

## Speciation Analysis of Mercury in Cereals by Liquid Chromatography Chemical Vapor Generation Inductively Coupled Plasma-Mass Spectrometry

LIANG-YEN LIN, LAN-FANG CHANG, AND SHIUH-JEN JIANG\*

Department of Chemistry, National Sun Yat-sen University, Kaohsiung 80424, Taiwan

A simple and rapid procedure for the separation and determination of inorganic, methyl, and ethyl mercury compounds was described using liquid chromatography (LC) followed by vapor generation inductively coupled plasma-mass spectrometry (VG-ICP-MS). Well resolved chromatograms were obtained within 5 min by reversed-phase liquid chromatography with a C8 column as the stationary phase and a pH 4.7 solution containing 0.5% v/v 2-mercaptoethanol and 5% v/v methanol as the mobile phase. The separated mercury compounds were converted to mercury vapors by an *in situ* nebulizer/vapor generation system for their introduction into ICP. The concentrations of NaBH<sub>4</sub> and HNO<sub>3</sub> required for vapor generation were also optimized. The method was applied for the speciation of mercury in reference materials NIST SRM 1568a Rice Flour and NIST SRM 1567a Wheat Flour and also rice flour and wheat flour samples purchased locally. The accuracy of the procedure was verified by analyzing the certified reference material NRCC DOLT-3 Dogfish Liver for methyl mercury. Precision between sample replicates was better than 13% for all the determinations. The detection limits of the mercury compounds studied were in the range 0.003–0.006 ng Hg mL<sup>-1</sup> in the injected solutions, which correspond to 0.02–0.06 ng g<sup>-1</sup> in original flour samples. A microwave-assisted extraction procedure was adopted for the extraction of mercury compounds from rice flour, wheat flour, and fish samples using a mobile phase solution.

**KEYWORDS:** Mercury speciation analysis; chemical vapor generation; liquid chromatography; inductively coupled plasma-mass spectrometry; rice flour; wheat flour

### INTRODUCTION

Mercury is one of the most toxic elements that are present naturally in the environment (1). The release of mercury into the atmosphere is by degassing from the Earth's crust, oceans, and also due to human activities such as burning household, industrial waste and fossil fuel such as coal (2). The toxicity, biogeochemistry, and bioavailability of elements depend not only on their total concentration but also on their chemical form (3, 4). Mercury exists in inorganic and various organic forms of varying toxicities (5). Mercury released into the air is deposited on land and water, which is transformed into more toxic methyl mercury by bacteria (6, 7). Methyl mercury thus generated can be accumulated through the aquatic food chain and concentrated hundreds/thousands of times posing a potential risk to humans and wild life that consume fish (8). Apart from fish, mercury can also be accumulated from water and soil into different types of crops that produce grains and vegetables (9). As the food, grains, vegetables, and fish are consumed by human beings, periodic monitoring of these samples for toxic species is of great concern. Though it is well known that methyl mercury is more toxic, it is important

to know its fraction among inorganic and other possible organic forms of mercury with greater accuracy. The knowledge of speciation of mercury is also important for assessing their mobility in the environment.

A variety of methods have been reported for the speciation of mercury by coupling GC and LC with element specific techniques such as AAS, AES, AFS, and ICP-MS (10–13). However, LC techniques are better suited for the separation of polar compounds such as inorganic and organic mercury (14). Coupling of LC to ICP-MS gained much attention because of its ease of sample preparation, simplicity of interface to the detector, availability of the isotope ratio information, and specificity of the signal intensity of the determined element (15). Vapor generation of mercury after LC separation is found to give better sensitivities (16–18). The longer elution times and the high concentrations of organic solvents and acids are some of the drawbacks reported in the literature, which increases analysis times and decreases the signal-to-noise ratios that dictate the detection limits. The aim of the present work is to develop a rapid and simple mercury speciation method with better detection limits and lower retention times than the existing methods for routine monitoring of food samples. In this respect, the developed procedure is a better alternative to existing procedures.

\* Corresponding author. Fax: 886-7-5253908. E-mail: sjjiang@faculty.nsysu.edu.tw.

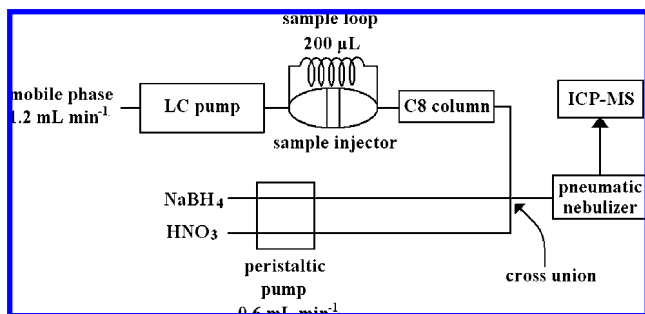


Figure 1. Schematic diagram of LC-VG-ICP-MS.

Table 1. Operating Parameters of ICP-MS, LC, and Vapor Generation

ICP-MS instrument	Perkin-Elmer SCIEX ELAN 6100 DRC II
ICP parameters	
RF power	1350 W
plasma gas flow rate	15.0 L min <sup>-1</sup>
auxiliary gas flow rate	1.10 L min <sup>-1</sup>
nebulizer gas flow rate	0.98 L min <sup>-1</sup>
Mass Spectrometer Settings	
resolution	0.7 amu at 10% peak maximum
dwelt time	100 ms
sweeps	10
readings	500
replicates	1
autolens	on
isotopes monitored	<sup>200</sup> Hg, <sup>202</sup> Hg
LC System	
pump	Hitachi model L-6000 and L-6200
injector	Rheodyne 7215i
column	Perkin-Elmer C8, 3 µm diameter particles, 3 mm i.d. × 30 mm length
mobile phase	0.5% v/v 2-mercaptoethanol, 5% v/v methanol, pH 4.7
flow rate	1.2 mL min <sup>-1</sup>
sample loop volume	200 µL
VG Conditions	
NaBH <sub>4</sub>	0.1% m/v in 0.02 mol L <sup>-1</sup> NaOH
NaBH <sub>4</sub> solution flow rate	0.6 mL min <sup>-1</sup>
HNO <sub>3</sub>	0.1% v/v
HNO <sub>3</sub> solution flow rate	0.6 mL min <sup>-1</sup>

The present study deals with the separation of inorganic mercury [Hg(II)], methyl mercury [methyl-Hg], and ethyl mercury [ethyl-Hg] compounds by using a small LC column followed by their determination using ICP-MS after vapor generation. The mercury compounds are converted into vapors of mercury (hydride and/or Hg<sup>0</sup> vapor) by vapor generation (VG) using NaBH<sub>4</sub> prior to introduction into ICP-MS (17). The procedure was applied for the speciation of mercury in reference samples NRCC DOLT-3 Dogfish Liver, NIST SRM 1568a Rice Flour, and 1567a Wheat Flour as well as cereal samples obtained locally. The mercury compounds are extracted from the samples using a microwave extraction procedure using 2-mercaptoethanol solution.

## MATERIALS AND METHODS

**LC-VG-ICP-MS Device and Conditions.** An ELAN 6100 DRC II ICP-MS (Perkin-Elmer SCIEX, Concord, ON, Canada) was used for these experiments. Sample solutions were introduced by a pneumatic nebulizer with a Scott-type spray chamber. In this study, a simple *in situ* nebulizer/vapor generation sample introduction system was coupled with LC-ICP-MS for the mercury speciation analysis (17, 18). The schematic diagram of the setup is shown in Figure 1. The vapor generation system used in this study is a minor and inexpensive modified version of the system described in detail elsewhere (17). Vapor generated from the vapor generation system was delivered to the ICP-

MS for mercury determination. The operating conditions of ICP-MS were optimized by continuous introduction of a solution containing 1 ng mL<sup>-1</sup> Hg in mobile phase into the vapor generation system. The vapor generation ICP-MS operating conditions used in this work are summarized in Table 1. The optimum conditions could vary slightly with time.

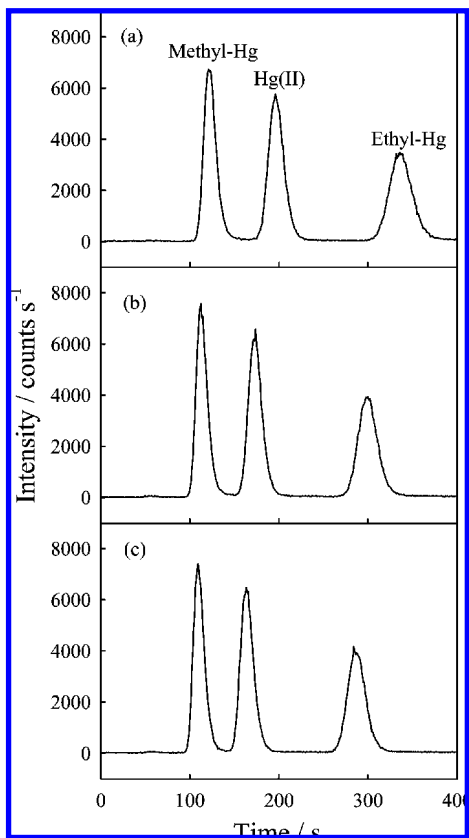
The LC assembly comprised LC pumps (Hitachi, Model L-6000 and L-6200), an injector (Rheodyne 7255i), and a reversed-phase column (Perkin-Elmer C8, 3 µm diameter particles, 3 mm i.d. × 30 mm length). Samples were loaded with a syringe into a 200 µL sample loop. The PEEK cross union was used for mixing the sample, NaBH<sub>4</sub>, and HNO<sub>3</sub>. The mixture was then introduced into the *in situ* nebulizer/vapor generation sample introduction system through polytetrafluoroethylene (PTFE) tubing, where mercury vapor was separated, and introduced into ICP-MS. All separations were performed at room temperature. The conditions listed in Table 1 were those that yielded the best chromatogram of the various sets tested.

**Chemicals.** Analytical-reagent grade chemicals were used without further purification. Purified water (18.2 MΩ-cm) from a Milli-Q water purification system (Millipore, Bedford, MA, USA), was used to prepare all the solutions. HNO<sub>3</sub> (Suprapur), NaBH<sub>4</sub>, NaOH, L-cysteine, and methanol were procured from E. Merck (Darmstadt, Germany). CH<sub>3</sub>HgCl and C<sub>2</sub>H<sub>5</sub>HgCl were from TCI (Tokyo, Japan). Hg(NO<sub>3</sub>)<sub>2</sub> element standard solution was obtained from Spex CertiPrep (Metuchen, NJ, USA). 2-Mercaptoethanol was obtained from Riedel-deHaen (Seelze, Germany). NaBH<sub>4</sub> (0.1% m/v) was prepared daily in 0.02 mol L<sup>-1</sup> NaOH.

**Sample Preparation and Extraction.** The applicability of the developed procedure on the real samples was carried on NIST SRM 1568a Rice Flour and 1567a Wheat Flour reference materials as well as rice flour and wheat flour samples purchased at a local market. The accuracy of the procedure was verified by analyzing the certified reference material NRCC DOLT-3 Dogfish Liver. NRCC DOLT-3 selected as the sample is certified for methyl mercury concentration along with total mercury.

A simple and rapid microwave-assisted extraction procedure was used for the extraction of inorganic, methyl, and ethyl mercury from dogfish, rice flour, and wheat flour samples. A CEM MARS (Matthews, NC, USA) microwave digester equipped with temperature and pressure sensors was used for cereal sample extraction and digestion using HP500 vessels. Approximately 1 g each of rice and wheat flour samples (2 g wheat flour SRM; 0.1 g DOLT-3 fish liver) was accurately weighed into 15 mL polyethylene centrifuge tubes, and 10 mL of 0.5% v/v 2-mercaptoethanol in 5% methanol was added. The centrifuge tube was then placed into the HP500 containing 60 mL of water. The microwave system was programmed to maintain the water temperature at 60 °C for 3 min with a ramp time of 2 min. After microwave heating, the samples were allowed to cool and directly centrifuged for 10 min at 3500 rpm (Hettich, Germany). The supernatant of dogfish liver was diluted by another 40-fold with mobile phase solution, and all the supernatants were filtered through a PVDF filter (Millipore) of 0.22 µm porosity prior to LC separation. The concentrations of mercury compounds were determined by an external calibration method based on peak area. The spike recoveries of individual compound were determined by spiking various samples with suitable amount of inorganic, methyl, and ethyl mercury, dried and then extracted by the extraction solution. The inorganic, methyl, and ethyl mercury standards spiked were 3.0, 3.0, and 1.5 ng g<sup>-1</sup> in rice flours, 0.5, 0.5, and 0.5 ng g<sup>-1</sup> in NIST wheat flour, 3.0, 3.0, and 1.5 ng g<sup>-1</sup> in Taiwan wheat flour, and 1, 1, and 1 µg g<sup>-1</sup> in NRCC dogfish liver.

The extraction efficiency of the mercury compounds was verified by comparing the total mercury concentrations in the extracts using the present procedure with the certified values and/or those obtained from the dissolution method. The dissolution of the samples was carried out in a closed vessel at 80 psi using HNO<sub>3</sub>. To approximately 0.5 g of samples taken in a PTFE vessel, 5 mL HNO<sub>3</sub> was added and closed. The vessel was then heated at 80 psi for 20 min. The digest was diluted to 10 mL and analyzed by pneumatic nebulization ICP-MS with 1 ng mL<sup>-1</sup> of rhodium as the internal standard.

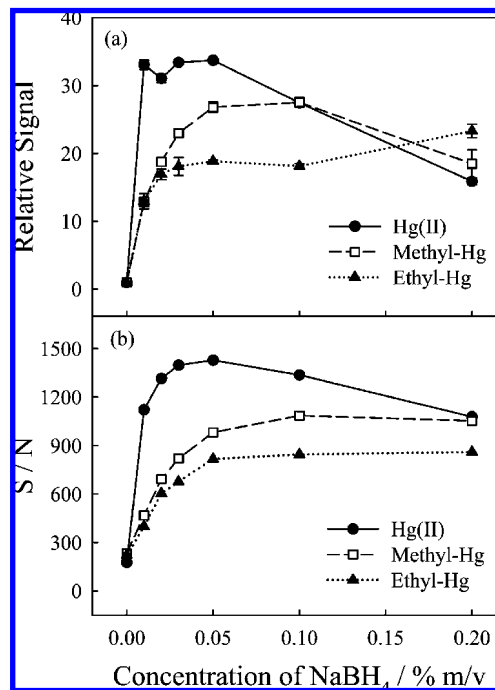


**Figure 2.** Effect of concentration of 2-mercaptoethanol in the mobile phase (5% v/v methanol) on separation of inorganic, methyl, and ethyl mercury compounds: (a) 0.3% v/v, (b) 0.5% v/v, and (c) 0.7% v/v. Hg(II), methyl-Hg, and ethyl-Hg were present at 10 ng Hg mL<sup>-1</sup> each using LC-ICP-MS.

## RESULTS AND DISCUSSION

**Selection of LC Operating Conditions.** The ionic compounds of mercury, namely, Hg(II), methyl-Hg, and ethyl-Hg were separated using a reversed-phase C8 column. To achieve the best chromatogram, 0.5% m/v L-cysteine (17) and 0.5% v/v 2-mercaptoethanol (19) were compared as the mobile phase, and we found that 0.5% v/v 2-mercaptoethanol was better because of better resolution and lower background. 2-Mercaptoethanol was selected as the complexing agent in the following experiments. The flow rate of mobile phase used for all of the studies was 1.2 mL min<sup>-1</sup>. Conventional pneumatic nebulization was employed for the optimization of LC conditions without vapor generation. The effect of the addition of methanol to the mobile phase has been carried out in the range 1–8% v/v. It was found that the retention times decreased with the increase of methanol concentration. However, the resolution of methyl-Hg and Hg(II) was found to decrease when methanol concentration was higher than 5%. Hence, 5% v/v methanol was selected. **Figure 2** shows the effect of 2-mercaptoethanol concentration on the chromatogram. As shown, the retention times decreased with the increase of 2-mercaptoethanol concentration. To achieve better separation and shorter separation times, 0.5% v/v 2-mercaptoethanol was selected in the following experiments. A summary of the optimum LC operating conditions is listed in **Table 1**.

**Optimization of Vapor Generation Conditions.** The mercury compounds were subjected to vapor generation using NaBH<sub>4</sub> after separation for their determination by ICP-MS. The concentration of NaBH<sub>4</sub> required was studied in the range



**Figure 3.** Effect of NaBH<sub>4</sub> concentration on (a) relative signal (peak area) and (b) S/N of mercury compounds studied. Flow rate of NaBH<sub>4</sub> was 0.6 mL min<sup>-1</sup>, and the concentration of HNO<sub>3</sub> was 0.1% v/v. All the data were measured relative to the first point (0% m/v NaBH<sub>4</sub>). The values are the means of three measurements ± standard deviation.

0–0.2% m/v. The results are shown in **Figure 3**. As can be seen, 0.1% NaBH<sub>4</sub> was found to give a better signal-to-noise ratio (S/N) and hence was selected. The flow rate of NaBH<sub>4</sub> used was 0.6 mL min<sup>-1</sup>. The effect of HNO<sub>3</sub> concentration on the vapor generation of mercury compounds has also been studied in the range 0–0.5% v/v. From the experiments, it was found that better signal-to-noise ratio and good reproducibility could be obtained when 0.1% HNO<sub>3</sub> was used. HNO<sub>3</sub> (0.1% v/v) was selected in real sample analysis. A summary of the conditions used for vapor generation is listed in **Table 1**. Typical chromatograms of 1 ng mL<sup>-1</sup> each of Hg(II), methyl-Hg, and ethyl-Hg mixture solution using LC-VG-ICP-MS are shown in **Figure 4**. As shown, the background of mercury (about 620 counts s<sup>-1</sup>) increased slightly when vapor generation sample introduction was used. Repeatability was determined using five consecutive injections of a test mixture containing 1 ng Hg mL<sup>-1</sup> each of Hg(II), methyl-Hg, and ethyl-Hg. The relative standard deviation of the peak areas and peak height was better than 2.6%, and the repeatability of retention time was better than 1.8% for all the compounds. The signals were linear in the concentration range 0.1–10 ng mL<sup>-1</sup> for Hg(II), methyl-Hg, and ethyl-Hg with correlation coefficients better than 0.9997. The detection limit was estimated from the peak height versus concentration plot and based on the concentration (as element) necessary to yield a net signal equal to three times the standard deviation of the background. The LC-ICP-MS detection limits were 0.004, 0.003 and 0.006 ng mL<sup>-1</sup> for Hg(II), methyl-Hg and ethyl-Hg, respectively. Because of the use of a simple LC system, in this study the background was reduced significantly to get better detection limits. The limits of detection are better than the reported procedures (4, 11, 14–22).

**Sample Analysis.** The proposed procedure was applied for the speciation analysis of mercury in reference samples NIST SRM 1568a Rice Flour, 1567a Wheat Flour, and NRCC

**Table 2.** Concentrations and Recoveries of Mercury Compounds in Reference Materials As Measured by LC-VG-ICP-MS<sup>a</sup> (*n* = 3)

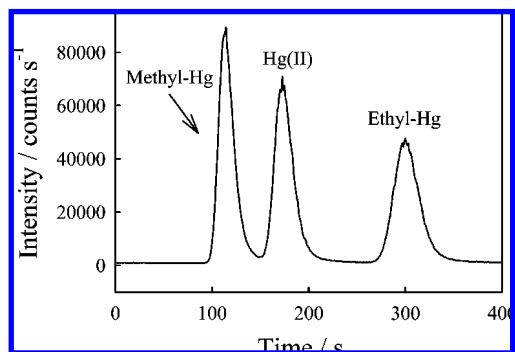
mercury compounds	DOLT-3 Dogfish Liver		1568a Rice Flour		1567a Wheat Flour	
	measured ( $\mu\text{g g}^{-1}$ as Hg)	spike recovery (%)	measured ( $\text{ng g}^{-1}$ as Hg)	spike recovery (%)	measured ( $\text{ng g}^{-1}$ as Hg)	spike recovery (%)
Hg(II)	1.71 $\pm$ 0.09	93 $\pm$ 5	3.61 $\pm$ 0.29	95 $\pm$ 6	0.61 $\pm$ 0.02	94 $\pm$ 6
methyl-Hg	1.55 $\pm$ 0.08	95 $\pm$ 4	2.25 $\pm$ 0.04	94 $\pm$ 4	0.032 $\pm$ 0.004	92 $\pm$ 7
ethyl-Hg	nd <sup>b</sup>	96 $\pm$ 3	nd <sup>b</sup>	98 $\pm$ 3	nd <sup>b</sup>	97 $\pm$ 4
sum of mercury compounds	3.26 $\pm$ 0.12		5.96 $\pm$ 0.29		0.64 $\pm$ 0.02	
methyl-Hg (certified) <sup>c</sup>	1.59 $\pm$ 0.12					
total Hg (certified) <sup>c</sup>	3.37 $\pm$ 0.14		5.8 $\pm$ 0.5		(0.5)	

<sup>a</sup>The values are the means of three determinations  $\pm$  standard deviation. <sup>b</sup>nd = not detected. <sup>c</sup>NIST and NRCC certified values.

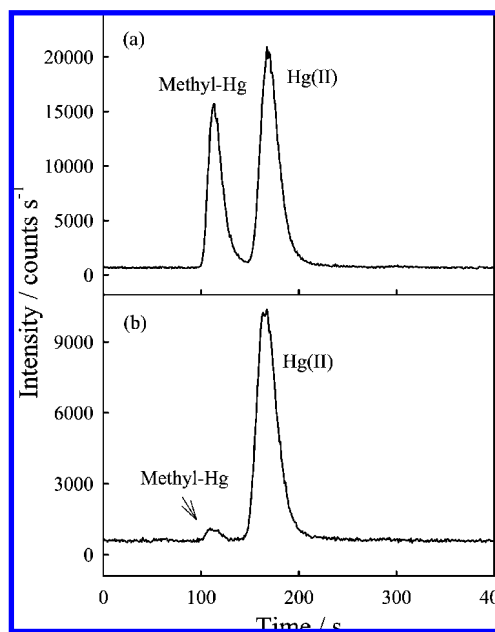
**Table 3.** Concentrations and Recoveries of Mercury Compounds in Rice Flour and Wheat Flour As Measured by LC-VG-ICP-MS<sup>a</sup> (*n* = 3)

mercury compounds	rice flour		wheat flour	
	measured ( $\text{ng g}^{-1}$ as Hg)	spike recovery (%)	measured ( $\text{ng g}^{-1}$ as Hg)	spike recovery (%)
Hg(II)	3.06 $\pm$ 0.12	93 $\pm$ 4	2.09 $\pm$ 0.11	96 $\pm$ 5
methyl-Hg	1.57 $\pm$ 0.11	94 $\pm$ 6	1.15 $\pm$ 0.07	93 $\pm$ 4
ethyl-Hg	nd <sup>b</sup>	97 $\pm$ 3	nd <sup>b</sup>	97 $\pm$ 4
sum of mercury compounds	4.63 $\pm$ 0.16		3.24 $\pm$ 0.13	
total hg (Digestion) <sup>c</sup>	4.87 $\pm$ 0.22		3.37 $\pm$ 0.24	

<sup>a</sup>The values are the means of three determinations  $\pm$  standard deviation. <sup>b</sup>nd = not detected. <sup>c</sup>Flour (0.5 g) in 5 mL of HNO<sub>3</sub> and digestion in closed system, and then determined by pneumatic nebulization ICP-MS.

**Figure 4.** Chromatogram obtained for 1 ng Hg mL<sup>-1</sup> each of Hg(II), methyl-Hg, and ethyl-Hg at the optimum conditions listed in Table 1 using LC-VG-ICP-MS.

DOLT-3 Dogfish Liver. It was also applied on rice and wheat flour purchased from the local market. The samples were subjected to microwave extraction using a solution of 0.5% v/v 2-mercaptoethanol and 5% v/v methanol to extract the mercury compounds of interest. The quantifications have been carried out using peak area mode using external calibration. The recoveries and assessment of the chromatogram peaks were carried out by spiking the sample solution with suitable concentrations of all the mercury compounds. The recoveries of the mercury compounds were in the range 92–98%. The recovery of ethyl mercury was better than the previous result (23). The Hg-selective chromatograms of NIST SRM 1568a Rice Flour and 1567a Wheat Flour are shown in Figure 5. The concentrations obtained for individual mercury compounds and total mercury in the samples are shown in Tables 2 and 3. As shown, the sum of the concentrations of individual compounds was in agreement (at 95% confidence level) with the total concentration of Hg certified/determined in all of the samples despite the levels in low ng g<sup>-1</sup> affecting both accuracy and

**Figure 5.** Chromatogram for the separation of mercury compounds using LC-VG-ICP-MS after microwave assisted extraction. (a) NIST SRM 1568a Rice Flour and (b) NIST SRM 1567a Wheat Flour reference materials. The concentrations of Hg(II) and methyl-Hg in (a) and (b) are about 0.36 ng mL<sup>-1</sup> and 0.24 ng mL<sup>-1</sup>, and 0.12 ng mL<sup>-1</sup> and 0.007 ng mL<sup>-1</sup>, respectively.

precision. The value of methyl mercury obtained in NRCC DOLT-3 was validated with the certified value. Inorganic mercury was the major mercury compound with detectable levels of methyl mercury in all the flour samples (23, 24). It is interesting to see that there was a significant difference in the fraction of methyl mercury between rice flour and wheat flour. Ethyl mercury could not be detected in all the samples. Precision between sample replicates was better than 13% (methyl-Hg in NIST wheat flour) for all the determinations. According to the regulations of the Taiwan government, the maximum allowed concentration of Hg in rice is 0.05  $\mu\text{g g}^{-1}$ . The concentration of mercury in the analyzed rice sample is below the maximum allowable concentration. The concentrations obtained were corrected for moisture content for comparison with the certified value of the rice and wheat reference materials.

**Conclusions.** The advantage of online separation of mercury compounds for their determination using VG-ICP-MS was demonstrated. Because of the use of a simple LC system, the background was reduced to achieve better detection limits for mercury compounds. The rapid and simple microwave extraction procedure used yielded near quantitative recoveries of all of the mercury compounds without any decomposition of individual compounds. This offers a fast and easy operating

extraction method. The system could provide a rapid and sensitive separation technique for mercury speciation in food samples.

#### ABBREVIATIONS USED

ICP-MS, inductively coupled plasma mass spectrometry; LC, liquid chromatography; VG, vapor generation.

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