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Simultaneous determination of arsenic and mercury species in rice by ion-pairing reversed phase chromatography with inductively coupled plasma mass spectrometry

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ABSTRACT

An analytical method using reversed phase chromatography–inductively coupled plasma mass spectrometry for arsenic and mercury speciation analysis was described. The effect of ion-pairing reagent on simultaneous separation of four arsenic (arsenite, arsenate, monomethlyarsonate and dimethylarsinate) and three mercury species (inorganic mercury (Hg^{II}), methylmecury and ethylmercury) was investigated. Parameters including concentrations and pH of the mobile phase were optimized. The separation and re-equilibration time was attained within 20 min. Meanwhile, a sequential extraction method for arsenic and mercury in rice was tested. Subsequently, 1% HNO_3 microwave-assisted extraction was chosen. Calibration curves based on peak area measurements were linear with correlation coefficient greater than 0.9958 for each species in the range studied. The detection limits of the species were in the range of 0.84–2.41 μg/L for arsenic and 0.01–0.04 μg/L for mercury, respectively. The proposed method was then successfully applied for the simultaneous determination of arsenic and mercury species in rice flour standard material and two kinds of rice from local markets.

1. Introduction

Arsenic (As) and mercury (Hg) are well known as toxic elements because of their potential risks to human health. Exposure to As or Hg has been linked to an increased risk of many physiological disorders and various types of cancer (Mir et al., 2007). With the rapid development of modern industry, As and Hg are often present as a result of human activities, such as mining/processing of ore and wood treatment (Afton, Kubachka, Catron, & Caruso, 2008). Since As and Hg can be taken up and accumulated by crops, the safety and quality of agricultural products are subject to serious threats from contaminated sources.

Unfortunately, rice, a staple crop for Chinese people, is very efficient in As and Hg accumulation (Ma, Shen, Wu, Tang, Shen, & Zhao, 2014; Ren, Sun, Wang, Luo, & Ma, 2014; Rothenberg et al., 2011; Sommella et al., 2013). Along with drinking water and seafood, the consumption of rice has become the major contributor to As and Hg, thereby causing potential health risks (Sun, Williams, & Zhu, 2009). Studies have shown a positive correlation between health risks and the total As and Hg concentrations of rice (Fang et al., 2014; Qian et al., 2010). The toxicities of As and Hg depend on their chemical speciation. Generally, inorganic As is more toxic than organic As, and the levels of toxicity of As compounds are as follows: arsenite (As^{III}) > arsenate (As^{V}) > monomethlyarsonate (MMA) > dimethylarsinate (DMA) (Gurkan, Kir, & Altunay, 2015; Moreda-Piñeiro, Moreda-Piñeiro, & Romarís-Hortas, 2011). Interestingly, some organic As species such as arsenobetaine and arsenosugars have been proved to be non-toxic (Gómez-Ariza, Lorenzo, & Garcıa-Barrera, 2004; Nam, Oh, Min, & Lee, 2010). Evidence has shown that the major Hg species generally found in biological samples are either methylmecury (MeHg) or inorganic mercury (Hg^{II}) (Doker & Bosgelmez, 2015; Li et al., 2007). The levels of toxicity of Hg compounds are as follows: MeHg > ethylmercury (EtHg) > Hg^{II}. Therefore, measuring the total As and Hg concentration alone is not enough to assess the hazards of As and Hg.

In recent years, a number of analytical techniques have been widely employed for the speciation analysis of As or Hg, including gas chromatography (GC) or high performance liquid chromatography (HPLC) coupled with element specific techniques such as atomic absorption spectroscopy (AAS), atomic emission

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spectrometry (AES), atomic fluorescence spectroscopy (AFS), and inductively coupled plasma mass spectrometry (ICP-MS) (Do, Robinet, Pradeau, & Guyon, 2001; Pasias, Thomaidis, & Piperaki, 2013; Pelcova, Docekalova, & Kleckerova, 2015; Zmozinski, Llorente-Mirandes, Lopez-Sanchez, and Silva (2015)). HPLC techniques are better suited for the separation of As or Hg due to the relatively wide compatibility of mobile phase composition and the easiness of sample preparation (Lin, Chang, & Jiang, 2008; Liu, Zhang, Hu, & Cheng, 2013). ICP-MS also has the advantages of high sensitivity, wide linearity, low detection limit, and multi-elemental analysis (Iserte, Roig-Navarro, & Hernández, 2004). Therefore, coupling of ICP-MS with HPLC is the most widely used technique for the individual speciation of As or Hg that has been employed successfully on a number of different matrices (Khan et al., 2015; Moreno, Garcia-Barrera, & Gomez-Ariza, 2013; Raber et al., 2012; Souza, Campiglia, & Barbosa, 2013). C_{18} reversed-phase chromatography with the ion-pairing reagent tetrabutylammonium hydroxide (TBAH) has been employed in the separation of four selenium and four As species within 18 min by HPLC coupled with ICP-MS (Afton et al., 2008). Currently, however, a single method for the simultaneous separation of common As and Hg species in one chromatographic run has not yet been reported. Gómez-Ariza et al. (2004) established a method for the simultaneous determination of Hg and As species in natural freshwater by HPLC (hydrides generation and cold vapor) coupled with a home modified AFS and a standard AFS system. However the use of modified AFS makes this method somewhat complex, and it cannot be widely used for other matrices. To the best of our knowledge, no information is available regarding the simultaneous speciation analysis of As and Hg in rice using HPLC–ICP-MS.

The main objective of this work was to develop a method for the simultaneous speciation analysis of As and Hg species using HPLC–ICP-MS. Seven common environmentally and biologically observed As and Hg species standards were baseline separated on a C_{18} chromatography column *via* ion-pairing reversed phase chromatography. To illustrate the potential applicability of the proposed method, we successfully optimized the chromatographic method applied to the certified reference materials (CRMs) NIST 1568a rice flour, GBW 10043 rice flour, and two kinds of rice flour from local markets.

2. Materials and methods

2.1. Reagents and materials

All the solutions were prepared with doubly deionized water (DDW) (18 MΩ cm^{-1}, Millipore-Q American). The following commercial products were used: Nitric acid (65%) was purchased from Merck, methanol (HPLC grade) was obtained from Kermel, ammonium dihydrogen phosphate (GR) was purchased from Simopharm Chemical Reagent Co., Ltd, L-cysteine (Reagent Grade) from Solarbio Science & Technology Co., Ltd, and both ammonium acetate (GR) and tetrabutylammonium hydroxide (TBAH) 40% in water (ion-chromatography grade) from Shanghai Aladdin.

The rice flour CRM is GBW 10043 rice flour from the National Standard Substance Center in Beijing, China. As (1 mg/mL) in 1 mol/L HNO_3 and Hg (1 mg/mL) in 1 mol/L HNO_3 for the quantification of total As and Hg were acquired from Aladdin. Standards used for sample spiking and identification are as follows: arsenic acid solution (AsO_4^{3-}; As^V, 32.4 ± 0.7 μg/g); arsenious acid solution (AsO_3^{3-}; As^{III}, 124.3 ± 2.0 μg/g); monomethylarsonic acid solution (MMA, 46.2 ± 1.5 μg/g); dimethylarsinic acid solution (DMA, 97.4 ± 3.3 μg/g); methylmercury solution (MeHg, 65.5 ± 2.5 μg/g); ethylmercury solution (EtHg, 75.3 ± 2.7 μg/g); mercuric chloride ($HgCl_2$ ⩾ 99.5%). All the standard materials were purchased from the National Standard Substance Center (Beijing, China). All standard solutions were prepared by dissolving compounds in DDW at 100 μg/mL As and 10 μg/mL Hg. Working standard solutions (1–20 ng/mL for As species and 0.1–2 ng/mL for Hg species) were prepared daily by diluting the 100 μg/mL As species and 10 μg/mL Hg species mixed standard stock solutions.

2.2. Instrumentation

A microwave (CEM, USA) was used for digesting and extracting samples. For total As and Hg determination, all measurements were carried out with an 7700× ICP-MS (Aglient USA). An Aglient 1260 HPLC with a reverse-phase C_{18} column (150 mm × 4.6 mm, 5 μm, Agilent Eclipse plus, USA) were used for the separation of As and Hg species. The outlet of the chromatographic column was directly connected to the nebulizer of the ICP-MS with a small piece of perfluoroalkoxy (PFA). The instrumental operating conditions are shown in Table 1.

2.3. Sample collection and preparation

The proposed method was validated using NIST 1568a rice flour, GBW 10043 rice flour and two kinds of rice samples from local markets. Sample A was Hunan-grown rice with a relatively high concentration of As, are determined in on our previous investigation (Fang et al., 2014). Sample B was common rice purchased from Liaoning province. All the rice samples were ground to powder, passed through an 80-mesh sieve, and oven-dried at 60 °C for 5 h. The rice flour samples were packaged in clean plastic bags and placed in a refrigerator at 4 °C until analysis.

2.4. Determination of total As and Hg

The total content of As and Hg in rice flour was determined by ICP-MS. The rice flour samples were digested according to the method described by Fang et al. (2014). Approximately 0.500 g of rice four was weighed into digestion vessels, and then added with 5 mL of HNO_3. After soaking for 1 h, 1 mL of H_2O_2 was added, and then placed in the microwave digester. The temperature was raised first to 120 °C in 5 min, then to 160 °C in 5 min and held for 5 min. Finally the digestion temperature was elevated to 180 °C in 5 min and held for 10 min.

Table 1
Instrumental operating conditions.

ICP-MS parameters						
Power	1550 W					
Plasma Ar flow	15.0 L min^{-1}					
Carrier Ar flow	1.05 L min^{-1}					
Isotopes monitored	^{75}As, ^{200}Hg, ^{201}Hg, ^{202}Hg					
Quadrupole bias	−16.0 V					
Octopole bias	−18.0 V					
Dwell time for each isotope	0.1 s					
HPLC conditions						
Ion-pairing RP-HPLC	Agilent Eclipse plus C_{18} (150 mm × 4.6 mm, 5 μm)					
Mobile phase A	5 mmol/L TBAH, 10 mmol/L $NH_4H_2PO_4$					
Mobile phase B	5% (v/v) methanol, 0.1% (m/v) L-Cysteine, 0.06 mmol/L CH_3COONH_4					
pH	7.1					
Flow rate	1.0 mL min^{-1}					
Injection volume	40 μL					
Gradient program						
Time (min)	0	0.5	1.5	10	12	20
%A	100	100	0	0	100	100
%B	0	0	100	100	0	0

2.5. Extraction of As and Hg species

2.5.1. Microwave-assisted extraction

Rice flour samples were extracted with 1% HNO_3 as described by Sun et al. (2008). Approximately 0.500 g of rice flour was accurately weighed into microwave vessels, added with 15 mL of 1% HNO_3, and pre-extracted at room temperature for 1 h, then placed in the microwave oven. The temperature was raised first to 55 °C in 5 min and held for 10 min, then to 75 °C in 5 min and held for 10 min, and finally to 95 °C in 5 min and held for 30 min. After cooling, the digests were transferred to a centrifuge tube, and centrifuged at 5000 rpm for 20 min. The supernatants were diluted to 25 mL with DDW, and filtered through a 0.22 μm cellulose acetate membrane, then stored in a cold location at 4 °C until analysis. For analysis, three subsamples and blanks were prepared in parallel.

2.5.2. Ultrasonic-assisted extraction

Rice flour samples were extracted by ultrasonic-assisted extraction with different extraction solvents according to the methods of Narukawa, Suzuki, Inagaki, & Hioki (2014). Approximately 0.500 g of rice flour was accurately weighed into centrifuge tubes. Five milliliter of extraction solvent (methanol: water (v:v/1:1) or 10 mmol/L $NH_4H_2PO_4$ in 0.1% (m/v) L-cysteine) was added, sonicated at 60 °C for 4 h, and then centrifuged at 5000 rpm for 20 min. The supernatants were diluted to 25 mL with DDW, and then filtered through a 0.22 μm cellulose acetate membrane. All the samples were stored in a cold location at 4 °C until analysis. For analysis, three subsamples and blanks were prepared in parallel.

The extraction efficiency of As and Hg compounds was defined as the ratio of total As and Hg concentrations in extracts to those in samples.

2.6. Separation of As and Hg species by HPLC-ICP-MS

An Agilent Eclipse plus C_{18} (150 mm × 4.6 mm, 5 μm) was chosen in combination with an ion-pairing reagent. Several parameters including concentrations of TBAH, $NH_4H_2PO_4$, L-cysteine, methanol, and the pH were optimized with regards to separation time and resolution, as well as signal intensity in ICP-MS. The optimized instrumental settings for the ICP-MS detection and the chromatographic conditions used for species separation are listed in Table 1.

2.7. Analytical performance characteristics

Since there was no reference value available for the sample obtained locally, the accuracy of the extraction method was checked by comparing the sum of the concentration of individual As and Hg species with the total concentration obtained from the complete digestion method. The quantifications were carried out based on peak area by external standard calibration. Recoveries and precision of the method were examined by spiking the sample solution with suitable concentrations of each of As and Hg mixture species. An amount of 10 g rice flour samples were spiked with 1 mL of standard solutions (1 μg/mL As species and 0.05 μg/mL Hg species), dried and then extracted with the extraction solution. The limits of detection (LOD) were calculated taking into account three times the standard deviation of seven replicates for the blank peak areas divided by the slope of the calibration curves. The reproducibility of the chromatographic retention times and peak areas were calculated by taking the relative standard deviation (RSD) of eleven replicates of the mixed standards (As: 50 ng/mL, Hg: 4 ng/mL). The column recovery is expressed as the ratio of the peak area of a single As (20 ng/mL) or Hg specie (2 ng/mL) eluted from the chromatographic column to the peak area eluted without the chromatographic column.

3. Results and discussion

3.1. Optimization of chromatographic separation conditions

Previous studies showed that pH 5.5–6.5 was optimal for As species and successfully separation of As species was achieved at pH 6.0 on a C_{18} column with ion-pairing reagent (Afton et al., 2008). Therefore, according to previous publications (Afton et al., 2008; Li et al., 2007), a preliminary study was attempted to select the mobile phase at pH 6.0 using Agilent Eclipse plus C_{18} column without using any ion-pairing reagents (mobile A, 15 mmol/L $NH_4H_2PO_4$ for As; mobile phase B, 3% (v/v) methanol, 0.1% (m/v) L-cysteine and 0.06 mmol/L CH_3COONH_4 for Hg). Unfortunately, As species could not be separated and the shape of Hg peaks was welter under this condition. Therefore the further optimization of chromatographic separation based on the preliminary results was carried out.

3.1.1. Selection of analytical column and ion-pairing agent

The acid dissociation constants (pK_a) of As species are spread over a large range: As^{III} (pK_{a1}, 9.2), As^{V} (pK_{a1}, 2.3; pK_{a2}, 6.8; pK_{a3}, 11.6), MMA (pK_{a1}, 2.6; pKa2, 8.2), and DMA (pK_{a1}, 6.2). Most of the As species compounds may be anionic at in the appropriate pH (As^{III} is neutral molecules) (Ammann, 2011). Considering the ionic characteristics of As and Hg (As species are anionic charged, while Hg species are positively charged), reversed-phased chromatography was necessary for the simultaneous separation of As and Hg (Gómez-Ariza et al., 2004). Several studies showed that reversed-phased column exhibited good performance for the separation of Hg species (Doker & Bosgelmez, 2015; Pelcova et al., 2015; Souza et al., 2013). Compared with Hg species, As species, with stronger polarization are more weakly retained on the reversed-phased column due to their negative charges. Ion-pairing agents have been evaluated for their ability to bind with As ions to form reserved molecules in order to enhance hydrophobic retention on the reversed-phased column (He, Cook, Littlepage, Cundy, & Mangalathillam, 2015). Ion-pairing reagents including hexanesulfonate, tetrabutylammonium phosphate (TBA) (Do et al., 2001), TBAH, and tetraethyl ammonium perchlorate (TEAP) were often used for the simultaneous separation of As and selenium (Afton et al., 2008). Among the ion-pairing agents, TBAH has been provided sufficient retention necessary for the baseline separation of As (Afton et al., 2008). For this reason, TBAH was chosen as the ion-pairing reagent for As. In this study TBAH was used only in the first 1.5 min, and the Hg species were first eluted after 5 min. The retention time of the Hg species did not change with the concentration of TBAH. Fig. 1A illustrates the influence of TBAH on As separation. When the concentration of TBAH < 5 mmol/L, the four As compounds could not be completely separated, but when TBAH > 5 mmol/L, the four As peaks were thoroughly separated. Higher concentrations of TBAH increased the retention time of the As species. To obtain better separation and appropriate retention times, 5 mmol/L TBAH was selected for use in the following experiments. Finally the elution order of the As species was as follows: As^{III}, DMA, MMA and As^{V}.

3.1.2. Effect of phosphate on HPLC separation

Phosphate is widely used as an eluent due to its ability adjust the pH of the sample injected onto a column to that of the eluent pH (Ammann, 2011). Furthermore, phosphate has a charge that can compete for the ion-pairing reagent with the As species. In this study, ammonium dihydrogen phosphate ($NH_4H_2PO_4$) was chosen

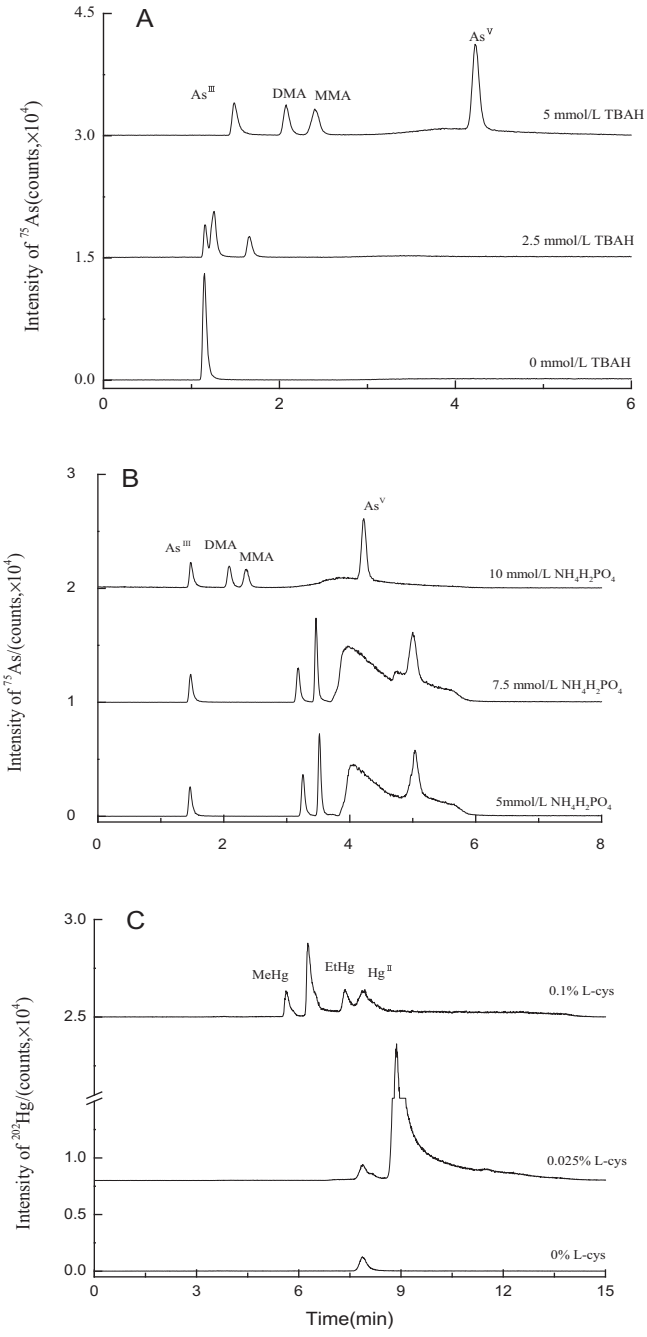

Fig. 1. The effect of (A) TBAH, (B) $NH_4H_2PO_4$, (C) L-cysteine on chromatograms of As or Hg species (100 ng/mL each of As^{III}, DMA, MMA, As^{V}, and 20 ng/mL each of Hg^{II}, MeHg, EtHg; pH 6.0).

as the mobile phase to separate As compounds. We used the ion-pairing reagent only in the first 1.5 min because of the delay of alternated mobile phase. As shown in Fig. 1B, 1.5 min shows good separation of As species was achieved in 1.5 min, which enabled the simultaneous separation. With an increase of $NH_4H_2PO_4$, the retention times of the As compounds decreased, except for that of As^{III} (Fig. 1B). When the concentration was higher than 10 mmol/L, DMA and MMA could not be well separated, presumably because with the increase of $NH_4H_2PO_4$, there were more charges competing with the As species. To obtain a better peak shape and to avoid the overlap of the As and Hg compounds, 10 mmol/L of $NH_4H_2PO_4$ was chosen in the confirmation experiment.

3.1.3. Effect of L-cysteine

The use of a complexing agent can enhance the interaction of Hg species and the non polar stationary phase (Gómez-Ariza et al., 2004). The use of some compounds such as 2-mercaptoethanol, diethyldithiocarbamate and L-cysteine were necessary to avoid the memory effect of Hg due to their complexing capability (Chang, Jiang, & Sahayam, 2007). Compared to other complexing agents, L-cysteine showed good performance in eluting Hg compounds (Lopez, Cuello, Camara, & Madrid, 2010). The separation of Hg species is based on the formation of cysteine-mercury complexes (Batista, Rodrigues, Souza, Souza, & Barbosa, 2011b). L-cysteine was therefore chosen in this study. The evaluated concentrations of L-cysteine in the mobile phase solution were 0, 0.025, 0.050, 0.075, 0.100, and 0.125%. As shown in Fig. 1C, when no L-cysteine, little Hg was eluted. When the concentration of L-cysteine was 0.025%, the Hg compounds that could not be eluted initially, could be all eluted, forming a huge peak. By increasing of the L-cysteine, three Hg compounds could be separated and their retention time did not change with L-cysteine concentration. However, an L-cysteine concentration of >0.1% could create an elevated signal baseline at the end of mercury separation. As shown in Figs. 1C and 2B, when 0.1% L-cysteine was used, three Hg species were separated well. Therefore, 0.1% L-cysteine was chosen in the confirmation experiments for better peak shape.

3.1.4. Effect of methanol

Appropriate addition of methanol can improve the detection sensitivity of As and Hg (Nam et al., 2010). In this study, we

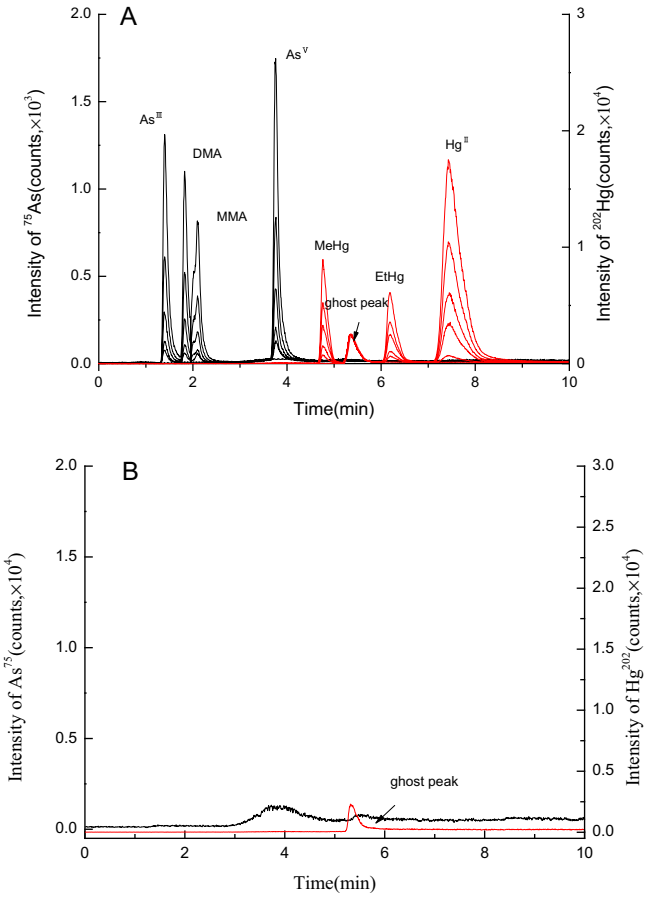

Fig. 2. HPLC-ICP-MS chromatograms of (A) a mixture of As and Hg standards and (B) double-deionized water (1–20 ng/mL each of As species, and 0.1–2 ng/mL each of Hg species). The analysis was performed according to the optimum conditions shown in Table 1.

observed the effect of the addition of methanol to the mobile phase in the range 0–6% (v/v), and found the retention times to decrease with an increased methanol concentration until the methanol concentration reached 5%. Another important observation is that when the addition of methanol in the mobile phase was 5% (v/v), three Hg compounds were separated from the ghost peak (Fig. 2A). Accordingly, 5% (v/v) methanol was selected in the confirmation experiments.

3.1.5. Effect of pH

The wide pK_a range of the analytes determined that pH would influence their ion concentration and in turn affect the elution time and selectivity of As and Hg species (Afton et al., 2008; Ammann, 2011). In our preliminary experiment, when the pH was below 6, the baselines of As and Hg were confused. When the pH was below 4, the major As and Hg species were unstable. Therefore, the pH of the mobile phase in the range of 6.0–8.0 was checked (mobile phase A: 5 mmol/L TBAH and 10 mmol/L $NH_4H_2PO_4$; mobile phase B: 5% (v/v) methanol, 0.1% (m/v) L-cysteine and 0.06 mmol/L CH_3COONH_4). For the As compounds, the retention time of DMA decreased with an increase in pH. At pH < 6.70, the retention time of MMA was also decreased with increased pH, but it exhibited the opposite trend at pH > 6.70, the retention time of MMA was increased. For Hg compounds, the retention times of MeHg and EtHg decreased with increased pH. The effect of pH was more pronounced for organic As and Hg species. When the pH was greater than 7.10, the EtHg and Hg^{II} peaks were merged. At pH < 7.10, the DMA and MMA peaks were merged and the shape of the Hg peaks was welter. Finally, pH 7.1 was selected to obtain better separation and resolution of As and Hg species. The pH of both mobile phases A and B was similar.

The optimal chromatographic separation conditions are shown in Table 1. Under this condition four As and three Hg species were separated within 20 min (including 10 min re-equilibration time). The concentration of each As and Hg compounds were 100 ng/mL and 20 ng/mL, respectively. As shown in the Fig. 2A, however, there was an unknown peak between MeHg and EtHg. Therefore doubly deionized water was chosen as the sample and injected into the HPLC-ICP-MS (Fig. 2B), and it turned out that an unknown peak with the same peak area was appeared in the same retention time. To clarifications of this unknown peak, three Hg isotopes (200, 201, 202) were monitored. However, the ghost peak was observed in all elution conditions, and also showed up during later application of rice extracts. Therefore, this Hg ghost peak was not eluted from standards or rice extracts. As L-cysteine is often contaminated with inorganic Hg, the ghost peak might be due to the addition of L-cysteine in the mobile phase during gradient elution. Either residue or the state of the ICP-MS may also account for the reasons for the ghost peak.

Table 2
The comparison of As and Hg extraction efficiency of various extraction procedures.

Extraction procedure	Percent of the total As (%)	Percent of the total Hg (%)
Mobile phase ultrasonic extraction	25.42 ± 1.76	65.62 ± 1.27
50% (v/v) Methanol ultrasonic extraction	76.01 ± 3.17	8.90 ± 0.43
1% HNO_3 Microwave-assisted extraction	74.31 ± 2.42	59.80 ± 4.47

3.2. Analytical figures of merit

Fig. 2A shows the separation of As and Hg species at standards concentrations ranging from 1 to 20 ng/mL and 0.1–2 ng/mL, respectively. Calibration curves based on peak area measurement were linear with a correlation coefficient (R^2) greater than 0.9958 for each species in the range studied. Under the optimized conditions, the limit of detection (LOD) were 1.04, 0.84, 0.95, 2.41, 0.03, 0.01, and 0.04 μg/L for As^{III}, DMA, MMA, As^{V}, MeHg, EtHg, and Hg^{II}, respectively. The LODs of this method for the As species were lower than those reported by Heitkemper, Vela, Stewart, & Westphal (2001). The RSD of the retention time ranged from 0.1 to 0.4 and the RSD of the peak area was between 2.2 and 5.1. All the column recoveries were more than 87.0%. These values are in agreement with those reported by Zmozinski et al. (2015), who achieved column recoveries from 58% to 104% using LC-ICP-MS. All the results were considered acceptable at these low levels.

3.3. Evaluation of the extraction efficiency

Generally, phosphate buffer is used as solvent for the extraction of As species from biological tissue (Liu et al., 2013; Reyes et al., 2009) and a mixture of L-cysteine is used for extracting reagent for Hg species (Chang et al., 2007; Lopez et al., 2010). In previous studies, extraction techniques such as shaking or sonication with either acid or alkali as solvent have been commonly used for extracting As and Hg from biological tissues (Narukawa et al., 2014; Souza et al., 2013). Kim et al. (2013) used 1% HNO_3 to extract As from rice grains and achieved good recoveries and minimal inter-conversion of As species. However, the use of 1% HNO_3 has the potential risk to reduce As^{V} to As^{III}, and it has been reported that mixtures of methanol and water are relatively mild extractants, which could maintain the integrity of the As species in the

Table 3
Speciation of arsenic and mercury in rice flour extracted by 1% HNO_3 microwave-assisted extraction.

Species	NIST 1568a		GBW10043	Sample A	Sample B
	Referenced value [a]	Founded			
As^{III} (mg/kg)	0.06 ± 0.01	0.07 ± 0.03	0.06 ± 0.01	0.10 ± 0.02	0.03 ± 0.01
DMA (mg/kg)	0.17 ± 0.03	0.10 ± 0.03	–	0.10 ± 0.03	–
MMA (mg/kg)	0.011 ± 0.002	–	–	–	–
As^{V} (mg/kg)	0.04 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.18 ± 0.04	0.10 ± 0.03
Sum of As species (mg/kg)	0.28	0.20	0.09	0.38	0.13
Total As (mg/kg)	0.29 ± 0.03	0.25 ± 0.02	0.11 ± 0.01	0.50 ± 0.01	0.18 ± 0.31
MeHg (μg/kg)	2.25 ± 0.04	2.76 ± 0.21	3.90 ± 0.37	4.99 ± 0.57	3.61 ± 0.23
EtHg (μg/kg)	–	–	–	–	–
Hg^{II} (μg/kg)	3.61 ± 0.29	0.78 ± 0.11	–	–	–
Sum of Hg species (μg/kg)	5.86	3.54	3.90	4.99	3.61
Total Hg (μg/kg)	5.8 ± 0.5	5.98 ± 0.51	6.65 ± 1.10	10.35 ± 1.60	6.20 ± 0.90

– Below the LOD.

[a] The values for total As and Hg are extracted from the Certificate of Analysis, while the values of individual As or Hg species are according to Juskelis, Li, Nelson, and Cappozzo (2013) and Lin et al. (2008).

sample (Mir et al., 2007). Finally, three modified classical extraction techniques: mobile phase ultrasonic extraction, 1% HNO_3 microwave-assisted extraction and 50% (v/v) methanol ultrasonic extraction were chosen for preliminary exploration of their applicability in the simultaneous extraction of As and Hg species. As shown in Table 2, the microwave-assisted 1% HNO_3 extraction gave a relatively higher extraction efficiency of As and Hg (74.31% for As, 59.80% for Hg), which was acceptable. The concentration of As^{III} and As^{V} was higher than that achieved by 50% (v/v) methanol ultrasonic extraction (Table 3) and the As and Hg species did not vary as indicated by their recoveries. The extraction efficiency of As by 50% (v/v) methanol ultrasonic extraction in this study agree with that of a previous study (Narukawa, Inagaki, Kuroiwa, & Chiba, 2008), but there was little extraction of the Hg compounds when using 50% methanol. As Hg has a high affinity to sulphydryl groups, 50% methanol, a relatively mild organic solvent, may not dissolve. Hg compounds from rice, especially considering that the total Hg concentration of our rice flour was low (Lopez et al., 2010). Finally, the 1% HNO_3 microwave-assisted extraction was chosen for the simultaneous analysis of As and Hg species in rice by HPLC-ICP-MS, and a comparison with 50% methanol ultrasonic-assisted extraction was performed for As species.

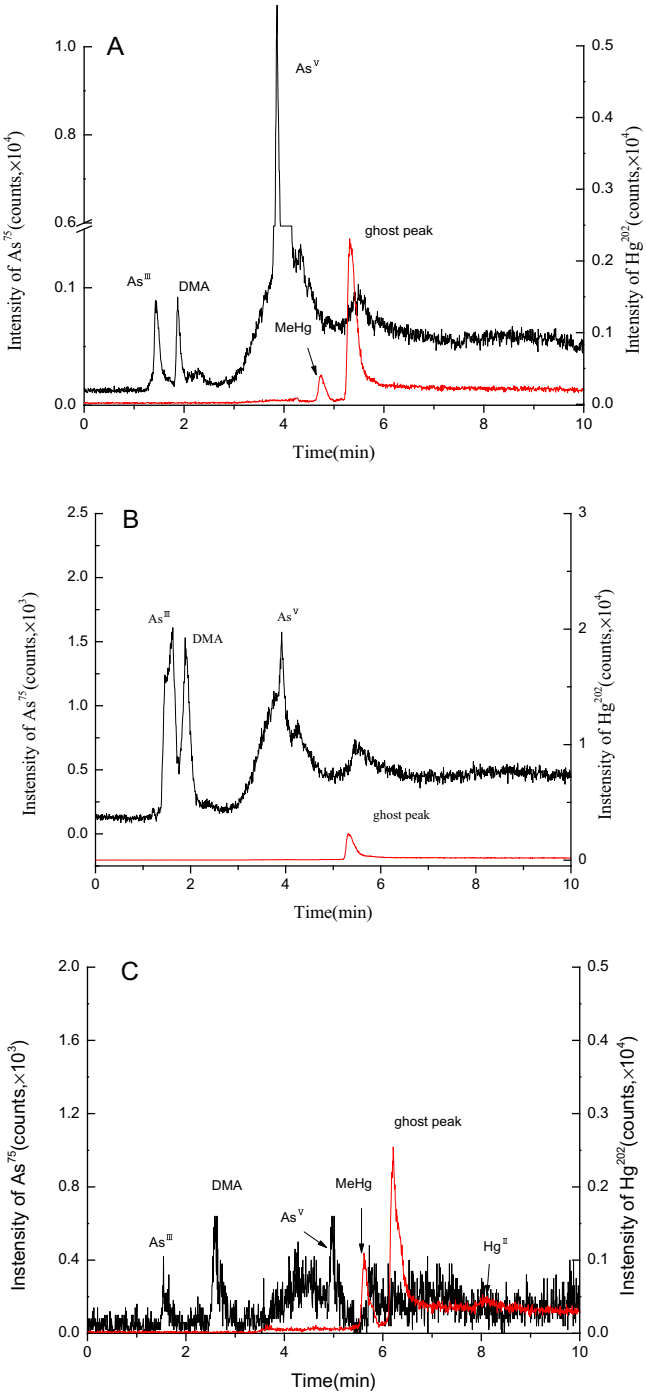

Fig. 3. HPLC-ICP-MS chromatograms of As and Hg species extracted from sample A rice flour by (A) 1% HNO_3 microwave-assisted extraction, (B) 50% methanol ultrasonic processor extraction and (C) NIST 1568a rice flour by 1% HNO_3 microwave-assisted extraction.

3.4. Application to rice samples

The total As and Hg concentrations in rice were determined by ICP-MS. The method was validated against the certified reference material NIST 1568a rice flour (As: 0.290 ± 0.030 μg/g, Hg: 5.8 ± 0.5 ng/g) and GBW 10043 rice flour (As: 0.114 ± 0.018 μg/g, Hg: 4.8 ± 0.8 ng/g). The results for the NIST 1568a and GBW 10043 rice flour (Table 3) agreed with the certified values. Finally, Table 3 shows the concentrations of total As and Hg in all rice flour samples. The simultaneous determination method was validated using rice flour. As shown in Fig. 3, As^{III}, As^{V} and MeHg were found in all rice flour samples. DMA was found in both NIST 1568a and sample A, while Hg^{II} was found only in the NIST 1568a rice flour. These results are consistent with previous studies indicating that As^{III}, DMA, and As^{V} were the dominant species identified in rice products (Heitkemper et al., 2001;Mar, Reyes, Rahman, & Kingston, 2009), and that MeHg was found as the main species identified in rice (Rothenberg et al., 2011). Moreover, Lin et al. (2008) reported that the major Hg species in NIST SRM 1568a rice flour were Hg^{II} (3.61 ng/g) and MeHg(2.25 ng/g). Batista, Souza, Souza, & Barbosa (2011a) summarized the concentration of each As species in NIST 1568a rice flour from previous studies: As^{III} 52–129 ng/g, DMA 31–180 ng/g, MMA 0–14 ng/g, As^{V} 0–53 ng/g. The analysis results of this study for the reference material NIST 1568a rice flour (Table 3) were agreed with those of previous studies.

Spike recovery experiments with As^{III}, DMA, MMA, As^{V}, MeHg, EtHg, and Hg^{II} showed the stability of the species in the extraction processes. The recoveries of the As and Hg compounds extracted by 1% HNO_3 microwave-assisted extraction ranged from 85.33 to 119.35% and 72.57 to 96.82%, respectively. The recoveries of Hg species were relatively lower, presumably because Hg compounds are unstable and volatile. Compared to 50% (v/v) methanol ultrasonic extraction, 1% HNO_3 microwave-assisted extracted more inorganic As, and had a better resolution with a relatively smooth baseline. Searching for a new extraction solution that is suitable for both As and Hg in rice is still desirable. However, until a new extraction solution is established, 1% HNO_3 microwave-assisted extraction is still practical, which provides good recoveries and minimal inter-conversion of both As and Hg.

4. Conclusions

In this study, an analytical technique for simultaneous speciation of As and Hg in rice flour by HPLC-ICP-MS was established, and 1% HNO_3 microwave-assisted extraction was chosen as the simultaneous extraction for As and Hg species in rice flour. All As and Hg species could be separated within 20 min (including 10 min re-equilibration time) by using TBAH as the ion-pairing reagent on a C_{18} column. The approach is simple and sensitive and can be used for the simultaneous speciation analysis of As and Hg species in rice flour.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2016.07.003.

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