

A New Candidate Reference Material for Inorganic Arsenic and Arsenosugars in Hijiki Seaweed: First Results from an Inter-laboratory Study

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An inter-laboratory study was carried out to characterize a candidate hijiki seaweed for its concentrations of total arsenic and water-soluble arsenic compounds, particularly arsenosugar compounds. The candidate material, a dried hijiki seaweed powder, was analyzed by individual techniques in two laboratories. The water-soluble arsenic compounds were separated by anion exchange, and reversed-phase columns, and As(V), DMA and four kinds of arsenosugars, namely glycerol (-OH), phosphate (-PO₄), sulfonate (-SO₃), and sulfate (-SO₄) types were detected by HPLC-ICP-MS. The methods applied were validated by analyzing a second sample, the NMIJ CRM 7405-a hijiki seaweed, which is certified for both total arsenic and As(V). Techniques for the inter-laboratory study, extraction efficiencies under different extraction conditions, some chromatographic techniques and sequential extraction were investigated. The results from the two laboratories for the candidate hijiki material showed good agreement within the measurement uncertainties for total and water-soluble arsenic compounds.

Keywords Hijiki seaweed, speciation, inorganic arsenic, arsenosugar compounds, validation

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Introduction

Arsenic (As) is widely distributed in the environment and biosphere where it exists in a range of chemical compounds.¹⁻³ Organoarsenic compounds, including monomethylarsonic acid (MMA), dimethylarsinic acid (DMA) and various arsenosugars are usually the major As forms in seaweeds.⁴ Seaweed takes-up arsenate from seawater, possibly because of an incapacity to distinguish it from the essential macronutrient phosphate. Most algal compounds transform the arsenate into various arsenosugars, a process thought to protect the algae from potentially toxic arsenate.⁵⁻⁹

Some seaweeds, like *Fucus serratus* and *Laminaria spp.* are known to contain mainly arsenosugar compounds, whereas inorganic As (iAs) compounds, such as As(III) and As(V), are found in these algae only at trace levels.¹⁰ Arsenosugar compounds typically found in algae are differentiated by an oxygen (O), comprising glycerol (-OH), phosphate (-PO₄), sulfonate (-SO₃), and sulfate (-SO₄) end groups. It is suspected that the toxicity of arsenosugar compounds depends on their structures,¹¹⁻¹³ and their components depend on the type of algae, such as brown, green, and red algae.^{14,15} Furthermore, seafood and seaweed are regarded as crucial parts of a healthy diet because they contain several nutrients associated with beneficial health effects. However, in the case of hijiki, it is

likely to contain high concentrations of iAs, which is the most toxic As species. In addition, arsenosugar compounds are one of the most important research targets to facilitate the evaluation and discussion on their metabolism and associated risks.

To identify of As metabolism and to evaluate their toxicity, the identification and quantification of each arsenosugar are essential. High-performance liquid chromatography with inductively coupled plasma-mass spectrometry (HPLC-ICP-MS) and electrospray mass spectrometry (ESI-MS) are the most widely used analytical methods for the speciation of As.¹⁶⁻²⁵ Quality control and quality assurance for ensuring the reliability of laboratory studies, and certified reference materials (CRMs) can be effective in this regard. The National Metrology Institute of Japan (NMIJ) has produced CRM 7405-a hijiki powder, a dried seaweed powder with a certified mass fraction of As(V); information related to other As compounds, however, is not available.²⁶ The National Institute of Standards and Technology (NIST) has supplied SRM 3232 kelp (*Thallus laminariae*) powder as an alga matrix, including the values for three arsenosugar compounds, but those values are reference mass fraction values.²⁷ To compensate for the lack of a suitable CRM for arsenosugars, other strategies, including an addition-recovery test, a standard addition method, and/or multiple analysis methods, are carried out to support the accuracy of the results.^{28,29} But without a CRM, it is difficult to confirm the accuracy of independent results or to compare them with other results.

For a development of hijiki CRM, quantification techniques of water-soluble arsenic compounds including arsenosugars were established by HPLC-ICP-MS and LC-MS. The details of the

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extraction conditions, separation condition were investigated, and the mass balances of water-soluble As compounds were discussed. Then, an inter-laboratory study was conducted to analyze the candidate hijiki CRM material 7405-b hijiki powder.

Experimental

Instrumentation

Laboratory 1: An ICP-MS (Agilent 7900, Agilent Technologies, Waldbronn, Germany) system was employed using He as the collision cell gas for removing polyatomic interferences, such as $^{40}\text{Ar}^{35}\text{Cl}$ on ^{75}As . HPLC-ICP-MS measurements were carried out using an Agilent 1100 series HPLC (Agilent) system equipped with a binary pump, a vacuum degasser, a column oven, and an autosampler with a variable 100 μL injection loop connected by 0.125 mm polyetheretherketone (PEEK) tubing (Upchurch Scientific, Oak Harbor, WA) to ICP-MS system equipped with a Micromist nebulizer and a Scott double-pass spray chamber. A Thermo AS14a anion-exchange column (Thermo Fisher Scientific Inc., Dionex, CA, hereafter referred to as IC-Anion: filler particle size of 5 μm , i.d. of 3.0×150 mm) and an ACE phenyl-hexyl reversed-phase column (RPC, Advanced Chromatography Technologies Ltd., Aberdeen, Scotland, hereafter referred to as the phenyl-hexyl: filler particle size of 5 μm , i.d. of 3.0×250 mm) were used for the separation of As compounds.

An Ultraclave microwave system (MLS GmbH, Leutkirch, Germany) was used for microwave-assisted acid digestion, and a GFL-1083 shaking water bath (Gesellschaft für Labortechnik, Burgwedel, Germany) was used for extraction.

The limits of detection (LOD, $\sigma = 3$) of As for ICP-MS and of As(V) for HPLC-ICP-MS based on As standard solutions using the IC-Anion column were 0.01 ng g^{-1} for both; the LODs of DMA and arsenosugar compounds for HPLC-ICP-MS using the IC-Anion column were 0.07 ng g^{-1} ; and the LOD of Sugar-328 for HPLC-ICP-MS using the phenyl-hexyl column was 0.04 ng g^{-1} .

Laboratory 2: An ICP-MS (7500c, Agilent Technologies, Tokyo, Japan) system equipped with a Micromist nebulizer (0.1 mL) and a Scott spray chamber (2°C) was used. ICP-MS was typically operated using He as the collision cell gas (3 mL min^{-1}) to minimize polyatomic interferences. An HPLC system with an ODS column was used to separate As compounds using an automatic sample injector (Nanospace SI-2, Osaka Soda Co., Ltd., Osaka, Japan). The HPLC column exit was directly connected to the ICP-MS nebulizer with PEEK tubing (HPLC-ICP-MS). A CAPCELL PAK C_{18} MG column (hereafter referred to as ODS C_{18} ; filler particle size of 3 μm , i.d. of 4.6×150 mm, polymer-coated column, Osaka Soda) and a Sunrise C_{28} column (hereafter referred to as ODS C_{28} ; filler particle size of 3 μm , i.d. of 4.6×150 mm, TMS end-capped column, ChromaNik Technologies Inc., Osaka, Japan) were used as an eluent flow rate of 0.5 mL min^{-1} . The LOD ($\sigma = 3$) of As for ICP-MS was 0.02 ng g^{-1} . The LOD ($\sigma = 3$) of As(V) for HPLC-ICP-MS was 0.06 ng g^{-1} ; the LOD ($\sigma = 3$) of DMA and arsenosugar compounds for HPLC-ICP-MS were 0.08 ng g^{-1} .

UPLC-MS/MS analyses were carried out using an UPLC/ Xevo TQD system (Waters, Bedford, MA). Separation with ODS C_{18} was carried out with a 10 mmol L^{-1} ammonium dihydrogen phosphate aqueous solution (pH 8.25, A) and methanol (B), with isocratic elution at 5% B for 6 min. The flow rate was 750 $\mu\text{L min}^{-1}$. Column separations were performed at 40°C , and 8 μL of the sample was injected. The MS conditions (ESI⁺) were as follows: capillary voltage, +3.0 kV; cone-gas flow rate, 50 L h^{-1} ; desolvation-gas flow rate,

1000 L h^{-1} ; desolvation temperature, 600°C . Data were obtained under a selected ion monitoring (SIM) mode at m/z 409 and 329 for Sugar-408 and Sugar-328, respectively.

An MLS 1200 mega (Milestone-MLS, Leutkirch, Germany) was used for microwave digestion. An ultrasonic bath was used to accelerate the extraction of the As compounds, and a heating block system (Digi PREP, SCP Science Inc., Quebec, Canada) was also used for heat extraction.

Reagents and standards

Laboratory 1: A single-element As standard (As in 2% HNO_3 , $1000 \pm 3 \mu\text{g As mL}^{-1}$, CPI International, Santa Rosa, CA) was used as the calibration source for the analysis of total As. Stock solutions of As(V) and DMA were prepared from $\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$ (Merck, Darmstadt, Germany) and sodium dimethylarsinate (Fluka, Buchs, Switzerland), respectively, and each solution was dissolved in water to prepare a stock standard solution containing 1000 mg As kg^{-1} . The concentrations of prepared As(V) and DMA solutions were determined based on NMIJ CRM As(V) and DMA solutions at Laboratory 2. Also, the As(V) solution was used for the calibration of As(V), and the DMA solution was used for the calibration of DMA and arsenosugar compounds in hijiki sample; 5 mmol L^{-1} of formic acid adjusted to pH 4.0 with aqueous ammonia (Suprapur, Merck, Darmstadt, Germany) was used as the mobile phase in the IC-Anion column (Lab 1 condition 1), and 10 mmol L^{-1} of acetic acid adjusted to pH 5.0 with aqueous NH_3 was used as the mobile phase for the phenyl-hexyl column (Lab 1 condition 2).

Laboratory 2: The Japan Calibration Service System (JCSS) As standard solution (*ca.* 1000 mg L^{-1} As, which was prepared from high-purity As_2O_3) (Kanto Chemical Industries Ltd., Tokyo, Japan) was used as the source standard solution for the total As analysis; it was also used as the As(III) source standard solution on the speciation analysis. As(V) CRM (NMIJ CRM 7912-a) and DMA CRM (NMIJ CRM 7913-a), issued by the NMIJ, were used as source standard solutions for the determination of As(V) and DMA, respectively. These JCSS and CRMs materials are traceable to SI. As(V) was also used for the calibration of arsenosugar compounds in Lab 2 condition 1.

Source solutions of Sugar-328 [Sugar-OH] and Sugar-408 [Sugar- SO_4] were used: synthesized Sugar-OH (Hayashi pure chemical Ind., Ltd., Osaka, Japan),³⁰⁻³² and synthesized Sugar- SO_4 (Hayashi), respectively. After the desired arsenosugars compounds were purified by using ODS C_{18} and IC-Anion exchange columns with 10 $\text{mM NH}_4\text{H}_2\text{PO}_4$ (pH 8.25), their stock solutions were prepared. The concentrations of total As in the isolated arsenosugar solutions were determined by ICP-MS based on the JCSS As standard solution. The prepared Sugar-328 and -408 solutions were used as the calibrant for Sugar-328 and -408 in hijiki sample in Lab 2 condition 2. There are no pure materials of Sugar-392 and -482; the mixture of Sugar-392 and -482, which had been prepared by University of Graz, was just used for their identification. Working mixed standard solutions were prepared daily by mixing the stock solutions and appropriately diluting with water.

The mobile phases used for the ODS C_{18} and ODS C_{28} columns included 10 mmol L^{-1} sodium 1-butanefulfonate/4 mmol L^{-1} malonic acid/4 mmol L^{-1} tetramethylammonium hydroxide (TMAH)/0.05% methanol (pH 3.0) (Lab 2 condition 1) and 10 mmol L^{-1} sodium 1-butanefulfonate/4 mmol L^{-1} malonic acid/4 mmol L^{-1} TMAH/2 mmol L^{-1} $\text{NH}_4\text{H}_2\text{PO}_4$ /0.05% methanol (pH 2.7) (Lab 2 condition 2).

Ultrapure-grade acids and alkalis were used (Kanto Chemical Industries, Ltd., Tokyo, Japan). A high-purity enzyme of pepsin (from porcine gastric mucosa) was obtained from Sigma-Aldrich

(St. Louis, MO). Methanol (LC/MS analysis grade, Wako Pure Chemical Industries, Osaka, Japan) and dibutylammonium acetate (*ca.* 0.5 mol L⁻¹ in water, Tokyo Chemical Industries, Tokyo, Japan) were obtained. Water purified from a Milli-Q-Labo filter (resistivity of 18 MΩ·cm, Nippon Millipore, Ltd., Tokyo, Japan) was used throughout the experiment.

Sample and certified reference material

A candidate material of hijiki seaweed (*Hizikia fusiforme*) was prepared as a common sample: for inter-laboratory validation. Hijiki seaweed (*ca.* 22 kg) was collected from the west coast of Japan and washed with water to remove seawater and loosely attached debris. It was dried, freeze-pulverized, sieved (<100 μm), and homogenized. Then, the homogenized powder was transferred to amber glass bottles (*ca.* 20 g each) and sterilized by ⁶⁰Co gamma radiation (20 kGy). The bottles were individually sealed in aluminum-coated nylon packages. The candidate material was prepared in 640 bottles as the common sample.

The NMIJ CRM 7405-a hijiki seaweed powder (NMIJ/AIST) was used as the control material. The certified values (*k* = 2) for the total As and As(V) in NMIJ CRM 7405-a are (35.8 ± 0.9) mg kg⁻¹ and (10.1 ± 0.5) mg kg⁻¹, respectively.

Procedure for total arsenic determination

Laboratory 1: 200 mg of the sample was directly weighed into 12 mL quartz tubes, followed by the addition of 5 mL of concentrated nitric acid. The tubes were transferred to a Teflon[®] rack of the Ultraclave microwave system and covered with Teflon[®] caps. After the system was closed, an argon pressure of 4 × 10⁶ Pa was applied, and the mixture was heated to 250°C for 30 min before being allowed to cool to room temperature. After mineralization, the samples were transferred to 50 mL polypropylene tubes, followed by the addition of 500 μL of the internal standard (1 mg L⁻¹ Ge in 1% HNO₃) and water to 50 mL. All standards for the total As determination were prepared using 10 (v/v)% of concentrated HNO₃ and the same concentration of the internal standard to match the matrix of the digested samples. The concentrations of As in the digests were determined by ICP-MS.

Laboratory 2: A 0.50-g aliquot of the sample was precisely weighed and added to a PFA vessel, followed by the addition of 5 mL of HNO₃, 2 mL of H₂O₂, and 1 mL of HF. The samples were heated at 250 W for 5 min; 250 W for 10 min; 400 W for 10 min; 650 W for 5 min; and 250 W for 10 min, followed by cooling to room temperature. After cooling, the vessel containing the dissolved sample was placed on a hot plate to evaporate the sample to dryness. The residue was dissolved with 2 (w/w)% HNO₃ to make up 50 g of a sample solution. After appropriate dilution to contain 5 ng g⁻¹ of Rh, which was used as the internal standard, the resulting solution was used for determining the total As by ICP-MS. A gravimetric preparation method was employed in all preparations. Blank tests were carried out to investigate any possible As contamination; none was detected.

Extraction procedure for water-soluble arsenic compounds

Laboratory 1: Each sample of 100 mg was weighed with a precision of 0.1 mg and added to 15 mL polypropylene tubes, followed by the addition of 10 mL of water. Samples were extracted overnight on a rotating wheel at room temperature. The extracts were subjected to centrifugation at 4700g for 15 min. After filtration, the supernatant was directly used for HPLC-ICPMS analysis.

Laboratory 2: A portion of the sample (0.5 g) was accurately

weighed into a 50-mL polypropylene tube and then 10 g of water was added. The sample was subjected to ultrasonication in a bath for 1 h. The suspensions were subjected to centrifugation at 4000 rpm for 10 min, and the supernatant was passed through a 0.45-μm syringe-type PVDF membrane filter, and the filtrate was analyzed. For comparison, enzyme extraction was also carried out; 0.07 mol L⁻¹ of HCl containing 0.01 (w/w)% pepsin was added as the extracting solvent, followed by heating using a heating block system at 37°C for 2 h.

Sequential extraction

Sequential extraction was carried out on the candidate material in Laboratory 2. To increase the extraction efficiencies of water-soluble As compounds, water extraction was replicated three times. Then, the liquid and solid phases obtained at each stage were decomposed by microwave-assisted acid digestion. The total As concentrations in the samples were determined by ICP-MS.

Results and Discussion

Identification of arsenosugar compounds

The arsenosugar compounds in the isolated arsenosugar solutions were identified using UPLC-MS/MS equipped with an ODS C₁₈ column. Sugar-328 and Sugar-408 were confirmed by their protonated ions (*m/z* 329 for Sugar-OH and *m/z* 409 for Sugar-SO₄) and product ions (*m/z* 237 and 97 from *m/z* 329 for Sugar-OH and *m/z* 329 and 97 from *m/z* 409 for Sugar-SO₄).³³ Figure S1 (Supporting Information) shows the chromatograms of Sugar-328 and -408 solutions and an extract of candidate hijiki material. Both of Sugar-328 and -408 were detected from the extract of the candidate hijiki material.

The Sugar-328 and -408 solutions were analyzed and monitored by HPLC-ICP-MS, and no As containing impurity could be detected. The Sugar-328 and -408 solutions were spiked with the extract of the candidate hijiki material, and the peak positions on HPLC-ICP-MS with ODS column was confirmed (Figs. S2 and S3, Supporting Information).

Effect of extraction conditions

Extraction of the candidate material with water was carried out under various liquid/solid ratios ranging from 20 to 100 and extraction times of 1 to 10 h. Table S1 (Supporting Information) summarizes the results obtained. By monitoring the extracted As compounds using the ODS column, 9 peaks including some unknown peaks were observed (Fig. 1). The total As content of the extracts, recorded as the sum of the 9 As compounds, was constant (RSD 1.56%), as were the relative amounts of each compound, over the whole range of extraction conditions. These results demonstrated that the quantification of each As compound was not dependent on the extraction conditions.

Distribution of arsenic compounds and their mass balances

Sequential water extractions were carried out on the candidate material; and the results obtained of the sequential extraction are summarized in Table S2 (Supporting Information).

The total As extracted from the 1st extraction with water was (27.18 ± 0.40) mg kg⁻¹, which was in good agreement with the sum of As compounds from speciation analysis in Table S1. Arsenic was not detected from the 2nd and 3rd extractions with water, when the residual interstitial water in the solid phase after the first extraction was corrected. The non-extractable As (24.42 ± 0.60 mg kg⁻¹) was detected from the residue. The recovery, including water-soluble As compounds and the residue,

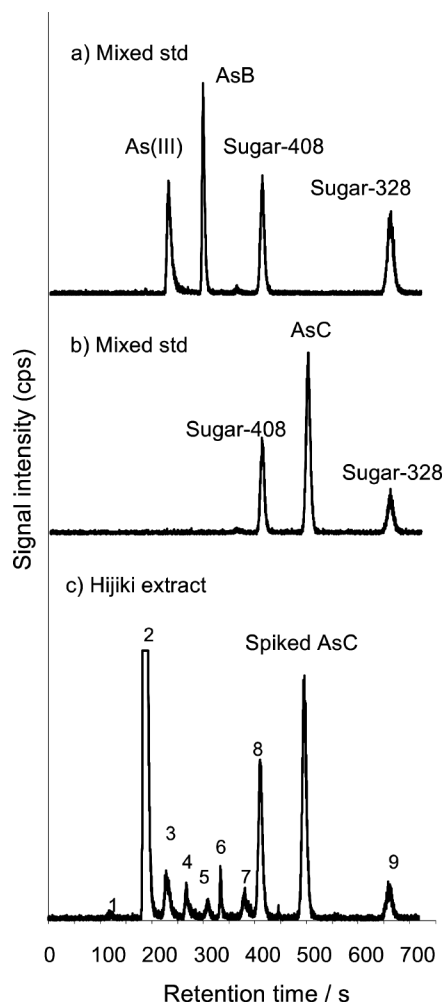


Fig. 1 Chromatograms of the synthesized Sugar-328, -408, and the extract. Extraction, water; column, ODS C_{28} ; mobile phase, 10 mmol L^{-1} sodium 1-butanefulfonate/4 mmol L^{-1} malonic acid/4 mmol L^{-1} TMAH/2 mmol L^{-1} $NH_4H_2PO_4$ /0.05% of methanol (pH 2.7); flow rate, 0.5 $mL\ min^{-1}$; injection volume, 20 μL ; c) 1, unknown-1; 2, As(V); 3, As(III) or unknown-2; 4, DMA; 5, Sugar-392; 6, Sugar-482; 7, unknown-3; 8, Sugar-408; 9, Sugar-328.

was $(104.2 \pm 1.9)\%$ based on the concentration of total As in the candidate material. Thus, the mass balances of water-soluble and insoluble As compounds based on the total As concentration are 53% and 47%, respectively.

Inter-laboratory validation

As mentioned above, candidate hijiki material contained Sugar-328, and -408 and some more water-soluble As compounds. In addition, the water-soluble As compounds can be completely extracted by water. Although some unknown peaks were detected, the inter-laboratory study was carried out by using the candidate hijiki material to validate the common As speciation techniques and the certification of As compounds.

Total arsenic determination

Since all data are required in terms of the dry mass fraction, the moisture content of the candidate material (*ca.* 1.0 g) was measured by drying it in an oven at $105^\circ C$ for 5 h as the condition typical for the dry mass correction factor. The moisture contents of 10 bottles varied from 7.29 to 8.25%. The assigned value for the moisture content was $(7.84 \pm 0.13)\%$

(Mean $\pm u$).

Table 1 summarizes the results obtained from the determination of the total As in the NMIJ CRM 7405-a and the candidate material after a correction for the moisture content. The total As from Laboratory 1 and 2 tests for the CRM 7405-a were $(35.2 \pm 0.3)\ mg\ kg^{-1}$ and $(35.5 \pm 0.3)\ mg\ kg^{-1}$, respectively; the results were in good agreement with the certified value. The results (six bottles, three sub-samples) obtained for the total As in the candidate material were $(49.2 \pm 0.4)\ mg\ kg^{-1}$ at Laboratory 1 and $(49.6 \pm 0.3)\ mg\ kg^{-1}$ at Laboratory 2, and they were in good agreement.

Speciation analysis for water-soluble arsenic compounds

In Laboratory 1 tests, water-soluble As compounds were determined by HPLC-ICP-MS using the IC-Anion and phenyl-hexyl columns. Table 1 summarizes the results, and Fig. 2 shows the obtained chromatograms. By using the IC-Anion column, As(V) in the CRM 7405-a was determined to be $(9.7 \pm 0.1)\ mg\ kg^{-1}$, a result well within the certified value of As(V). In addition, DMA, Sugar-408, Sugar-392 and Sugar-482 were also separated and detected. Cationic and neutral compounds, however, were not retained on this column, and thus it was not possible to separate Sugar-328 from other cationic and/or neutral compounds. Hence, the phenyl-hexyl column was employed for the separation of Sugar-328. The results obtained for As(V), Sugar-408, Sugar-328, Sugar-482, Sugar-392 and DMA in the candidate material were $(24.3 \pm 0.4)\ mg\ kg^{-1}$, $(1.42 \pm 0.03)\ mg\ kg^{-1}$, $(0.46 \pm 0.01)\ mg\ kg^{-1}$, $(0.20 \pm 0.01)\ mg\ kg^{-1}$, $(0.158 \pm 0.005)\ mg\ kg^{-1}$ and $(0.233 \pm 0.005)\ mg\ kg^{-1}$, respectively.

As(V), DMA, and arsenosugar compounds, namely Sugar-328, Sugar-408, Sugar-392 and Sugar-482, from the CRM using the ODS C_{18} column were determined in Laboratory 2. Table 1 summarizes the results, and Fig. 3 shows the chromatograms obtained. As(V) in the CRM was determined to be $(10.1 \pm 0.2)\ mg\ kg^{-1}$, which was in good agreement with the certified value. The results obtained from condition 1 for As(V), Sugar-408, Sugar-328, Sugar-482, Sugar-392 and DMA in the candidate material were $(24.5 \pm 0.4)\ mg\ kg^{-1}$, $(1.41 \pm 0.02)\ mg\ kg^{-1}$, $(0.45 \pm 0.01)\ mg\ kg^{-1}$, $(0.205 \pm 0.005)\ mg\ kg^{-1}$, $(0.154 \pm 0.003)\ mg\ kg^{-1}$ and $(0.25 \pm 0.01)\ mg\ kg^{-1}$, respectively. Also, the results obtained from condition 2 for As(V), Sugar-408, Sugar-328, Sugar-482, Sugar-392 and DMA in the candidate material were $(24.4 \pm 0.5)\ mg\ kg^{-1}$, $(1.40 \pm 0.04)\ mg\ kg^{-1}$, $(0.43 \pm 0.01)\ mg\ kg^{-1}$, $(0.200 \pm 0.001)\ mg\ kg^{-1}$, $(0.158 \pm 0.006)\ mg\ kg^{-1}$ and $(0.24 \pm 0.01)\ mg\ kg^{-1}$, respectively.

Certified values of arsenosugars in the CRM 7405-a were not available; therefore, it is difficult to discuss their accuracies; however, all results obtained for the candidate material in Laboratories 1 and 2 showed good agreement. Further, all water-soluble As compounds in the candidate material showed good agreement within each the measurement uncertainty.

Uncertainties and combined values³⁴

The property values of each As species in the candidate material were calculated as a weighted mean of the results of HPLC-ICP-MS at Laboratories 1 and 2 in Table 1, where $1/u_i$ was used as the weighting factor, because there was no significant anomaly among the data. The property values of each As species are summarized in Table 2.

Uncertainties were estimated uncertainties related to the standard solution (u_{std}): those related to the measurement results ($u_{anal}(x)$), and those related to the dry mass correction factor (u_{dry}).

The results obtained from each laboratory comprised three sub-samples from six bottles ($n = 18$); therefore, inhomogeneity

Table 1 Determination results of total As and water-soluble As compounds

		NMIJ CRM 7405-a			Candidate material		
		Mean/mg kg ⁻¹ (as As)	<i>u</i> /mg kg ⁻¹	Relative <i>u</i> , %	Mean/mg kg ⁻¹ (as As)	<i>u</i> /mg kg ⁻¹	Relative <i>u</i> , %
Total As	Lab 1	35.2	0.3	0.9	49.2	0.4	0.7
	Lab 2	35.5	0.3	0.9	49.6	0.3	0.6
As(V)	Lab 1 condition 1	9.7	0.1	1.1	24.3	0.4	1.6
	Lab 2 condition 1	10.1	0.2	2.0	24.5	0.4	1.8
	Lab 2 condition 2				24.4	0.5	2.0
Sugar-408	Lab 1 condition 1	3.23	0.03	1.0	1.42	0.03	1.9
	Lab 2 condition 1	3.36	0.03	1.0	1.41	0.02	1.3
	Lab 2 condition 2				1.40	0.04	2.9
Sugar-328	Lab 1 condition 2	0.26	0.01	5.2	0.46	0.01	1.9
	Lab 2 condition 1	0.32	0.01	2.6	0.45	0.01	1.3
	Lab 2 condition 2				0.43	0.01	1.5
Sugar-482	Lab 1 condition 1	1.18	0.02	1.5	0.20	0.01	3.0
	Lab 2 condition 1	1.24	0.01	1.1	0.205	0.005	2.4
	Lab 2 condition 2				0.200	0.001	0.5
Sugar-392	Lab 1 condition 1	0.64	0.01	2.0	0.158	0.005	2.9
	Lab 2 condition 1	0.63	0.01	1.0	0.154	0.003	2.1
	Lab 2 condition 2				0.158	0.006	3.7
DMA	Lab 1 condition 1	0.59	0.03	5.7	0.233	0.005	2.2
	Lab 2 condition 1	0.60	0.02	2.9	0.25	0.01	2.2
	Lab 2 condition 2				0.24	0.01	4.0

Lab 1 condition 1: IC-Anion column, water extraction, Lab 1 condition 2: phenyl-hexyl column, water extraction. Lab 2 condition 1: ODS C₁₈ column, water extraction, Lab 2 condition 2: ODS C₂₈ column, extraction 0.07 mol L⁻¹ containing 0.01 (w/w)% of pepsin.

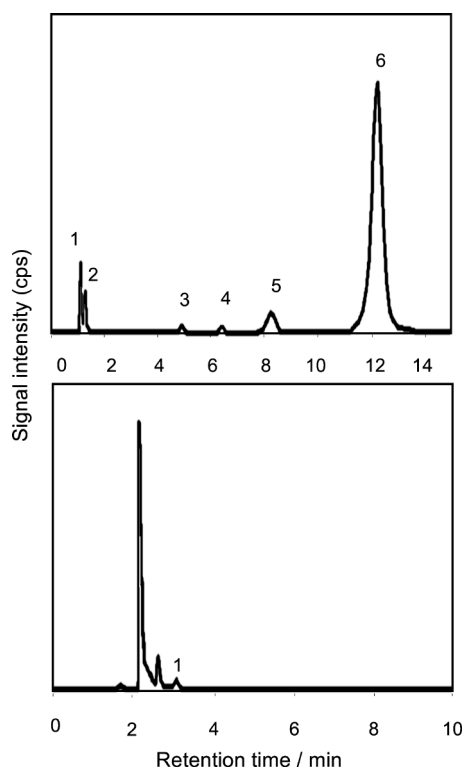


Fig. 2 Chromatograms of As compounds in the candidate material at Laboratory 1. (Upper) Column, AS14a anion-exchange column; mobile phase, 5 mmol L⁻¹ formic acid adjusted to pH 4.0 with aqueous ammonia; flow rate, 0.7 mL min⁻¹; injection volume, 10 μ L; extraction, water; 1, cationic and neutral species; 2, DMA; 3, Sugar-482; 4, Sugar-392; 5, Sugar-408; 6, As(V). (Lower) Column, phenyl-hexyl reversed-phase column; mobile phase, 10 mmol L⁻¹ of acetic acid adjusted to pH 5.0 with aqueous NH₃; flow rate, 0.7 mL min⁻¹; injection volume, 10 μ L; extraction, water; 1, Sugar-328.

depending on the bottles is contained in the measurement results. Thus, an uncertainty related to inhomogeneity is not estimated. The combined standard uncertainty (u_c) was calculated based on all of the uncertainty components in the budget (Table 2). As results, the combined mass fractions of each As species in the candidate material were estimated with the expanded uncertainty (U), where k is a coverage factor and U corresponds to the 95% confidence intervals when k is set at 2.

Conclusions

In this study, an inter-laboratory study was carried out using a prepared common hijiki seaweed sample. Independent extraction procedures and different chromatographic techniques were applied for the As species in the candidate material; however, when the data were evaluated based on each value reported, the results obtained from the quantitative determination of As(V), DMA, and arsenosugar compounds were consistent within its measurement uncertainties. Also, the relative combined standard uncertainties of the results of the inter-laboratory study were 1.0% for total As, 1.3% for As(V), 1.7% for Sugar-408, 1.8% for Sugar-328, 1.7% for Sugar-482, 1.7% for Sugar-392 and 1.8% for DMA.

To evaluate the results of the inter-laboratory study, the extraction efficiency and multiple measurement methods were investigated. A comparison between the total water-soluble As and the sum of the concentrations of the chromatographed water-soluble As compounds gave a 99% mass balance. Water-soluble and insoluble compounds in the candidate material were investigated and the stepwise extraction provided 53 and 47% extraction of the total As from the candidate materials, respectively. The recovery of the entire mass balance was confirmed to be \sim 100%. Those results indicated that the inter-laboratory study was performed accurately based on measurements.

Table 2 Combined mass fractions of total As and water-soluble As compounds in the candidate material and its uncertainty values

As compound	Mass fraction/ mg kg ⁻¹ (as As)	Uncertainty/ mg kg ⁻¹ (k = 2)	Relative standard uncertainty, %			Combined standard uncertainty	
			u_{std}	u_{anal}	u_{dry}	u_c (Relative), %	u_c /mg kg ⁻¹
Total As	49.4	1.0	0.20	0.46	0.86	1.0	0.5
As(V)	24.4	0.6	0.84	0.50	0.86	1.3	0.3
Sugar-408	1.41	0.05	1.39	0.43	0.86	1.7	0.02
Sugar-328	0.45	0.02	1.39	0.80	0.86	1.8	0.008
Sugar-482	0.202	0.007	1.39	0.59	0.86	1.7	0.004
Sugar-392	0.157	0.005	1.39	0.57	0.86	1.7	0.003
DMA	0.239	0.009	1.39	0.75	0.86	1.8	0.004

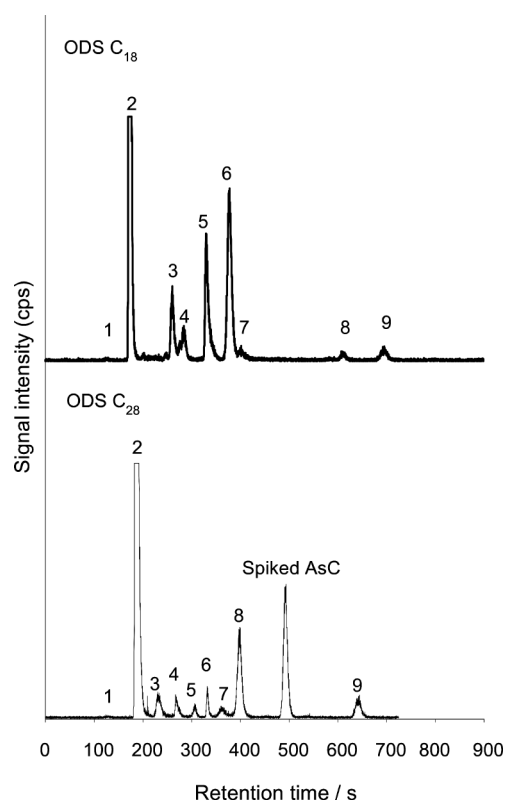


Fig. 3 Chromatograms of As compounds in the candidate material at Laboratory 2. (Upper) Column, ODS C₁₈ column; mobile phase, 10 mmol L⁻¹ sodium 1-butanefulfonate/4 mmol L⁻¹ malonic acid/4 mmol L⁻¹ TMAH/0.05% methanol (pH 3.0); flow rate, 0.5 mL min⁻¹; injection volume, 20 μL; extraction, water; 1, unknown-1; 2, As(V); 3, DMA; 4, Sugar-392; 5, Sugar-482; 6, Sugar-408; 7, unknown-2; 8, unknown-3; 9, Sugar-328. (Lower) Column, ODS C₂₈; mobile phase, 10 mmol L⁻¹ sodium 1-butanefulfonate/4 mmol L⁻¹ malonic acid/4 mmol L⁻¹ TMAH/2 mmol L⁻¹ NH₄H₂PO₄/0.05% methanol (pH 2.7); flow rate, 0.5 mL min⁻¹; injection volume, 20 μL, extraction, 0.07 mol L⁻¹ HCl containing 0.01 (w/w)% of pepsin; 1, unknown-1; 2, As(V); 3, unknown-2; 4, DMA; 5, Sugar-392; 6, Sugar-482; 7, unknown-3; 8, Sugar-408; 9, Sugar-328.

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Supporting Information

Details of the chromatograms. This material is available free of charge on the Web at <http://www.jsac.or.jp/analsci/>.

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