Inorganic arsenic speciation analysis in food using HPLC/ICP-MS: Method development and validation

Yu-Cheng Lai, Yi-Chen Tsai, Yu-Ning Shin, Ya-Chun Chou*, Ying-Ru Shen, Nu-Ching Lin, Shu-Han Chang, Ya-Min Kao, Su-Hsiang Tseng, Der-Yuan Wang

Division of Research and Analysis, Taiwan Food and Drug Administration, Ministry of Health and Welfare, No.161-2, Kunyang St, Nangang District, Taipei City, 11561, Taiwan, ROC

Abstract

Arsenic (As) compounds can be classified as organic or inorganic, with inorganic arsenic (iAs) having significantly higher toxicity than organic As. As may accumulate in food materials that have been exposed to As-contaminated environments. Thus, the "Sanitation Standard for Contaminants and Toxins in Foods" published by the Ministry of Health and Welfare set the standard limits for iAs content in rice, seaweed, seafood, and marine oils to safeguard public health. Therefore, a robust analytical method must be developed to selectively and quantitatively determine iAs content in rice, seaweed, seafood, and marine oils. Herein, we reported and verified the method of combined high-performance liquid chromatography/inductively coupled plasma-mass spectrometry (HPLC/ICP-MS) to determine iAs content in a wide variety of food. The fish oil samples were spiked with different concentrations of the As(III) standard solution, and their iAs analyzes were obtained via extraction procedures using the 1% (w/w) nitric acid (HNO_3) solution containing 0.2 M hydrogen peroxide (H_2O_2) under sonication. The extracts were subsequently analyzed for their As(V) contents using HPLC/ICP-MS with aqueous ammonium carbonate as the mobile phase. The As(III) species had completely oxidized into the As(V) species, which prevented interferences between organic and iAs during chromatography. The method showed good extraction efficiencies (generally >90%) for the iAs samples, and their limits of quantification in fish oil were 0.02 mg/kg. The method was verified via the iAs speciation analytes of rice, seaweed, seafood, and marine oil matrices. The average recoveries for the fortified samples of each matrix ranged from 87.5 to 112.4%, with their coefficients of variation being less than 10%. Surveillance studies were conducted on the iAs contents of food samples purchased from local Taiwanese markets. The results showed that the only Hijiki (*Sargassum fusiforme*) higher than the maximum limit of the sanitation standard for iAs in seaweed, whereas the remaining samples met their corresponding requirements. This method is quick and straightforward, and it can be applied for the routine analysis of iAs content in a wide variety of food products to ensure public health safety.

Keywords: Arsenic speciation, HPLC/ICP-MS, Inorganic arsenic in food

1. Introduction

Arsenic (As) is a naturally occurring toxic element that is ubiquitous in the environment and classified as a human carcinogen (Group I) [1]. As can exist as an inorganic or organic compound, and its speciation analysis is essential because the different As species exhibit different toxicity levels. Inorganic As (iAs) species such as arsenite (As(III)) and arsenate (As(V)) exhibit higher toxicities than the organic As species of dimethylarsinic acid (DMA), monomethylarsonic acid (MMA), arsenobetaine (AsB), and arsenocholine (AsC). As(III) and As(V) harm human and animal health; sustained exposures to As(III) and As(V) result in both carcinogenic and non-carcinogenic problems. Exposure to iAs increases the risks of developing numerous physiological disorders and a variety of detrimental health effects, including cardiovascular diseases, neurological effects, diabetes mellitus, skin disorders, and various cancers (bladder, skin, lung, and kidney) [2–4]. Human exposure to iAs occurs predominantly through drinking As-contaminated groundwater and

consuming food products grown in natural As environments (i.e., rock weathering) and near anthropogenic processes (i.e., mining activities). Rice is a staple crop consumed in considerable quantities in Asia, which makes the intake of iAs via rice consumption a significant risk factor [5,6]. Elevated concentrations of iAs have been detected in rice in several South-East Asian countries, which increases the risk of iAs intake for consumers [7−10]. In addition to rice, the consumption of seaweed, seafood, and marine oils has become major contributors to iAs intake, thereby posing potential health risks and attracting extensive attention to their exposure through consumption. However, the iAs concentration in food does not represent the total As concentration [11,12], e.g., marine foods generally contain low concentrations of iAs because As predominantly exists as non-toxic organic As species such as arsenobetaine (AsB) [13,14]. Several proficiency tests on the iAs contents of different food commodities (e.g., rice, seaweed, and seafood) were conducted recently to assess the validity of the methods employed [15,16]. The test materials were sent to the participating laboratories, where their iAs concentrations were measured according to the laboratory's method of choice. The iAs concentrations measured for the rice samples showed consistency; however, those measured for the seafood and seaweed samples showed inconsistencies [17]. The expert laboratories established the iAs concentrations for the different food commodities investigated in the proficiency tests, for which no certified values existed. As stated above and in contrast to terrestrial food, seafood can contain high concentrations of total As but generally relatively low concentrations of iAs. The remainder of the As content may consist of the non-toxic AsB and numerous other organic As compounds [18]. Herein, the challenge is to separate the analytes of interest, i.e., to separate the toxic iAs from the multitude of organic As compounds.

Numerous techniques have been applied for the speciation analysis of As in food, with the highlighted approach of the high-performance liquid chromatography inductively coupled plasma mass spectrometry (HPLC/ICP-MS) method having gained significant attention. HPLC/ICP-MS is currently the most frequently applied method for the speciation analysis of As in food owing to its outstanding advantages of superior separation performance and high sensitivity. The essential step preceding the instrumental analysis is the preparation of the analyte sample via the extraction of the targeted species using extractants such as an acid, base, enzyme, or methanol. It is essential that As is

extracted from the sample without changing its chemical speciation during speciation analysis. Numerous literature reviews regarding various chemical and enzymatic extractions have been consulted for the extractions of As species from plant and animal tissues [19−26]. Moreover, measuring As(III) and As(V) individually in food was not considered necessary because human risk assessment is based on iAs and does not make distinction between As(III) and As(V) [27]. Therefore, it has become common practice to add an oxidant (commonly hydrogen peroxide (H_2O_2)) [28] to convert all As(III) into As(V) and to express the As(V) concentration as iAs. This approach also removes the need to quantify As(III) in the presence of other closely eluting As species when using an anion-exchange column. The most common method for species extraction uses diluted (1−2%) nitric acid (HNO_3), which is an effective extractant for plants [29,30] and marine tissues [31], and a wide variety of other food matrices [32].

With the increased risk of iAs intake for consumers, it is necessary to develop a rapid method for the routine analysis of iAs content in food. Therefore, this study aims to establish a rapid and simple method for extracting and determining iAs content in a wide variety of food materials. Herein, we develop the analytical strategy for extracting iAs from food samples using HNO_3/H_2O_2 and measuring the extract using HPLC/ICP-MS with an ammonium carbonate buffer that has been optimized for the rapid determination of iAs in different food matrices. Further, we apply the method to the samples of rice, seaweed, seafood, and marine oils to determine their iAs contents and examine the validity of this method for the establishment of regulatory experiments.

2. Materials and methods

2.1. Reagents and chemicals

All reagents were of analytical grade and ultrapure grade unless specified otherwise. Ultra-pure water (H_2O; 18.2 MΩ cm^{-1}) was purified using the Millipore water purification system (Billerica, MA, USA). HNO_3 (purity ≥67%) and ammonium carbonate were purchased from J.T. Baker (Phillipsburg, NJ, USA). Methanol (purity ≥99.9%), *n*-hexane (purity ≥95%), H_2O_2 (purity ≥30%), and acetic acid (CH_3COOH; purity ≥99%) were purchased from Merck Ltd. (Darmstadt, Germany).

Analytical standards of As(III) and As(V) were purchased from Inorganic Ventures (Christiansburg, VA, USA). AsB (purity ≥95%) was purchased

from Sigma—Aldrich (Louis, MO, USA). AsC bromide (purity $\geq$98%) was supplied by Toronto Research Chemicals Inc. (Toronto, ON, Canada). MMA (purity $\geq$95%) was purchased from Chem Service (West Chester, PA, USA). DMA (purity $\geq$95%) was obtained from Wako (Osaka, Japan).

2.2. Samples and reference samples

The matrices evaluated in this study included rice, seaweed, seafood, and marine oil samples as follows. Rice samples of polished rice, brown rice, and rice cereal. Seaweed samples of hijiki (Sargassum fusiforme), kelp bud, kelp knot, kombu, laver, and processed seaweed. Seafood samples of lobster, mud crab, flower crab, neritic squid, squid, clams, fresh oysters, shrimps, and spiny shrimp. Muscles of salmon, tilapia, swordfish, grass carp, flounder, milkfish. Marine oil samples of salmon oil, tuna oil, refined fish oil, and deep-sea fish oil. All samples were purchased from traditional local markets or supermarkets in Taipei, Taiwan. Each sample type was finely cut using ceramic scissors, homogenized using the electric food processor, and placed in the refrigerator at 4 $^{\circ}$C before analysis. The National Institute of Standards and Technology (NIST) Certified Reference Material (CRM) NIST SRM 1568b (Gaithersburg, MD, USA) for rice flour; CRM-TORT3 and CRM-DORM4 from National Research Council of Canada (NRC); CRM-7402a, CRM-7403a and CRM 7405-a from National Metrology Institute of Japan (NMIJ) for marine samples was used as the reference materials.

2.3. Sample preparation

The iAs analytes were obtained via extraction procedures performed on the samples spiked with different concentrations of the As(III) standard solution. The optimal extraction process was as follows: 1.0 g sample of the homogeneous material were weighed accurately and placed into separate 50 mL polypropylene centrifuge tubes. The samples were spiked with 1, 5, and 10 μg mL^{-1} As(III) standard solutions at room temperature for 10 min. The addition of 10 mL extraction solvent (1% (w/w) HNO$_3$ containing 0.2 M H$_2$O$_2$) was made into each tube under the ultrasonic bath at 80 $^{\circ}$C for 30 min. The samples were cooled and centrifuged at 3000$\times$g for 10 min, and their supernatants were subsequently transferred into 50 mL storage bottles. The addition of 5 mL extraction solvent was made into the residue, and the above steps were repeated to conduct one extraction. The supernatants were combined with the first extracts and

diluted to 20 mL using the extraction solvent. If there was residual fish oil in the solution, 1 mL n-hexane was added for liquid—liquid extraction, and the bottom layer was taken for chromatography. All solutions collected from the procedure were filtered through a 0.22 μm PVDF membrane filter before their HPLC/ICP-MS analyses. The corresponding blank sample was prepared using the same procedure described above. In addition, a solvent blank was prepared to check for background contamination.

2.4. HPLC/ICP-MS instrumentation

ICP-MS measurements were performed using the Agilent 7700x, and HPLC measurements were performed using the Agilent 1260 series instrument (Santa Clara, CA, USA). HPLC separations were performed under the anion-exchange conditions at 25 $^{\circ}$C with PRP-X100 columns (4.6 $\times$ 150 mm, 5 μm; Hamilton Company, Reno, Nevada, USA) and the mobile phase of ammonium carbonate (0.5 or 200 mmol L^{-1} at pH 8.5, adjusted with 50% acetic acid). Mobile phase A consisted of 200 mmol L^{-1} ammonium carbonate in 3% (v/v) methanol, and mobile phase B consisted of 0.5 mmol L^{-1} ammonium nitrate in 3% (v/v) methanol. The gradient program was set as follows: the initial condition was 0—2 min: 0% A; 2—3 min: 0—50% A; 3—8 min: 50—50% A; 8—9 min: 50—100% A; 9—12 min: 100—100% A; 12—13 min: 100—0% A; and 13—15 min: 0% A at a flow rate of 1 mL min^{-1}. The sample injection volume was 20 μL. All As compounds were successfully separated within 8 min, and the total run time for this analysis was 15 min. Maximum sensitivity and quantitative measurements of the peak areas were achieved by selecting the optimum detection for each As compound. Quantification was performed using external calibration and the peak area measurements. The instrumental operating conditions are shown in Table 1.

2.5. Method validation

The standard calibration curves were obtained by diluting the standard solutions to final concentrations ranging from 1 to 20.0 mg L^{-1} using the 1% (w/w) HNO$_3$ solution containing 0.2 M H$_2$O$_2$. The limit of quantification (LOQ) was estimated at a signal-to-noise (S/N) ratio $\geq$10. The accuracy, precision, and reproducibility were assessed by determining the As(V) content in the fortified samples containing 0.02, 0.1, and 0.2 mg kg^{-1} of the As(III) standard solution, using HPLC/ICP-MS analysis for five replicates.

Table 1. *Operational conditions for HPLC/ICP-MS analysis of As speciation.*

Parameter	HPLC condition
Instrument	1260 Infinity Series
Column	Hamilton PRP-X100 (4.6 × 150 mm, 5 μm)
Injection volume	20 μL
Column temperature	Ambient
Mobile phase	A : 200 mM (NH4)2CO3 in 3% (v/v) methanol, pH 8.5
	B : 0.5 mM (NH4)2CO3 in 3% (v/v) methanol

Gradient program	Time (min)	Flow rate (mL/min)	A (%)	B (%)
	0	1	0	100
	2	1	0	100
	3	1	50	50
	8	1	50	50
	9	1	100	0
	12	1	100	0
	13	1	0	100
	15	1	0	100

Parameter	ICP-MS condition
Spectrometer	Agilent 7700x ICP-MS
Interface	Sampler and skimmer cones of Pt
R.F.[a] power	1550 W
Sampling depth	8 mm
Ar flow, L/min	Plasma, 15; Auxiliary, 0.9; Nebulizer, 1.2
Optimization	59 Co, 89 Y, 205 TI
Analysis mode	He mode, He gas 4.0 mL/min
Isotopes monitored	^{75}As, ^{35}Cl, ^{37}Cl
Dwell time	1.0 s/^{75}As; 0.1 s/^{35}Cl, ^{37}Cl

[a] R.F. = Radio frequency.

3. Results and discussion

3.1. Optimization of chromatographic separation conditions

Previous studies have shown that pH is an essential factor for the speciation and successful separation of As species on an anion-exchange column with the mobile phase. Therefore, a preliminary study was conducted to select the mobile phase with a pH range of 5−9 according to previous publications [33−35] to ensure stable retention times of the As species upon the injection of acidic extracts onto the column, high elution power for As(V) to reduce analysis time, and high volatility to achieve long-term stability of the HPLC/ICP-MS measurements. Therefore, the well-established HPLC method prior to our research employed the Hamilton PRP-X100 column with the pH 8.5 carbonate buffer as the mobile phase. However, a significant problem encountered when using this method to measure low concentrations of As was interference from argon chloride ($^{40}Ar^{35}Cl$ on ^{75}As) as found in the acid extractions and matrix interferences. Thus, ICP-MS with He gas technology was applied to eliminate these interfering matrix components

according to the different chemical reactions that occur between the analytes and interfering ions. He (purity ≥99.999%) was chosen as the collision gas for the He mode because it deflected the interferences from the $ArC1^+$ at m/z 75 and enhanced the accuracy of the As^+. The flow rate of He was optimized and set at 4.0 mL/min in this study.

For As speciation analysis, the six As species were analyzed under gradient conditions using the mobile phase A mixture of 200 mmol L^{-1} ammonium carbonate and 3% (v/v) methanol, and the mobile phase B mixture of 0.5 mmol L^{-1} ammonium carbonate and 3% (v/v) methanol. The additional methanol improved peak shape with the adjustment of mobile phases and accelerated the emergence of the organic compounds. The detailed conditions for the HPLC method are shown in Table 1. The elution order was as follows: AsC, AsB, As(III), DMA, MMA, and As(V). Moreover, all the As species were separated in less than 8 min without affecting their resolutions when 1% HNO_3 was used on the samples spiked with 10 μg L^{-1} As standard solution, as shown in Fig. 1. The linearity and correlation coefficients (r) of the calibration curves for AsC, AsB, As(III), DMA, MMA, and As(V) were all higher than 0.9995 for the As speciation analysis, which can be
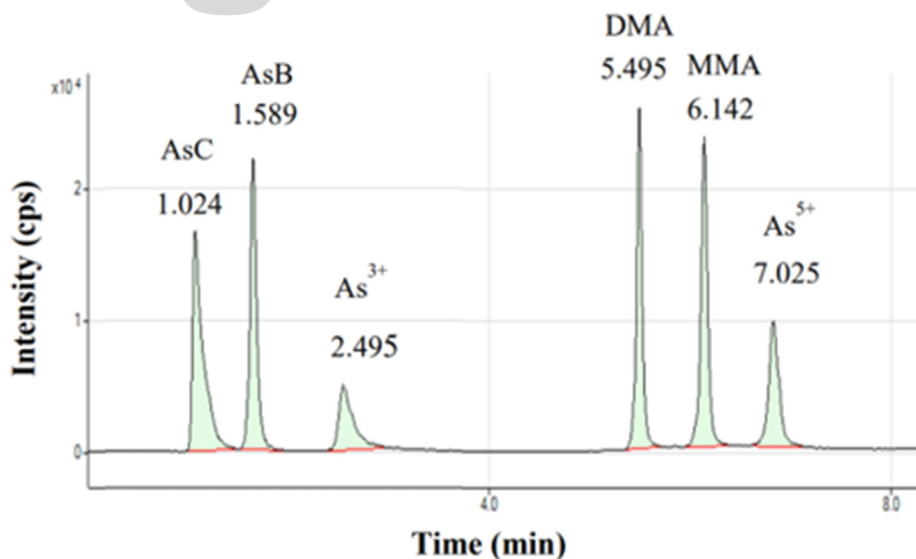
Fig. 1. HPLC/ICP-MS chromatogram of the standard solutions containing As compounds (AsC, AsB, As(III), DMA, MMA, and As(V)) at 10 μg L^{-1} dissolved in 1% (w/w) HNO$_3$ and separated on the anion-exchange column (PRP-X100).

considered satisfactory and within the acceptable variation range.

3.2. Optimization of extractant

Our study investigated the extraction of total As in food using the following extraction solutions: (i) deionized water, (ii) nitric acid solution, and (iii) 50% (v/v) ethanol. The extraction of nitric acid solution extracted the total As more efficiently than the deionized water and 50% (v/v) ethanol (Supplementary Table 1 (https://www.jfda-online.com/ cgi/viewcontent.cgi?filename=0&article=3432& context=journal&type=additional)). For fish oil, the extract efficiencies of different solvents were measured by spiking with 0.2 mg kg^{-1} each of various arsenic standards. The results shown in Supplementary Table 2 (https://www.jfda-online. com/cgi/viewcontent.cgi?filename=0&article=3432& context=journal&type=additional), nitric acid solution achieve better extraction rate with the sum of the extracted arsenic about 95.8%. Thus, we chose to prepare the standard solutions using the nitric acid solution, which resulted in better peak shapes and
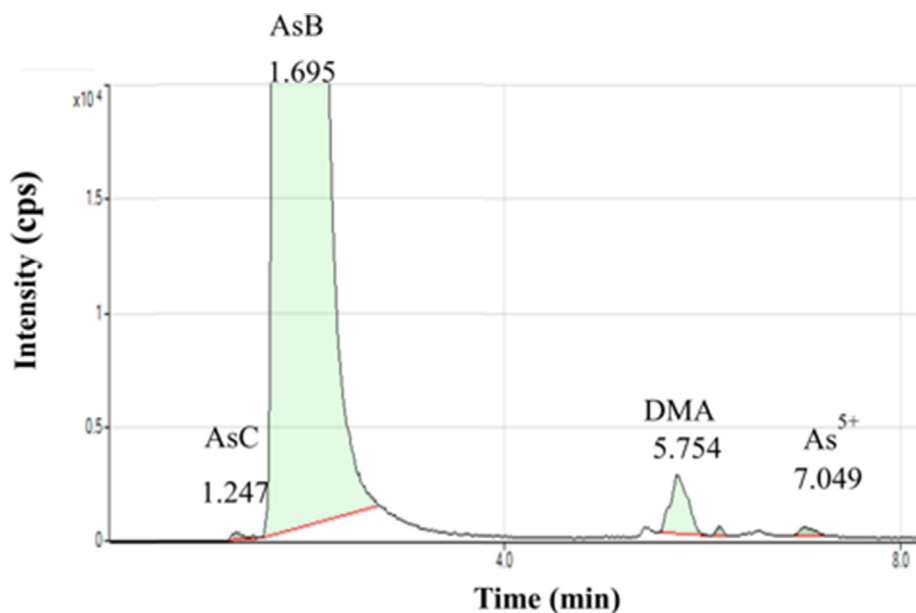

Fig. 2. HPLC/ICP-MS chromatogram of the As compounds in the lobster sample separated on the anion-exchange column (PRP-X100).

resolutions. Moreover, the effect of the extraction temperature by using sonication to extract 1 g NIST SRM 1568b rice flour studied at 50, 60, 70, and 80 °C. The recoveries were increased with the temperature, shown as $56.4 \pm 6.4\%$, $69.5 \pm 7.4\%$, $94.1 \pm 1.9\%$ and $99.8 \pm 1.8\%$. This demonstrated that 80 °C as the optimal extraction temperature selected for the following analyses.

The HPLC/ICP-MS chromatogram of the As speciation analysis of the lobster extract is shown in Fig. 2. However, AsB was the primary compound detected in the lobster samples analyzed, with As(III) enshrouded in the main organic compound of AsB, while As(III) was undetected. H_2O_2 proved to be an effective solvent for the extraction of iAs from food because it converted all of the As(III)

species into the As(V) species during extraction, which solved the challenges of difficult separations and quantifications between AsB and As(III). The highest extraction efficiency of all the samples was achieved using the HNO_3/H_2O_2 method, which correlated with the findings of this study.

For As speciation analysis, the As(III) species is commonly oxidized completely into the As(V) species before the HPLC procedure [36,37], to overcome the uncertainties associated with distinguishing between the measurements of As(III) and AsB on anion-exchange columns and prevent inter-conversions between the As(III) and As(V) species. The extraction procedure aimed to extract the iAs species from the organic matrix quantitatively without converting the organic As species into the iAs

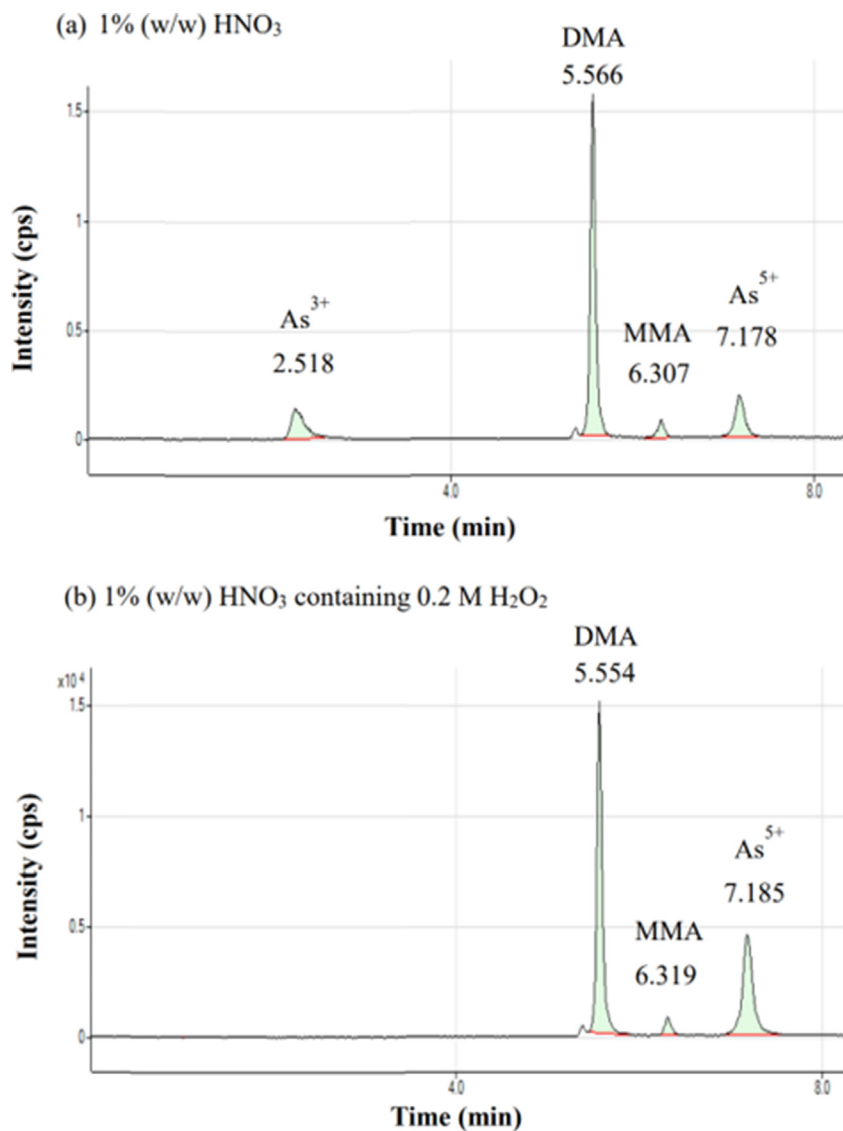Fig. 3. HPLC/ICP-MS chromatograms of the As compounds in the standard reference material 1568b (rice flour) extracted using 1% (w/w) HNO_3 (a) or 1% (w/w) HNO_3 containing 0.2 M H_2O_2 (b) and separated on the anion-exchange column (PRP-X100).

species. In reference to previous research [38], we extracted the iAs species from the NIST SRM 1568b rice flour using the 1% (w/w) HNO_3 solution containing 0.01, 0.05, 0.1, 0.2, and 0.4 M H_2O_2, as shown in Figs. 3 and 4. This was conducted to evaluate the concentration of H_2O_2 sufficient for the complete oxidation of As(III) into As(V) and test the stabilities of the organic As species (DMA and MMA) present in the solutions. This was verified to be true via the As speciation analysis of the NIST SRM 1568b rice flour (0.093 mg iAs kg^{-1} representing $101.1 \pm 1.9\%$ of total iAs) in the 1% (w/w) HNO_3 solution containing 0.2 M H_2O_2, whose results strongly agreed with the certified value (0.092 ± 0.010 mg iAs kg^{-1}). The consistent As(V) content showed that the DMA and MMA did not degrade under the reaction conditions. The procedure requires the complete oxidation of As(III) into As(V) and the absence of conversions (demethylations) by organic As species such as DMA and MMA into As(V). Furthermore, the previous studies reported that approximately 100% of the arsenic species were extracted by HNO_3/H_2O_2 acidic solvents [38,39]. According to the results, the 1% (w/w) HNO_3 extraction solution containing 0.2 M H_2O_2 was regarded as sufficient for the complete oxidation of As(III) into As(V).

3.3. Method validation

The LOQ was calculated at the S/N ratio of 10 in the sample blank (n = 5), where the S/N ratio represented those of the three concentrations of the As(III) standard solutions in the spiked fish oil samples at the estimated experimental LOQ shown in Table 2. The results obtained were calculated according to the Taiwan Food and Drug Administration (TFDA) guidelines for the "Test Method Validation Specification of Food Chemistry," and 0.02 mg kg^{-1} iAs was determined to be the basis for the method LOQ.

The sufficiency of the H_2O_2 concentration for the complete oxidation of As(III) into As(V) was investigated in more complex food matrices. The validation results obtained from the As speciation analytes of polished rice, seaweed, lobster, salmon, tilapia, and salmon oil are listed in Table 3. The results showed satisfactory recoveries within the acceptable range of 80–120% for all the samples, in regard to their spiked levels. At the spiked levels of 0.02, 0.10, and 0.20 mg kg^{-1} As(III) in the fortified samples (n = 5), the recovery performances were as follows: polished rice (89.7–103.9%), seaweed (97.1–108.1%), lobster (101.0–109.2%), salmon (87.5–93.6%), tilapia (103.5–112.4%), and salmon oil (90.4–99.8%). The iAs content was determined as the sum of As(III) and As(V); the average recoveries for the fortified samples of each matrix ranged from 87.5 to 112.4%, and their coefficients of variation were less than 10%. In addition, As(V) in marine CRMs and recovery experiments were analyzed. At the spiked levels of 0.10 mg kg^{-1}, the recovery of As(V) from the lobster hepatopancreas (CRM-TORT3), fish protein (CRM-DORM4), cod fish tissue (CRM-7402a) and swordfish tissue (CRM-7403a) was 95.5 ± 3.65, 113.9 ± 6.75, 110.1 ± 2.89, and $115.4 \pm 1.37\%$, respectively (Supplementary Table 3 (https://www.jfda-online.com/cgi/viewcontent.cgi?filename=0&article=3432&context=journal&type=additional)). These results clearly indicated that the analytical method exhibited good applicability for
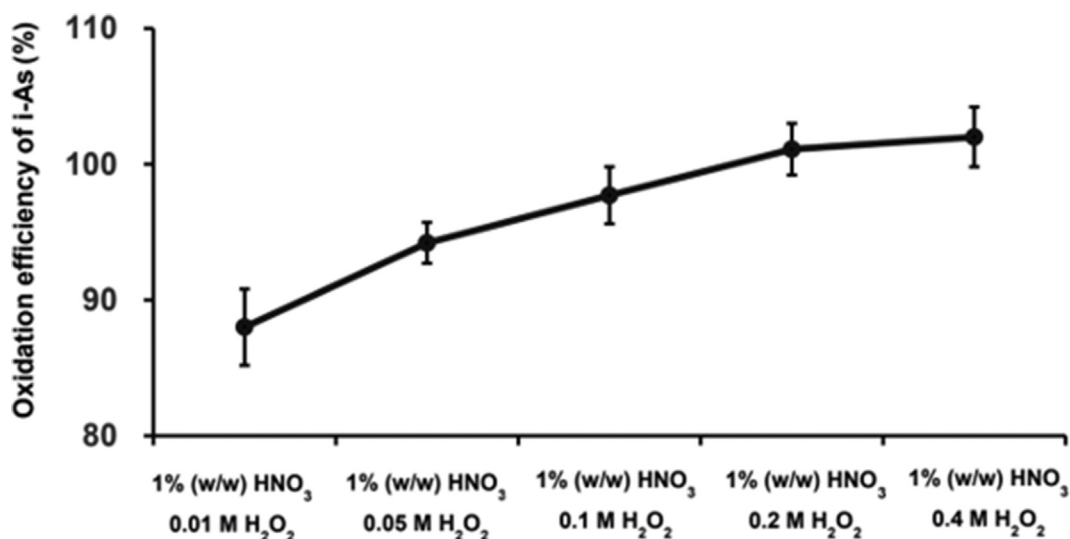

Fig. 4. Oxidation efficiency of the As(III) for different concentrations of H_2O_2 in the NIST SRM 1568b rice flour.

Table 2. *Assessment of the limits of quantitation (LOQ) of iAs analytes extracted from fish oil samples.*

Matrix	As compound	Spiked level (mg/kg)	S/N ratio[b]
Fish oil	iAs[a]	0.01	7.52 ± 1.55
		0.02	12.5 ± 2.19
		0.05	32.4 ± 3.71

[a] iAs = As(III) oxidized using H_2O_2.
[b] Mean ± SD, $n = 5$.

analyzing iAs in a wide variety of foods. To the best of our knowledge, for the first time, the developed method can be generalizable to nearly all food matrices, including rice (carbohydrate), seaweed (cellulose), seafood (protein) and fish oil (fat).

3.4. Determination of iAs in real food samples

This study investigated the iAs contents of commercial food products and established an analytical method to conduct small-scale market surveys to test 50 food samples, including rice, seaweed, seafood, and marine oil capsules, purchased from local markets in Taipei, Taiwan. The surveillance results showed that the average of iAs concentration ranged from 0.076 to 0.323 mg kg^{-1} for the rice, not detected to 25.6 mg kg^{-1} for the seaweed, not detected to 0.204 mg kg^{-1} for the seafood (Table 4). The highest concentration of iAs (25.6 mg kg^{-1}) was found in the sample of hijiki (*S. fusiforme*), and no arsenic was detected in the marine oils (Table 4 and Supplementary Table 4 (https://www.jfda-online.com/cgi/viewcontent.cgi?filename=0&article=3432&

Table 3. *Recovery of iAs in the spiked samples.*

Samples	As compound	Spiked level (mg/kg)	Recovery[b] (%)	CV (%)
Polished rice	iAs[a]	0.02	103.9 ± 6.11	5.88
		0.10	102.0 ± 5.62	5.51
		0.20	89.7 ± 4.55	5.07
Laver		0.02	97.1 ± 9.06	9.33
		0.10	101.8 ± 6.11	6.00
		0.20	108.1 ± 3.32	3.07
Lobster		0.02	101.0 ± 5.91	5.85
		0.10	103.1 ± 1.68	1.63
		0.20	109.2 ± 2.79	2.56
Salmon		0.02	91.0 ± 2.97	3.26
		0.10	87.5 ± 2.00	2.28
		0.20	93.6 ± 2.00	2.14
Tilapia		0.02	103.5 ± 5.41	5.23
		0.10	110.7 ± 3.62	3.27
		0.20	112.4 ± 0.77	0.68
Salmon oil		0.02	90.4 ± 2.37	2.62
		0.10	99.8 ± 0.78	0.78
		0.20	98.4 ± 1.65	1.67

[a] iAs = As(III) oxidized using H_2O_2.
[b] Mean ± SD, $n = 5$.

Table 4. *iAs concentration measured in rice, seaweed, seafood, and marine oil samples.*

Matrix	Sample	n	Average iAs[a] (mg/kg)
Rice	Polished rice	7	0.132 (0.084−0.177)[b]
	Brown rice	3	0.233 (0.162−0.323)[b]
	Rice cereal	1	0.076
Seaweed	Hijiki (*Sargassum fusiforme*)	1	25.6
	Kelp bud	1	0.158
	Kelp knot	1	N.D.[c]
	Kombu	1	0.162
	Laver	1	0.303
	Processed seaweed	3	0.033 (0.019−0.047)[b]
Seafood	Salmon	1	N.D.
	Tilapia	1	0.017
	Swordfish	1	0.018
	Grass carp	1	N.D.
	Flounder	1	N.D.
	Milkfish	1	N.D.
	Lobster	1	N.D.
	Mud crab	1	0.022
	Flower crab	1	0.021
	Neritic squid	2	N.D.
	Clams	1	0.204
	Fresh oysters	1	0.112
	Shrimps	2	0.033
Marine oils	Salmon oil	1	N.D.
	Tuna oil	2	N.D.
	Refined fish oil	2	N.D.
	Deep-sea fish oil	11	N.D.

[a] iAs = As(III) oxidized using H_2O_2.
[b] Average value (minimum - maximum).
[c] N.D., not detected.

context=journal&type=additional)). In this work, only hijiki (*S. fusiforme*) higher than the maximum limit of sanitation standard for iAs in seaweed, whereas the remaining samples entirely met requirements of iAs content set in "Sanitation Standard for Contaminants and Toxins in Foods" (Taiwan). We speculated that the hijiki result may have been attributed to its strong tolerance for iAs toxicity, which made it prone to a higher accumulation of iAs.

In summary, the analytical method for the determination of iAs (As(III) and As(V)) content in food was developed and optimized. The method consisted of extraction under sonication using the 1% (w/w) HNO_3 solution containing 0.2 M H_2O_2 and separation of the iAs compounds on the anion-exchange column (PRP-X100) using HPLC/ICP-MS. This method is simpler and quicker than the other processes that employ acid extraction as described in previous literature. Unknown As species were also detected in this study and further investigated to be identified using appropriate As standards and characterizations with HPLC/ICP-MS.

4. Conclusion

In this study, the analytical method for quantifying iAs in a wide variety of foods was developed. Moreover, the analytical method converted the As(III) species into the As(V) species via extraction using 1% (w/w) HNO_3 containing 0.2 M H_2O_2, showed sensitivity and selectivity toward the iAs, and detected six As compounds in less than 8 min using HPLC/ICP-MS. The proposed method afforded more accurate, fast, and excellent sample throughputs than the past methods and presented an easy procedure for the determination of iAs in food, which could be adopted by regulatory agencies for the routine quality controls of food for the public health protection.

Conflict of interest

The authors have disclosed no conflicts of interests.

Acknowledgments

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