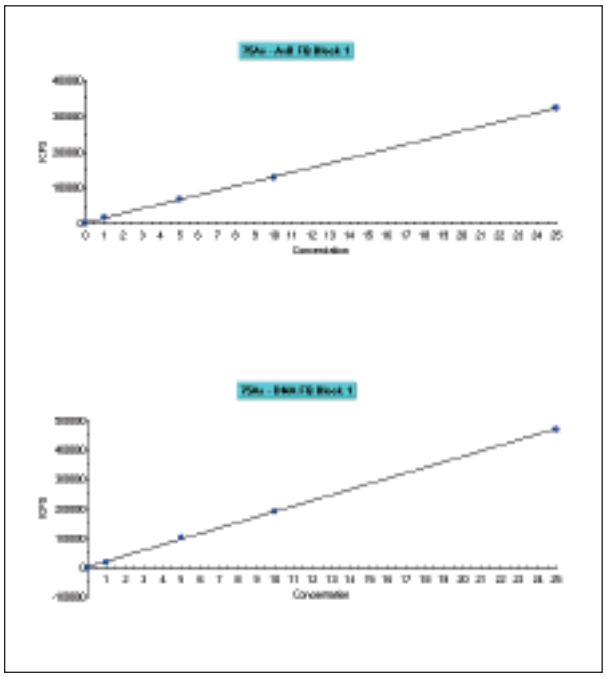


Figure 2. Calibration curves for the standards AsB and DMA

Two chromatographic peaks were observed in DORM-2 and were identified as AsB and DMA from correlation of retention time with standard calibrants. Appropriate dilutions of the commercially available fish extracts were necessary for quantification within the range of the calibration. The major species identified in each of the commercially available fish is AsB. The red tuna and canned tuna were found to contain AsV and MMA respectively and both contained DMA. However, these minor species comprised only 6 % of the total arsenic found in the fish samples. A fourth species observed only in the squid heads was uncharacterised as a standard was not available for this species. However, the Compound Independent Calibration technique was used (i.e. using the mean sensitivity of all the fully quantitative calibrations) to generate semi-quantitative data for this unknown. Figure 3 shows some example chromatograms of the commercial fish tissues.

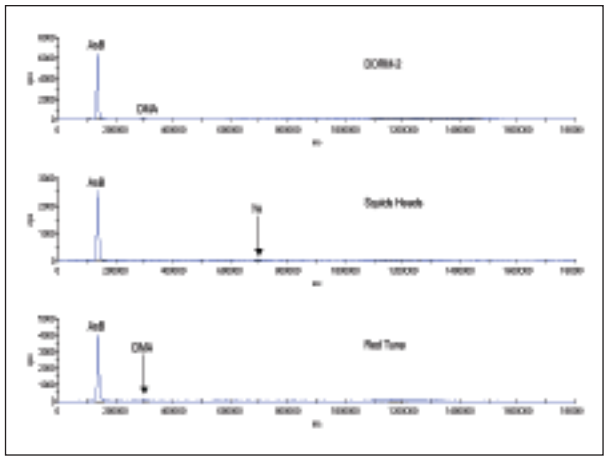


Figure 3. Chromatograms of DORM-2 and the fish samples 'Squid Heads' and 'Red Tuna'

The concentration determined in the aliquot of extract was extrapolated to the concentration found in the dry CRM and freeze-dried fish tissues by taking into account all dilution and weight factors. The CRM determinations were further corrected for moisture (determined as 5.2 %). The concentration of arsenic-containing species in the commercial fish samples was calculated from the moisture removed in the freeze-drying process. Table 2 summarizes the concentrations found in the CRM and seven fish samples. The fully quantified concentration of AsB found in the DORM-2 CRM was within 95 % of the certified value (16.4 ± 1.1). The agreement of the analytical data with the certified value in DORM-2 demonstrates the suitability of the sample preparation for arsenic speciation in fish and the accuracy of the HPLC-ICP-MS technique.

To further validate the analytical methodology, recovery of arsenic-containing standards into two of the commercially available fish samples was determined. Outlined in Table 2, the recovery was found to be at least 97 % for each species in both tissues.

ARSENIC CONTAINING SPECIES (µg As/g)

CRM	Source	AsB	AsIII	DMA	Unknown A	MMA	AsV
DORM-2 (Dogfish muscle)	IRMM, Belgium	15,6 ± 1,04	-	0,2 ± 0,03	-		
Sample	**Source**						
Red tuna	North-East Atlantic	3.9	-	0.11	-	-	0.13
Canned tuna	Ecuador	3.42	-	0.12	-	0.08	-
Fillet of Trout	Farmed, France	3.68	-	-	-	-	-
Freshwater Perch	France	0.06	-	-	-	-	-
Ling	North-East Atlantic	45.35	-	-	-	-	-
Alaskan Hake fillets*	Pacific Ocean	12.78	-	-	-	-	-
Squid Heads*	Indian Ocean	1.93	-	-	0.02	-	-

ARSENIC CONTAINING SPECIES RECOVERY (%)

Sample	Source	AsB	AsIII	DMA	Unknown A	MMA	AsV
Canned tuna	Ecuador	97.6	115.8	104.8	-	114.1	129.1
Squid Heads*	Indian Ocean	118.3	112.4	106.5	-	114.2	123.7

Table 2. Fully quantitative data for AsB, AsIII, DMA, MMA and AsV and semi-quantitative data for an unknown species in various fish samples and CRM DORM-2.

* Frozen Fish

Detection limits for the five arsenic-containing standards were determined in accordance with the 3σ model following fully quantitative analysis of the calibration blanks (n = 3). Absolute detection limits and sample detection limits were also determined and are also summarized in Table 3.

LIMITS OF DETECTION FOR ARSENIC-CONTAINING SPECIES

		AsB	AsIII	DMA	MMA	AsV
3σ	ng/g	0.33	0.56	0.55	0.95	1.08
Absolute	pg	6.6	11.3	11.0	18.9	21.6

Table 3. Figures of merit

Summary

The Thermo Scientific HPLC-ICP-MS instrument package offers a complete instrument solution for the sensitive and accurate determination of arsenic-containing species in fish. PlasmaLab software features allow rapid and automated integration, increasing productivity and in combination with the External Trigger Card permits failsafe automated instrument operation for routine speciation applications.

©2008 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific Inc. and its subsidiaries.

Specifications, terms and pricing are subject to change. Not all products are available in all countries. Please consult your local sales representative for details.

In addition to these offices, Thermo Fisher Scientific maintains a network of representative organizations throughout the world.

Africa
+43 1 333 5034 127
Australia
+61 2 8844 9500
Austria
+43 1 333 50340
Belgium
+32 2 482 30 30
Canada
+1 800 530 8447
China
+86 10 8419 3588
Denmark
+45 70 23 62 60
Europe-Other
+43 1 333 5034 127
France
+33 1 60 92 48 00
Germany
+49 6103 408 1014
India
+91 22 6742 9434
Italy
+39 02 950 591
Japan
+81 45 453 9100
Latin America
+1 608 276 5659
Middle East
+43 1 333 5034 127
Netherlands
+31 76 579 55 55
South Africa
+27 11 570 1840
Spain
+34 914 845 965
Sweden/Norway/ Finland
+46 8 556 468 00
Switzerland
+41 61 48784 00
UK
+44 1442 233555
USA
+1 800 532 4752

www.thermo.com

Thermo Fisher Scientific (Bremen) GmbH is certified DIN EN ISO 9001:2000

AN40741_E 01/08C