Analytica Chimica Acta 943 (2016) 41–49

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Species specific isotope dilution for the accurate and SI traceable determination of arsenobetaine and methylmercury in cuttlefish and prawn

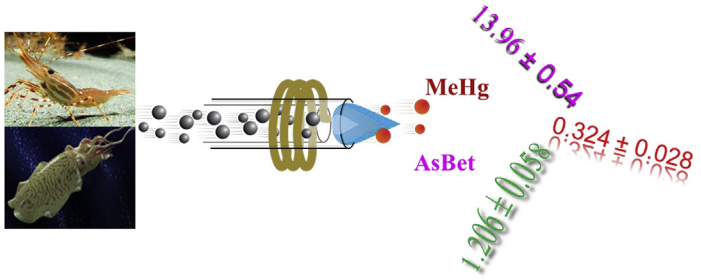
Paramee Kumkrong [a, b], Benjaporn Thiensong [b], Phuong Mai Le [a], Garnet McRae [a], Anthony Windust [a], Suladda Deawtong [b], Juris Meija [a], Paulette Maxwell [a], Lu Yang [a, *], Zoltán Mester [a]

[a] National Research Council Canada, 1200 Montreal Rd, Ottawa, Ontario, K1A 0R6, Canada
[b] Thailand Institute of Scientific and Technological Research, 35 Mu 3 Tambon Khlong Ha, Amphoe Khlong Luang, Pathum Thani, 12120, Thailand

HIGHLIGHTS

- The accurate and SI traceable determination of arsenobetaine (AsBet) and methylmercury (MeHg) in prawn and squid tissues.
- Primary standards of AsBet and MeHg characterized by ^{1}H-NMR ensure the final results traceable to SI.
- First report of SI traceable measurements of AsBet and MeHg in prawn and cuttlefish.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 29 July 2016
Received in revised form
9 September 2016
Accepted 21 September 2016
Available online 1 October 2016

Keywords:
Arsenobetaine
Methylmercury
Speciation
Isotope dilution
Certified reference material

ABSTRACT

Methods based on species specific isotope dilution were developed for the accurate and SI traceable determination of arsenobetaine (AsBet) and methylmercury (MeHg) in prawn and cuttlefish tissues by LC-MS/MS and SPME GC-ICPMS. Quantitation of AsBet and MeHg were achieved by using a ^{13}C-enriched AsBet spike (NRC CRM CBET-1) and an enriched spike of Me^{198}Hg (NRC CRM EMMS-1), respectively, wherein analyte mass fractions in enriched spikes were determined by reverse isotope dilution using natural abundance AsBet and MeHg primary standards. Purity of these primary standards were characterized by quantitative ^{1}H-NMR with the use of NIST SRM 350b benzoic acid as a primary calibrator, ensuring the final measurement results traceable to SI. Validation of employed methods of ID LC-MS/MS and ID SPME GC-ICPMS was demonstrated by analysis of several biological CRMs (DORM-4, TORT-3, DOLT-5, BCR-627 and BCR-463) with satisfying results.

The developed methods were applied for the determination of AsBet and MeHg in two new certified reference materials (CRMs) prawn (PRON-1) and cuttlefish (SQID-1) produced jointly by Thailand Institute of Scientific and Technological Research (TISTR) and National Research Council Canada (NRC). With additional measurements of AsBet using LC-ICPMS with standard additions calibration and external calibration at NRC and TISTR, respectively, certified values of 1.206 ± 0.058 and 13.96 ± 0.54 mg kg^{-1} for AsBet as As (expanded uncertainty, $k = 2$) were obtained for the new CRMs PRON-1 and SQID-1, respectively. The reference value of 0.324 ± 0.028 mg kg^{-1} as Hg (expanded uncertainty, $k = 2$) for MeHg was obtained for the SQID-1 based on the results obtained by ID SPME GC-ICPMS method only,

* Corresponding author.
E-mail address: Lu.yang@nrc-cnrc.gc.ca (L. Yang).

http://dx.doi.org/10.1016/j.aca.2016.09.031

whereas MeHg in PRON-1 was found to be < 0.015 mg kg^{-1}. It was found that AsBet comprised 69.7% and 99.0% of total As in the prawn and cuttlefish, respectively, whereas MeHg comprised 94.5% of total Hg in cuttlefish.

1. Introduction

Arsenic and mercury are toxic trace elements in the environment and the toxicity of arsenic and mercury depends on their chemical forms (speciation) [1,2]. For arsenic, inorganic As(III) and As(V) are considerably more toxic than the organic species such as monomethylarsonic acid (MMA) or dimethylarsinic acid (DMA) [1,3] whereas arsenobetaine (AsBet) and arsenocholine (AsC) are believed to be nontoxic [4], although this view is not held universally [5]. In contrast to arsenic, organic methylmercury is more toxic than inorganic Hg(II) [2]. Marine organisms are known to accumulate and biotransform arsenic and mercury, and this could provide a major route of exposure for humans to arsenic and mercury through consumption of marine food products [6–11]. As a result, many countries have set guidelines for seafood consumption to safeguard human health, and efforts have been devoted to the development of sensitive, accurate and rapid analytical methods for the speciation of As^{4-} [5,7,8,12–20] and Hg [10,11,21–26] in seafood products.

In general, quantitative speciation of arsenic and mercury in biological samples requires efficient extraction, good chromatographic separation and detection. Compromise in any of these steps can contribute to the difficulty of the analysis and degrading the accuracy of the results. More importantly, the measurement results are required to be traceable to the International System of Units (SI) [27], as this is the basis to achieve comparable measurement results from different laboratories to ensure fair trade globally. As defined in Vocabulary in Metrology [28], measurement traceability is the property of the result of a measurement or the value of a standard whereby it can be related to stated references, usually national or international standards, through an unbroken chain of comparisons, all having stated uncertainties. Therefore, SI traceable primary pure standard with known purity and associated uncertainty, as well as matrix-matched certified reference materials (CRMs) for method validation are required to establish the measurement traceability. Currently, the choice of SI traceable primary standards and matrix CRMs for speciation analysis of AsBet and MeHg (two major species in fish, prawn and cuttlefish) are limited. As shown in Table 1, only a few CRMs which have certified values for AsBet and/or MeHg from National Research Council Canada (NRC, Ottawa, ON, Canada), Institute for Reference Materials and Measurements (IRMM, European Commission, Geel, Belgium), National Institute of Standards & Technology (NIST, Gaithersburg, MD, USA) and National Metrology Institute of Japan (NMIJ, Tsukuba, Japan), are available on the market.

Table 1
Current available CRMs certified for MeHg and/or AsBet.

Name of CRM	Matrix	Analyte certified	Source
DORM-4	Fish	AsBet and MeHg	NRC, Canada
TORT-3	Lobster	AsBet and MeHg	NRC, Canada
DOLT-5	Dogfish liver	AsBet and MeHg	NRC, Canada
BCR-627	Fish	AsBet	IRMM, Belgium
ERM-CE464	Fish	MeHg	IRMM, Belgium
BCR463	Fish	MeHg	IRMM, Belgium
SRM1946	Fish	MeHg	NIST, USA
SRM2976	Mussel	MeHg	NIST, USA
CRM7402a	Fish	AsBet and MeHg	NMIJ, Japan
CRM7403a	Fish	AsBet and MeHg	NMIJ, Japan

In terms of primary (calibrant) standards for AsBet and MeHg, only BCR-626 (IRMM, Geel, Belgium) and NMIJ-7901a (NMIJ, Tsukuba, Japan) are available. Assessment of purities of these organometallic standards is far from trivial. As exemplified in the 2007 CCQM-P96 international intercomparison study [29] under the auspices of the International Committee of Weights and Measures (CIPM), it was discovered that the certified value of AsBet in both reference materials BCR-626 (IRMM, Geel, Belgium) and NMIJ-7901a (NMIJ, Tsukuba, Japan) which were used as primary calibrators for the determination of AsBet in fish, were biased and both of which shared a common traceability link through BCR-626. As a result of the CCQM-P96 study, the certified value for NMIJ-7901a was revised by 20% in 2009 [30] whereas only the expanded uncertainty was subsequently revised for the BCR-626 in 2009 from 0.6% to 7%.

To address these needs, this paper describes protocols for the accurate and SI traceable determination of AsBet and MeHg in two new prawn and cuttlefish reference materials, named PRON-1 and SQID-1, respectively. Isotope dilution (ID) calibration was applied for the determination of AsBet and MeHg by LC-MS/MS and SPME GC-ICPMS, respectively, with use of a ^{13}C-enriched arsenobetaine spike (NRC CRM CBET-1) and ^{198}Hg-enriched methylmercury (NRC CRM EMMS-1) spike, both synthesized at NRC. Purities of primary standards of the natural abundance arsenobetaine bromide (NRC CRM ABET-1) and methylmercury chloride (purchased from Sigma-Aldrich) were both characterized by quantitative ^{1}H-NMR with the use of NIST SRM 350b benzoic acid as a primary calibrator. This is first time reporting the use of quantitative ^{1}H-NMR for the determination of purity of MeHgCl primary standard, ensuring the MeHg results in cuttlefish and prawn CRMs traceable to SI. Two secondary methods of standard addition calibration by LC-ICPMS and an external calibration LC-ICPMS were used for the determination of AsBet at NRC and TISTR, respectively. Validation of the above employed methods at NRC was demonstrated by analysis of several biological Certified Reference Materials (DORM-4, TORT-3, DOLT-5, BCR-627 and BCR-463) with satisfying results.

2. Experimental

2.1. Instrumentation

A 5975C GC-MS and 7500 ICPMS from Agilent Technologies (Mississauga, ON, Canada), were used for the determination of MeHg. A DB-5 column (5% diphenyl, 95% polydimethylsiloxane, 30 m × 0.28 mm × 0.5 μm) was used for the separation of mercury species. A commercial heated GC transfer line for splitting the GC eluent to ICPMS and MS detectors was custom designed and made by Agilent Technologies as described in the previous work [26]. A manual SPME device, equipped with a fused silica fiber coated with a 100 μm film of polydimethylsiloxane (Supelco, Bellefonte, PA, USA), was used for the sampling of propylated MeHg from the headspace above its aqueous solutions. For convenience, SPME sampling was conducted in a regular fumehood. ICPMS