

Determination of Arsenic Species in Rice from Thailand and Other Asian Countries Using Simple Extraction and HPLC-ICP-MS Analysis

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ABSTRACT: Human exposure to arsenic (As) via rice consumption is of increasing concern. In the present study, the extraction and HPLC-ICP-MS analysis for As speciation in rice were investigated. A simple extraction with water and digestion with α -amylase followed by the analysis using ion-pairing mode HPLC separation was developed. The method showed good extraction efficiencies (generally >80%) and column efficiencies (>90%) for rice samples. The optimization of mobile phase showed well separated peaks of As species. The limits of quantification (LOQ) of As^{III}, As^V, MMA, and DMA that were calculated based on sample mass were 1.6, 2.0, 2.0, and 1.6 $\mu\text{g kg}^{-1}$, respectively. A total of 185 rice samples (various types of rice) collected from different four regions in Thailand and some other Asian countries were analyzed. The total As and inorganic As in rice samples were in the ranges of 22.51–375.39 and 13.89–232.62 $\mu\text{g kg}^{-1}$, respectively. The estimated weekly intake of inorganic As from rice by Thai people accounted for 13.91–29.22% of the provisional tolerable weekly intake (PTWI).

KEYWORDS: white and colored rice, arsenic speciation, HPLC-ICP-MS, arsenic weekly intake, Thailand

INTRODUCTION

Arsenic (As) is one of the most toxic elements that occurs in both inorganic and organic forms. Both forms of As can be found in the environment from natural sources^{1,2} and anthropogenic activities.^{3,4} Arsenic has received increased attention in recent years because of its carcinogenic and other toxic properties.^{5–7} Speciation of inorganic arsenic is often as important as total quantification because of its varying degrees of toxicity.^{8,9}

Arsenic speciation accumulated in rice is important because rice is a major dietary staple for large populations, especially in Asian countries, and elevated arsenic levels are found in rice and rice products available in the market places around the world.^{10–13} Therefore, rice consumption is considered to be one of the potential routes of dietary As exposure in many parts of the world.^{14–16} Arsenic speciation was investigated in rice grains,^{17–19} white rice,^{16,20,21} brown rice,²² and rice products.^{23,24} A number of studies have combined the good separation capabilities of HPLC with highly sensitive ICP-MS detection to identify and quantify As species in rice grains. HPLC separations were performed under anion-exchange conditions^{20,23,25–28} and ion-pairing conditions.^{21,29,30} During As speciation analysis, one of the key and difficult steps is the desirable extraction of As from the rice samples without any disturbance of the original As species.^{26–29} As^{III} is located inside the starch granules; therefore, it is probable that extraction efficiencies for As^{III} may be increased as the starch structure is broken down during heating.³⁰

The present study aimed to determine the levels of total As and four As species (arsenite; As^{III}, arsenate; As^V, dimethylated arsenic; DMA, and monomethylated arsenic; MMA) in various types of rice samples normally consumed in Thailand and also

purchased from other Asian countries (South Asia [India, Pakistan, and Sri Lanka], Malaysia, and Vietnam). This study also estimated the weekly intake and potential health risk of As species from rice consumption in four regions of Thailand (Central, South, Northeast, and North).

MATERIALS AND METHODS

Chemicals and Materials. High purity double distilled water (resistivity 18.2 M Ω cm) used in all experiments was obtained by using a Milli-Q water purification system (Millipore RiOs-DI, Bedford, MA). All reagents were of analytical-reagent grade. Nitric acid suprapure 65% (HNO₃), tetrabutylammonium hydroxide (TBAOH), ammonia solution 25% (NH₃), and HPLC grade methanol were purchased from Merck Chemicals (Darmstadt, Germany). Ammonium phosphate monobasic and α -amylase were purchased from Sigma-Aldrich (St. Louis, MO). Stock solution containing 1000 mg L⁻¹ each of the following species was prepared in water: arsenite and arsenate were prepared from NaAsO₂ and Na₂HAsO₄·7H₂O (Aldrich, St. Louis, MO); methylarsonate was prepared from Na₂CH₃O₃·As·6H₂O (Chem Service, West Chester); and dimethylarsinate was prepared from C₂H₇AsO₂ (Fluka, St. Louis, MO). The rice flour CRMs were NIST SRM 1568a (National Institute of Standards and Technology, Gaithersburg, Maryland) and NMJJ CRM 7503-a (National Institute of Advanced Industrial Science and Technology, National Metrology Institute of Japan).

Equipment. ICP-MS and HPLC systems were Agilent 7500c and Agilent 1100 series, respectively (Agilent, Tokyo, Japan). The column was X-Select (Charged Surface Hybrid; CSH) C18 column

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(4.6 × 150 mm of id and 5 μm of particle size) (Water Corporation, Milford, MA). Microwave digestion was performed with a MARS system (CEM, Mathews, NC). A shaking water bath from GFL type 1083 (Hanover, Germany) was used for sample extraction. Centrifugation was carried out to separate the supernatant from the sample extracts by a Thermo Scientific Sorvall Legend XTR centrifuge (Osterode, Germany).

Sample Collection. Several types of rice samples (white, red, nonpolished or brown, white sticky, and black sticky rice) were purchased from local markets and also collected from different provinces in Thailand. Some rice samples were purchased from other countries (Vietnam, Pakistan, India, Sri Lanka, and Malaysia). A total of 185 samples were collected during 2008–2012.

Sample Preparation. Rice samples were rinsed with double distilled water to remove dust and then dried by air flow at room temperature. Dried samples were milled to powder with a commercial blender with stainless steel blades. The powdered samples were stored in sealed plastic bags at 4 °C.

Total Arsenic Determination. Rice powder samples (0.20 g weighed with a precision of 0.0001 g) were weighed and transferred into polytetrafluoroethylene (PTFE) vessels. Then, 6.0 mL of concentrated nitric acid (65%) and 2.0 mL of double distilled water were added into the vessels. The vessels were closed, placed on the rotating turntable of a microwave oven, and the digestion process started. The digestion was allowed at 180 psi and 190 °C over 30 min and maintained at this condition for 30 min. The digested solutions were diluted to 50.00 g with double distilled water. The total As concentration in the digested solution was determined by ICP-MS using helium as collision gas for removing polyatomic interferences from argon chloride ($^{40}\text{Ar}^{35}\text{Cl}$).

Arsenic Speciation Extraction. Rice powder samples (0.25 g weighed with a precision of 0.0001 g) were weighed and transferred into 50 mL of polypropylene centrifuge tubes. Then, 10.0 mL of double distilled water was added into the tubes. The capped tubes were placed in a shaking water bath at 90 °C for 4 h. The rice–water suspension was centrifuged at 5000 rpm for 10 min. A portion of the resulting supernatant was further digested with 65% nitric acid at 90 °C for 1 h. The resulting clear supernatants were analyzed for total

As in the rice extract by using ICP-MS. Another portion of the resulting supernatant was mixed with α -amylase enzyme, incubated at 37 °C for 30 min, and then filtered through a 0.20 μm syringe-type PVDF membrane filter. The filtrate was analyzed for As species by using HPLC-ICP-MS.

The deterioration of the HPLC column (increase of column pressure) was observed after many samples were injected. The α -amylase enzyme was therefore added to digest the starch before HPLC analysis. The results showed that the pressure of the HPLC column was stabilized, and efficiencies of arsenic separation were very good for long-term running compared to those with the method without α -amylase enzyme. Moreover, there were no transformations of As species during extraction.

HPLC-ICP-MS Analyses. Separation of As species was performed under ion-pairing conditions at 35 °C of column temperature. The samples were loaded with a syringe into a 25 μL sample loop. The HPLC conditions such as pH and concentration of mobile phase were optimized. The effluent from the HPLC column was connected directly into the ICP-MS nebulizer through PFA (perfluoroalkoxy) tubing. To ascertain possible chloride interference in m/z 75, the signals at m/z 75 (^{75}As , $^{40}\text{Ar}^{35}\text{Cl}$), m/z 77 ($^{40}\text{Ar}^{37}\text{Cl}$), and m/z 35 (^{35}Cl) were monitored.

RESULTS AND DISCUSSION

HPLC Optimization. The HPLC conditions of the present study were modified from the previous study.³¹ The present study developed HPLC methods and provided good separation of four arsenic species and three unknown forms of arsenic (Figure 1c). The ^{35}Cl peak was also separated from other arsenic species; therefore, the interference from $^{40}\text{Ar}^{35}\text{Cl}$ did not occur during ICP-MS detection (Figure 1a). The optimized mobile phase solutions were 7.5 mM tetrabutylammonium hydroxide and 10 mM ammonium phosphate monobasic at pH 8.25 (95%) and methanol (5%), at a flow rate of 1.0 mL min⁻¹.

Effect of Extraction Time on Extraction Efficiency. A simple extraction technique using a shaking water bath was used in the present study. The extraction temperature was set at

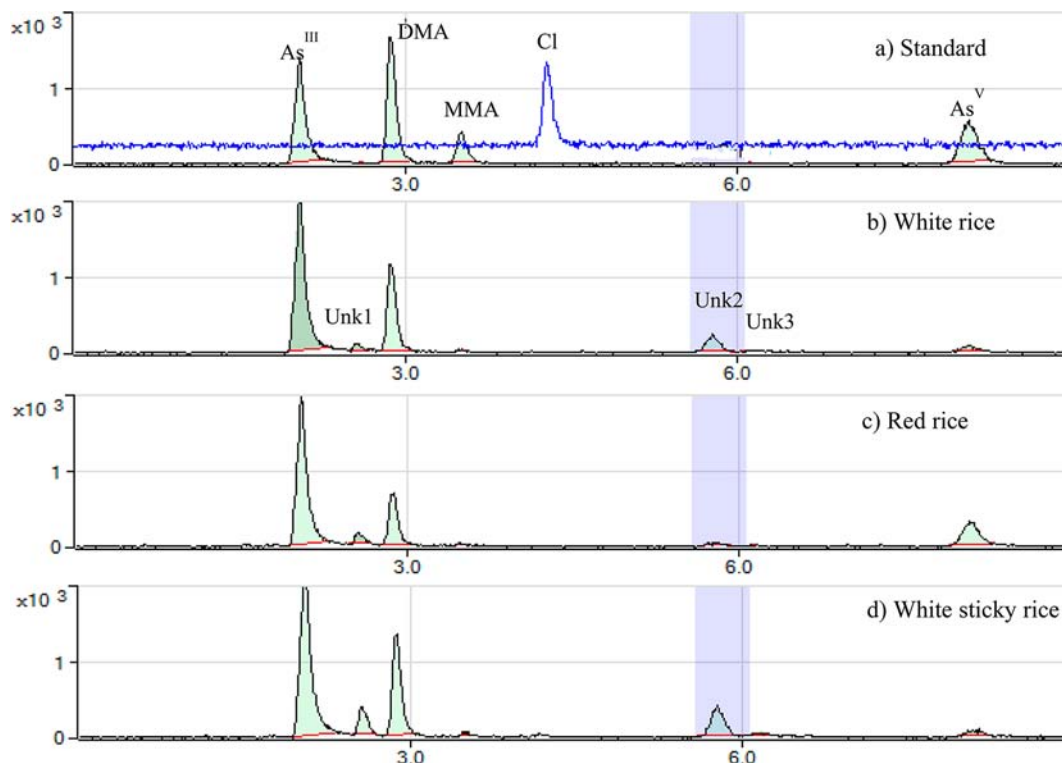


Figure 1. Chromatograms of As standards (a), white rice (b), red rice (c), and white sticky rice (d).

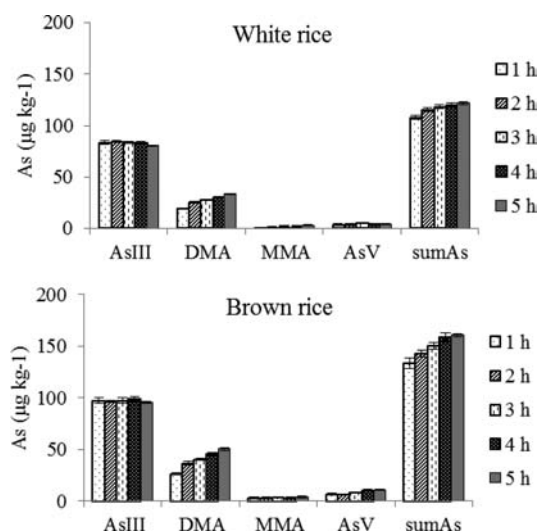


Figure 2. Effect of extraction time on arsenic speciation in rice samples.

90 °C modified from the method developed by Narukawa and Chiba.³⁰ The extraction times were investigated to achieve complete extraction of As species in rice samples. Figure 2 showed the effect of different extraction times (1, 2, 3, 4, and 5 h) at 90 °C on As speciation in rice. The results showed that extraction time affected the sum of four As species in both white and brown rice. Higher efficiency was obtained with higher extraction time, especially for DMA species. As^{III} was slightly decreased at 5 h of extraction. However, the sum of four As species at 5 h of extraction was not much different from that at 4 h of extraction; and therefore, it was considered that the best extraction time for As species was 4 h.

Effect of Solid/Liquid Ratio on Extraction Efficiency.

The effect of the solid/liquid extraction ratio was studied to ensure that the sample weight and the volume of extractant influence the thermal efficiency of the extraction process. Four solid/liquid ratios (0.10 g/10 mL, 0.25 g/10 mL, 0.5 g/10 mL, and 1.0 g/10 mL) were studied at 90 °C for 4 h. The results showed that changes in the solid/liquid ratio did not affect the extracted amount of four As species for both white and brown rice. The optimal solid/liquid ratio was 0.25 g/10 mL. The lower extraction ratio (0.10 g/10 mL) had lower sensitivity of As detection and the higher ratio (0.5 g/10 mL) contained higher matrices (starch) in the extract solution that interfered in HPLC analysis.

Analytical Performances. Different calibration curves were obtained by covering As^{III} and DMA concentrations at 0.1, 0.2, 0.5, 1.0, 2.0, and 4.0 $\mu\text{g L}^{-1}$, expressed as As, and As^V and MMA concentrations at 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 $\mu\text{g L}^{-1}$, also expressed as As. The limit of detection (LOD) and the limit of quantification (LOQ) were calculated based on 3 and 10 times the signal-to-noise ratios (3S/N and 10S/N, respectively). The LODs of As^{III}, As^V, MMA, and DMA that were calculated based on sample mass were 0.4, 0.8, 0.8, and 0.4 $\mu\text{g kg}^{-1}$, respectively, and the LOQs of As^{III}, As^V, MMA, and DMA that were calculated based on sample mass were 1.6, 2.0, 2.0, and 1.6 $\mu\text{g kg}^{-1}$, respectively.

The spike recovery was studied by adding each standard of each As species at three concentrations in three types of rice samples, and their recoveries were determined following the present extraction procedure ($n = 10$ for each concentration and each type of rice). Recoveries of all added species ranged from 86.68 to 119.94 (Table 1). The recovery values showed that there were no transformations of As species during extraction. The precision of the method was indicated by using standard deviation and relative standard deviation (for both concentration and retention time) of four types of rice: white rice, white sticky rice, brown rice, and red rice ($n = 10$ for each rice type). The precision of concentration was less than 5% except at low amounts of As^V ($<8 \mu\text{g kg}^{-1}$) in white rice, white sticky rice, and brown rice (6.78–9.59%). The precision of retention time was less than 0.5% for all As species in all rice samples.

The accuracy of the method was determined by analyzing SRM 1568a rice flour and NMIJ CRM7503a rice flour, and the results are shown in Table 2. There was no certified As species in SRM 1568a, but this SRM was used in a number of studies.^{11,15,28–30,32} The concentrations of As species found in SRM 1568a in the present study were in agreement with the previous report. The accuracies of As species found in NMIJ CRM 7503-a were in the ranges of 85–107% for all arsenic species (Table 2). The present results of analytical performances showed that the method was suitable for the determination of As speciation in rice samples.

Recoveries of Extraction, Column, and Method. Extraction recovery was calculated as $\text{As}_{\text{extracted}}/\text{As}_{\text{total}}$ ($\text{As}_{\text{extracted}}$ = total As extracted by H₂O and As_{total} = total As digested with HNO₃, both determined by ICP-MS). Column recovery was evaluated as the sum of $\text{As}_{\text{species}}/\text{As}_{\text{extracted}}$ (sum of $\text{As}_{\text{species}}$ = sum of As species determined by HPLC-ICP-MS). For overall method performance, the method recovery was calculated as

Table 1. Recoveries of Arsenic Species Spiked in Three Rice Types

As species	added As ($\mu\text{g kg}^{-1}$)	recoveries \pm SD ($n = 10$)		
		white rice	brown rice	black sticky rice
As ^{III}	4.00	93.75 \pm 15.18	119.94 \pm 16.05	95.37 \pm 14.74
	40.00	102.29 \pm 2.89	94.00 \pm 3.40	91.50 \pm 1.91
	160.00	99.13 \pm 1.22	98.42 \pm 1.96	91.71 \pm 3.04
As ^V	4.00	92.78 \pm 6.93	92.53 \pm 7.75	86.68 \pm 12.89
	20.00	111.82 \pm 7.10	102.95 \pm 10.10	105.32 \pm 10.01
	40.00	106.95 \pm 5.90	113.55 \pm 4.29	107.20 \pm 8.74
MMA	4.00	107.68 \pm 7.95	108.36 \pm 9.10	106.31 \pm 13.55
	20.00	107.76 \pm 4.07	104.12 \pm 4.99	105.62 \pm 3.56
	40.00	107.74 \pm 3.16	104.53 \pm 3.20	96.98 \pm 4.32
DMA	4.00	112.73 \pm 4.43	114.31 \pm 3.71	114.44 \pm 6.87
	40.00	102.16 \pm 1.61	100.64 \pm 1.44	97.70 \pm 1.52
	160.00	103.41 \pm 1.10	103.87 \pm 2.04	94.91 \pm 3.57

Table 2. Means and Ranges of Arsenic Species Concentrations in SRMs and Various Types of Rice Samples^a

sample name	N	As concentration ($\mu\text{g kg}^{-1}$)						total As	% inorganic As	% DMA
		As ^{III}	As ^V	MMA	DMA	sum As	30 ^c			
1568a rice flour	24	57.64 ± 2.77 (53–57) ^b	33.73 ± 4.77 (22–50.3) ^b	19.41 ± 3.12 (9–15) ^b	158.68 ± 6.06 (144.2–175) ^b	269.45 ± 9.91	290 ± 30 ^c	31.5 ± 2.0	54.7 ± 2.1	
7503-a rice flour	9	60.21 ± 2.48 (71.1 ± 2.9) ^c	14.50 ± 1.73 (13.0 ± 0.9) ^c	<2.0	12.47 ± 0.73 (13.3 ± 0.9) ^c	88.40 ± 3.99	98 ± 7 ^c	76.2 ± 3.8	12.7 ± 0.8	
white rice (South Asia) ^d	6	52.05 ± 9.90 (21.53–88.95)	5.49 ± 1.94 (<2.0–13.77)	<2.0	7.63 ± 1.31 (<1.6–10.96)	65.50 ± 11.70 (26.42–109.52)	68.91 ± 13.54 (25.35–125.20)	85.3 ± 4.0 (71.3–98.3)	11.1 ± 1.5 (5.9–15.9)	
white rice (Malaysia)	3	63.71 ± 2.63 (58.85–67.90)	7.06 ± 5.15 (<2.0–17.33)	<2.0 (<2.0–3.66)	24.94 ± 3.25 (19.30–30.56)	97.38 ± 9.33 (86.45–115.94)	102.91 ± 10.50 (88.31–123.28)	69.0 ± 1.4 (66.3–71.2)	24.3 ± 2.4 (19.9–28.3)	
white rice (Vietnam)	10	86.18 ± 8.60 (44.08–129.86)	5.02 ± 2.40 (<2.0–26.04)	<2.0 (<2.0–4.14)	16.25 ± 1.63 (5.94–25.08)	108.65 ± 9.23 (80.17–164.45)	136.31 ± 11.42 (93.08–204.08)	67.2 ± 2.5 (51.1–78.2)	12.0 ± 1.0 (5.4–15.4)	
white rice (Thailand)	79	81.44 ± 2.88 (14.29–153.73)	3.74 ± 0.19 (<2.0–7.85)	<2.0 (<2.0–6.40)	29.00 ± 1.77 (2.42–85.95)	115.73 ± 4.37 (17.96–250.23)	139.48 ± 5.94 (22.51–304.32)	63.2 ± 1.3 (42.5–96.1)	20.2 ± 0.8 (4.5–38.6)	
brown rice (Thailand)	14	110.45 ± 8.01 (79.69–171.61)	13.91 ± 2.21 (4.02–35.08)	3.08 ± 0.53 (<2.0–7.12)	25.93 ± 2.09 (9.08–34.94)	153.37 ± 7.64 (116.77–210.51)	239.02 ± 15.75 (118.16–343.12)	53.6 ± 3.1 (40.5–83.7)	11.5 ± 1.2 (4.0–18.9)	
brown rice (Vietnam)	2	190.50 ± 1.00 (189.51–191.50)	22.25 ± 3.30 (6.19–43.11)	<2.0	20.51 ± 14.53 (5.98–35.04)	235.66 ± 32.00 (203.66–267.66)	299.10 ± 45.40 (253.70–344.50)	72.7 ± 5.2 (67.5–77.9)	6.3 ± 3.9 (2.4–10.2)	
red rice (Thailand)	27	105.47 ± 7.13 (36.70–178.62)	20.91 ± 9.27 (3.45–72.36)	2.29 ± 0.33 (<2.0–5.34)	22.78 ± 2.17 (5.37–51.27)	152.78 ± 11.61 (88.55–250.72)	211.40 ± 15.05 (77.17–375.39)	63.3 ± 2.7 (39.9–95.6)	11.4 ± 1.0 (3.1–23.6)	
white sticky rice (Thailand)	38	54.66 ± 2.25 (25.62–80.30)	3.35 ± 0.59 (<2.0–23.42)	<2.0 (<2.0–5.35)	15.34 ± 1.49 (3.24–46.06)	74.11 ± 4.01 (29.63–155.12)	93.45 ± 4.79 (46.33–166.42)	63.5 ± 1.8 (44.2–89.4)	15.7 ± 1.1 (7.0–32.4)	
black sticky rice (Thailand)	6	58.31 ± 17.64 (12.63–132.79)	19.31 ± 11.39 (<2.0–74.61)	<2.0 (<2.0–4.18)	19.69 ± 6.98 (1.60–44.85)	98.84 ± 28.04 (15.48–193.27)	124.44 ± 41.52 (31.42–313.10)	66.1 ± 10.1 (43.8–103.6)	14.3 ± 3.2 (5.1–25.2)	

^aThe numbers in parentheses are the minimum and maximum concentrations. ^bResults reported by other studies. ^cCertified values. ^dIndia, Pakistan, and Sri Lanka.

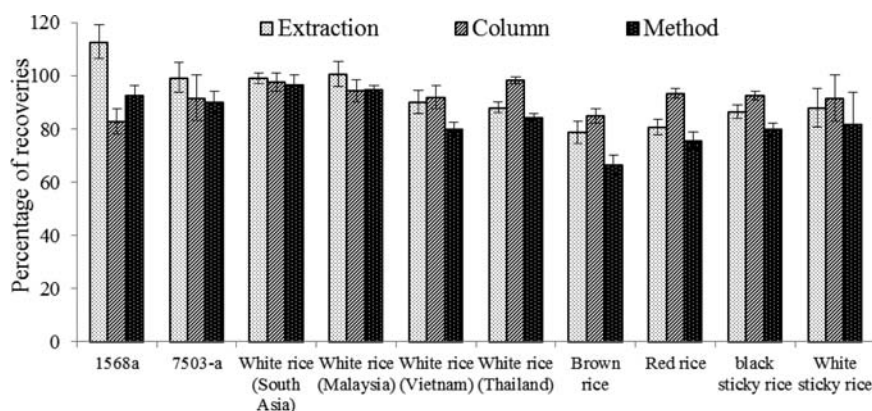


Figure 3. Extraction, column, and method efficiencies in various types of rice samples.

the sum of $As_{\text{species}}/As_{\text{total}}$. The individual sample was calculated for the extraction, column, and method recoveries (percentages). For each type of rice and SRMs, the means and standard error (SE) of the percentages were calculated (Figure 3). The averages of extraction efficiencies for all rice types were higher than 80%. However, extraction efficiencies of some brown rice samples were less than 80%. This may be due to the fact that brown rice contained high bran germs which had high protein, lipid, and fiber.³³ Our results agreed with the previous study that reported that high protein in rice samples resulted in a slight decrease in As extraction efficiencies.²¹ In addition, the extraction efficiency decreased with the increasing particle size of the sample.³⁰ The column efficiency of all rice types was higher than 85%, which showed good separation and detection of As species in the present study. Mean overall method recovery was higher than 80% for all rice types, except for brown rice (66.4 ± 3.6 , $n = 14$) and red rice (75.8 ± 3.3 , $n = 27$). The low recovery in some samples may result from unknown As species detected in the samples, which could not be calculated for their concentrations, especially in brown and red rice.

Sample Analysis. Total As and species in rice classified by color, type, and country of production are presented in Table 2.

Total As Concentration. The total As concentration in white rice from Thailand ($139.48 \pm 5.94 \mu\text{g kg}^{-1}$) was similar to that of white rice from Vietnam ($136.31 \pm 11.42 \mu\text{g kg}^{-1}$) but higher than that of white rice from Malaysia ($102.91 \pm 10.50 \mu\text{g kg}^{-1}$) and South Asia ($68.91 \pm 13.54 \mu\text{g kg}^{-1}$) (Table 2). The total As concentration in white rice from Vietnam in the previous study was 180–252 $\mu\text{g kg}^{-1}$,³⁴ which was higher than that in the present study. The total As concentration in white rice from Thailand was similar to those in the previous studies in white rice samples from Thailand reported by Meharg et al. ($140 \mu\text{g kg}^{-1}$)¹⁶ and Ruangwises et al. ($125 \mu\text{g kg}^{-1}$),³⁵ but it was higher than those reported by Nishimura et al. (90 – $100 \mu\text{g kg}^{-1}$).¹¹ Thai brown rice contained the highest total As concentration at a mean value of $239.02 \mu\text{g kg}^{-1}$, which was not different from that of Thai red rice ($211.40 \mu\text{g kg}^{-1}$), but it was much higher than that of Thai white rice ($139.48 \mu\text{g kg}^{-1}$). Studies of As concentrations in brown rice have reported that As was preferentially localized at the surface in the region corresponding to the pericarp and aleurone layer.^{10,21,25,36} Vietnam brown rice ($n = 2$) contained total As at the highest concentration ($299.10 \mu\text{g kg}^{-1}$ on average). White sticky rice contained total As at an average value of $93.45 \pm 4.79 \mu\text{g kg}^{-1}$ which was lower than that of black sticky rice ($124.44 \pm 41.52 \mu\text{g kg}^{-1}$)

(Table 2). The different As concentrations found in various types of rice depended on rice genotypes,^{37,38} and As concentration was found to increase as the size of grain increased.¹⁰ The sixth session of the Codex committee on Contaminants in Foods (CCCF) proposed a maximum level for As in brown rice at $300 \mu\text{g kg}^{-1}$ (inorganic or total As).³⁹ From total rice samples tested, there were eight rice samples (4% of total samples) that contained total As concentration higher than $300 \mu\text{g kg}^{-1}$; one sample of Thai white rice, two samples of Thai brown rice, one sample of Vietnam brown rice, three samples of Thai red rice, and one sample of Thai black sticky rice.

As Speciation. The present results of As speciation indicated that the major As species detected at high levels in all rice types were in the order $As^{\text{III}} > \text{DMA} > As^{\text{V}}$ (Table 2). Small amounts of MMA were detected in some samples (<2.0 – $7.12 \mu\text{g kg}^{-1}$). The highest concentration of As^{III} was found in Vietnam brown rice ($190.50 \pm 1.00 \mu\text{g kg}^{-1}$, $n = 2$) followed by Thai brown rice ($110.45 \pm 8.01 \mu\text{g kg}^{-1}$) and Thai red rice ($105.47 \pm 7.13 \mu\text{g kg}^{-1}$). The inorganic As concentration in white rice from Vietnam in the previous study was 111–175 $\mu\text{g kg}^{-1}$,³⁴ which was higher than that in the present study ($91.20 \mu\text{g kg}^{-1}$ on average). The sixth session of the Codex committee on Contaminants in Foods (CCCF) proposed a maximum level for inorganic As in polished rice at $200 \mu\text{g kg}^{-1}$.³⁹ From the total rice samples tested, only two rice samples contained inorganic As higher than $200 \mu\text{g kg}^{-1}$ (one sample of Thai red rice and one sample of Vietnam brown rice). The DMA concentration was found at the same averages for all types of rice (15.34 – $29.00 \mu\text{g kg}^{-1}$), except for white rice from South Asia ($7.63 \pm 1.31 \mu\text{g kg}^{-1}$). The As^{V} concentration was found at the highest average concentration of $22.25 \mu\text{g kg}^{-1}$ in Vietnam brown rice, which was at the same concentration as that of Thai red rice ($20.91 \mu\text{g kg}^{-1}$) but higher than that of Thai brown rice ($13.91 \mu\text{g kg}^{-1}$). The predominance of As^{III} in rice in the previous studies^{11,35} was in agreement with the present study. On average, inorganic As ($As^{\text{III}} + As^{\text{V}}$) in the present study accounted for 53.1–67.2% of the total As in all types of rice, except for white rice from South Asia and Vietnam brown rice (85.3 and 72.7%, respectively). Although total As in white rice from South Asia was at the lowest concentration, the percentage of inorganic As was found to be at the highest value. DMA was the second component of As species in rice with an average of 6.3–24.3%. The rice cultivar has an effect on the proportion of DMA in rice grain, and U.S. rice has a proportion of DMA in rice higher than Indian, Bangladesh, and European rice.⁴⁰

Table 3. Means and Ranges of Arsenic Species Concentration in Rice Samples Collected from Different Regions in Thailand (Numbers in Parentheses Are Minimum and Maximum Concentrations)

region	rice type	N	As concentration ($\mu\text{g kg}^{-1}$)						total As
			As ^{III}	As ^V	MMA	DMA	sum As		
central	white	29	94.74 \pm 4.22 (52.42–153.73)	4.29 \pm 0.21 (2.48–6.20)	2.29 \pm 0.28 (<2.0–6.40)	35.10 \pm 3.26 (13.50–85.95)	136.42 \pm 6.90 (75.15–250.23)	161.07 \pm 11.40 (90.00–304.32)	
	colored	19	115.90 \pm 7.43 (79.69–178.62)	16.01 \pm 1.78 (5.23–31.91)	2.67 \pm 0.46 (<2.0–7.12)	26.40 \pm 2.77 (5.90–51.27)	160.98 \pm 8.56 (118.38–243.22)	227.89 \pm 11.81 (132.57–302.73)	
south	white	18	80.83 \pm 5.66 (50.25–139.09)	3.74 \pm 0.44 (<2.0–7.85)	<2.0 (<2.0–3.70)	31.86 \pm 3.45 (15.09–79.19)	117.80 \pm 7.47 (82.88–214.84)	142.23 \pm 9.91 (94.74–270.61)	
	colored	5	116.66 \pm 9.21 (98.40–142.93)	26.54 \pm 12.13 (4.02–72.36)	2.66 \pm 0.93 (<2.0–5.34)	22.64 \pm 4.28 (9.08–32.59)	168.50 \pm 21.59 (132.26–250.72)	247.40 \pm 14.08 (219.80–284.18)	
northeast	white	22	65.14 \pm 5.03 (14.29–126.44)	3.02 \pm 0.42 (<2.0–6.76)	<2.0 (<2.0–2.94)	20.53 \pm 2.64 (2.42–40.73)	89.55 \pm 7.59 (17.96–172.03)	107.76 \pm 7.99 (22.51–161.00)	
	colored	9	98.75 \pm 12.47 (49.74–171.61)	14.97 \pm 3.49 (3.45–35.08)	2.28 \pm 0.62 (<2.0–4.32)	20.83 \pm 2.20 (5.37–26.63)	136.84 \pm 14.79 (58.55–207.59)	215.16 \pm 30.81 (77.17–343.12)	
north	white	8	86.46 \pm 5.44 (66.14–107.15)	3.87 \pm 0.51 (2.04–6.33)	<2.0 (<2.0–2.66)	26.04 \pm 3.53 (13.37–37.01)	117.97 \pm 7.41 (87.61–146.76)	151.86 \pm 13.44 (101.24–211.38)	
	colored	3	62.77 \pm 14.70 (36.70–87.58)	38.98 \pm 17.59 (11.12–71.52)	2.04 \pm 1.02 (<2.0–3.15)	20.76 \pm 4.40 (15.63–29.53)	124.56 \pm 16.14 (92.28–140.91)	159.14 \pm 45.80 (90.62–246.04)	

MMA was only found occasionally in rice, so this species was not considered for the relative coefficient. The relative coefficients were plotted using total As against inorganic As (As^{III} + As^V) and DMA. The concentrations of both inorganic As and DMA increased with increasing total As concentration ($R^2 = 0.316$ – 0.863), except for DMA in brown rice ($R^2 = 0.001$). The highest correlations of inorganic As with total As were observed in white rice from Thailand and Vietnam ($R^2 = 0.718$ and 0.863 , respectively). Inorganic As showed a stronger correlation than DMA with total As for all types of rice (R^2 of inorganic As > R^2 of DMA with total As). The relationship of inorganic As and DMA against total As has categorized rice into inorganic As (inorganic As > DMA concentrations) and DMA types (DMA > inorganic As concentrations).¹⁷ The present study showed that all rice samples can be categorized as the inorganic As type due to the high predominance of inorganic As in these rice samples.

Estimation of the Weekly Intake of As Species from Rice Consumption. Two types of rice, white rice and colored rice (red and brown), are the most popular rice consumed in Thailand. Therefore, we classified these two types of rice according to the four regions in Thailand (Central, South, Northeast, and North) following the collection areas to evaluate the As contamination in consumed rice in different regions (Table 3). The lowest concentrations of total As and As^{III} in white rice were found in the Northeastern region, while other regions had the same As concentration in white rice. The colored rice samples from the Northern region ($n = 3$) contained total As and As^{III} at the lowest concentrations, while other regions had similar concentrations for both total As and As^{III} in colored rice. However, only three colored rice samples from the Northern region were tested. The concentrations of total As and all As species found in colored rice were much higher than those of white rice in all regions in Thailand, except for DMA, which had the same concentrations for both white and colored rice.

The average daily consumption of milled rice by Thai people is 276 g per day.⁴¹ The average body weight of an adult Thai person is 63.12 kg (www.sizethailand.org/region_all.html). Estimated weekly intakes of each As species through white and colored rice consumption by Thai people in four regions in Thailand are shown in Table 4. The weekly intakes of As species were also compared to the provisional tolerable weekly intake (PTWI) of total As ($15 \mu\text{g kg}^{-1}$ body weight [BW]).⁴² The weekly intakes of inorganic As (As^{III} + As^V) from white rice by the Northeastern people ($2.086 \mu\text{g kg}^{-1}$ BW) was lower than those by other regions. The highest weekly intake of inorganic As from white rice was found in the Central region ($3.031 \mu\text{g kg}^{-1}$ BW) and from colored rice was found in the Southern region ($4.383 \mu\text{g kg}^{-1}$ BW). The calculation indicated that the average weekly intakes of inorganic As from white rice and colored rice by Thai people were 2.618 and $3.754 \mu\text{g kg}^{-1}$ BW, which were about 17.45 and 25.02% of PTWI, respectively. These values were similar to the average estimated weekly intake of inorganic As from rice by Chinese people ($2.86 \mu\text{g kg}^{-1}$ BW; 19.07% of PTWI)⁴³ but lower than that by Cambodian (0.602 – $12.355 \mu\text{g kg}^{-1}$ BW; 4.01–82.37% of PTWI),⁴⁴ Vietnamese ($7.389 \mu\text{g kg}^{-1}$ BW; 49.26% of PTWI),³⁴ and Indian people (3.71 – $11.13 \mu\text{g kg}^{-1}$ BW; 24.73–75.33% of PTWI).²² The estimated weekly intakes of inorganic As by Asian populations were higher than the intakes by populations in other countries which had less rice consumption such as Jamaican (7.8–10.2% of PTWI),⁴⁵ Brazilian (7.5% of PTWI),¹⁵ and Ghanaian (7.08–7.85% of PTWI).¹⁹

Table 4. Estimated Weekly Intake of As Species and Potential Health Risk Due to the Consumption of Rice

region	rice type	N	weekly intake ($\mu\text{g kg}^{-1}$ body weight) (% of the PTWI ^a)			
			As ^{III}	As ^V	MMA	DMA
central	white	29	2.900 (19.33)	0.131 (0.87)	0.070 (0.47)	1.074 (7.16)
	colored	19	3.548 (23.65)	0.490 (3.27)	0.082 (0.55)	0.808 (5.39)
south	white	18	2.474 (16.49)	0.114 (0.76)	0.042 (0.28)	0.975 (6.50)
	colored	5	3.571 (23.81)	0.812 (5.42)	0.082 (0.54)	0.693 (4.62)
northeast	white	22	1.994 (13.29)	0.093 (0.62)	0.026 (0.17)	0.628 (4.19)
	colored	9	3.023 (20.15)	0.458 (3.05)	0.070 (0.47)	0.638 (4.25)
north	white	8	2.646 (17.64)	0.118 (0.79)	0.049 (0.33)	0.797 (5.31)
	colored	3	1.921 (12.81)	1.193 (7.95)	0.063 (0.42)	0.636 (4.24)
Thai people ^b	white	77	2.504 (16.69)	0.114 (0.76)	0.047 (0.31)	0.869 (5.79)
	colored	36	3.016 (20.10)	0.738 (4.92)	0.074 (0.49)	0.694 (4.62)

^aProvisional tolerable weekly intake for total As ($15 \mu\text{g kg}^{-1}$ body weight).⁴² ^bAverages of four regions.

Conclusions. The HPLC-ICP-MS method for the determination of arsenic species was developed to achieve good separation and detection in the present study. The digestion of starch in the rice extracts with α -amylase enzyme could prevent the deterioration of the HPLC column. Therefore, the numbers of sample injections were increased resulting in a decrease cost of the running of analysis. The simple and high efficiencies of the method were in good agreement and can be used for routine analysis of As speciation in rice samples. The results of As speciation in rice samples showed that the major As species detected at high levels in all rice types were As^{III}, DMA, and As^V. The colored rice (red and brown) contained both inorganic As and total As higher than white rice. There were two rice samples of the total samples tested that contained inorganic As higher than the maximum level of inorganic As in rice (polished). The weekly intake of inorganic As from rice by Thai people was 13.91% (white rice from Northeastern region)–29.22% (colored rice from South region) of PTWI.

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