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Online YPA_4 Resin Microcolumn Separation/ Preconcentration Coupled with Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) for the Speciation Analysis of Mercury in Seafood

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A simple and effective method for the determination of trace amounts of methylmercury ($MeHg^+$) and inorganic mercury (Hg^{2+}) in seafood was developed by online microcolumn separation/ preconcentration combined with inductively coupled plasma optical emission spectrometry (ICP-OES). It was found that Hg^{2+} could be quantitatively adsorbed by YPA_4 resin from pH 7.0 to strong acidic medium (6 mol L^{-1} HCl) and that $MeHg^+$ was retained by the YPA_4 microcolumn only at pH 1.0−7.0. Therefore, a strong acidic medium (about 5 mol L^{-1} HCl), which could liberate mercury species from biological samples, was used to directly separate inorganic Hg^{2+} from total Hg, and $MeHg^+$ in effluent was retained by YPA_4 column after the effluent was adjusted to pH 1.5. The effects of acidity, sample flow rate and volume, elution solution, and interfering ions on recovery of the two mercury species have been systematically investigated. Under optimal conditions, the limits of detection (LODs) were 72 and 44 ng L^{-1} for Hg^{2+} and $MeHg^+$ (as Hg) with online concentration factors of 12.5 and 12.1, respectively. The relative standard deviations (RSDs) for nine replicate determinations at 5 ng mL^{-1} levels of mercury species were 2.7 and 2.0% for Hg^{2+} and $MeHg^+$, respectively. The calibration graphs were linear with a correlation coefficient of 0.9902 in the range of 0.5−100 ng mL^{-1} for Hg^{2+} and 0.9976 in the range of 0.1−100 ng mL^{-1} for $MeHg^+$, respectively. The developed method was successfully applied to the direct determination of $MeHg^+$ and Hg^{2+} in seafood samples, and the recoveries for the spiked samples were in the range of 89.9−102.4% ($MeHg^+$) and 87.0−104.6% (Hg^{2+}), respectively. The method was validated by analyzing a certified reference material DORM-2 (dogfish muscle), and the determined values were in good agreement with certified values.

KEYWORDS: Mercury; speciation; YPA_4 resin; solid phase extraction; inductively coupled plasma optical emission spectrometry; seafood

INTRODUCTION

Mercury has been widely recognized as one of the most hazardous of environmental pollutants and a highly dangerous element due to its accumulative and persistent character in the environment and biota (*1*). It is well-known that the toxicity, solubility, biogeochemical behavior, and transportation of mercury in the environment are heavily dependent on its chemical form (*2*). Hg^{2+} and methylmercury ($MeHg^+$) are the two major mercury species found in environmental and biological samples, and $MeHg^+$ is considerably more toxic than Hg^{2+} due to its high solubility in lipids (*3*). As the most commonly occurring organic mercury species in environmental and biological materials, methylmercury mainly comes from methylation of inorganic mercury in the environment by biotic processes, and its accumulation may lead to serious biomagnification through the aquatic food chains (*4*). The main exposure pathway of Hg to humans is the consumption of marine fishery products (fish, shellfish, crustaceans, etc.); therefore, the establishment of methodologies for speciation analysis of mercury in organisms is important for environmental protection and food safety (*5, 6*).

At present, hyphenated techniques are extensively used for the speciation analysis of mercury. These techniques are based on a powerful separation technique [such as gas chromatography (GC) (*7*), high-performance liquid chromatography (HPLC) (*6*), and ion chromatography (*8*)] combined with a sensitive atomic spectrometric detector. Although these hyphenated methods are attractive for mercury speciation analysis due to their excellent detection limits and selectivity, the complexity and high cost make them difficult to facilitate routine analysis and make the use of a simple nonchromatographic approach an attractive alternative for selective discrimination of mercury species (*5, 9–15*). The nonchromatographic techniques for mercury speciation analysis include liquid–liquid extraction (*9*), solid phase extraction

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(SPE) (*5, 10–14*), and other methods based on the differential behavior of Hg^{2+} and $MeHg^{+}$ versus the temperature of the measurement cell by cold vapor atomic absorption spectrometry (*15*). In recent years, SPE has been widely employed in flow injection online separation and preconcentration systems and has attracted great attention owing to its simplicity, rapidity, low cost, lower pollution level to the environment, and easy automation. Direct discrimination between inorganic mercury and methylmercury by SPE technique is based on the differential behavior of Hg^{2+} and $MeHg^{+}$ versus the analytical functional group of solid phase extraction material. Due to the similar chemical properties between inorganic mercury and methylmercury, it is difficult to find a suitable solid phase extraction material for direct separation of Hg^{2+} and $MeHg^{+}$. Several kinds of solid phase extraction materials have been reported for mercury speciation analysis (*10–14*). However, most of them aimed at only one of the mercury species, with the concentration of the other one obtained by subtraction (*11*); this differential approach often yielded highly imprecise values, especially when the concentration of one species was far higher than that of the other. Therefore, the exploration of new solid phase extraction materials for direct speciation analysis of mercury is a hotspot in the field of SPE (*5, 10–14*).

Nowadays, the detection methods for mercury speciation analysis include ultraviolet (UV) spectrophotometry (*16*), atomic fluorescence spectrometry (AFS) (*3, 6, 10, 13*), atomic absorption spectrometry (AAS) (*5, 11, 12, 15*), mass spectrometry (*7*), inductively coupled plasma mass spectrometry (ICP-MS) (*8, 9*), particle beam electron ionization mass spectrometry (PB/EI-MS) (*17*), inductively coupled plasma optical emission spectrometry (ICP-OES) (*18*), glow discharge optical emission detection (GD-OES) (*19*), and microwave-induced plasma optical emission spectrometry (MIP-OES) (*20*). It should be noted that, as an effective trace detection technique, ICP-OES has the merits of high sensitivity, simultaneous multielement determination, simple operation, and easy online determination and is thus extensively applied in trace element analysis. However, owing to matrix interference and insufficient instrumental detection limit for (ultra)trace mercury in real samples, direct determination of its species in complicated matrices is difficult. Therefore, to obtained accurate, reliable, and sensitive results, a separation/preconcentration method for speciation analysis of mercury is needed.

The chelating resin (YPA_4) is an aminoisopropylmercaptan type with a polythioether backbone; its adsorption characteristics for some metals have been studied in our previous work (*21–24*). Jiang et al. (*24*) employed this resin as microcolumn packing material and developed a separation/preconcentration approach for GFAAS determination of trace total Hg in natural water. In this work, a simple and effective method for speciation analysis of trace amounts mercury in seafood was developed by online microcolumn separation/preconcentration combined with ICP-OES. The retention and elution conditions for Hg^{2+} and $MeHg^{+}$ on YPA_4 resin have been studied, and the optimal experimental conditions were established. The developed method was applied to the determination of mercury species in real seafoods with satisfactory results.

INSTRUMENTATION AND REAGENTS

Instrumentation. An Intrepid XP Radial ICP-OES (Thermo, Waltham, MA) with a concentric model nebulizer and a cinnabar model spray chamber was used for the determination of analytes. The instrument operating conditions and wavelength used are given in **Table 1**. An SY1200 ultrasonic system (Shengyuan Ultrasonic Apparatus Equipment Co. Ltd., Shanghai, China) was used to accelerate the extraction of mercury species from fresh tissue. The pH values were controlled with a Mettler Toledo 320-S pH-meter (Mettler Toledo Instruments Co. Ltd., Shanghai, China) supplied with a combined electrode. A conical microcolumn (∅0.6 × 5, mm, 50 mm length, 200 μL pipet tip, Yuhua Experimental Instrument Factory, Haimen, Jiangsu, China) made of polypropylene material was used as YPA_4 holder. An HL-2 peristaltic pump (Shanghai Qingpu Huxi Instrument Factory, Shanghai, China) was employed to propel the sample, reagent, and eluent.

Table 1. Operation Parameters of Intrepid XP Radial ICP-OES

RF generator power (W)	1300
frequency of RF generator (MHz)	27.12
coolant gas flow rate (L min^{-1})	14
carrier gas (L min^{-1})	0.6
auxiliary gas flow rate (L min^{-1})	0.5
maximum integration times (s)	25
analytical wavelength (nm)	Hg, 184.950

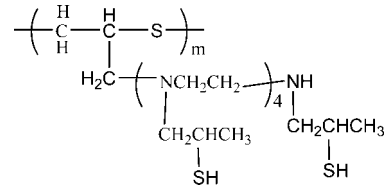

Figure 1. Structure of YPA_4 resin.

Reagents. A Hg^{2+} stock standard solution (1000 mg L^{-1}) was prepared from mercuric chloride (analytical grade, Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) dissolved in 0.5% (v/v) HCl. A $MeHg^{+}$ stock standard solution (100 mg L^{-1}, as Hg) was prepared from methylmercury chloride (Alfa Aesar, Ward Hill, MA) by dissolving an appropriate amount of the solid in methanol and making up to the required volume with 0.5% (v/v) HCl. The $MeHg^{+}$ stock standard solution was stored in a refrigerator and kept in the dark to avoid photodegradation (*25*). Working standards were prepared just before use by appropriate dilution of their stock standard solutions.

All chemicals were of analytical grade or high-purity grade. High-purity deionized water was used throughout this work.

All laboratory containers were made of glass or Teflon and thoroughly cleaned by soaking in nitric acid (10%, v/v) for at least 24 h. Prior to use, all acid-washed wares were rinsed with high-purity deionized water.

EXPERIMENTAL PROCEDURES

Preparation of YPA_4. The chelating resin YPA_4 was purchased from the Department of Polymer Chemistry, Wuhan University, and the preparation method has been described in the literature (*21*). The detailed structural properties of resin YPA_4 are shown in **Figure 1**. The chelating resin (YPA_4) is an aminoisopropylmercaptan type with a polythioether backbone, in which the total contents of S and N in the resin are 24.89 and 7.82%, respectively. YPA_4 with 140 mesh size was immersed in acetone and 1 mol L^{-1} HCl for 24 h, respectively, filtered, washed with high-purity deionized water, dried, and stored prior to use.

Microcolumn Preparation. A total of 10 mg of YPA_4 resin was filled into a 200 μL pipet tip (a conical microcolumn) plugged with a small portion of cotton at both ends. Before use, 1 mL of 1 mol L^{-1} HCl was passed through the microcolumn to elute the possible impurities, and a volume of high-purity deionized water was passed through the microcolumn to clean the acid. Then, the microcolumn was conditioned to the desired pH with appropriate buffer solution.

Extraction of Mercury Species. Fresh seafood samples were collected from local markets. The tissue was homogenized in a blender for subsequent extraction. According to the acid leaching procedure reported by Ortiz et al. (*26*), 7 mL of 5 mol L^{-1} HCl was added to

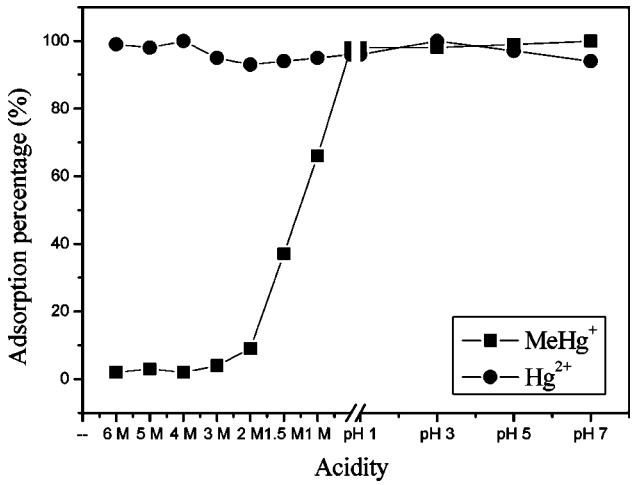

Figure 2. Effect of sample acidity (HCl) on the adsorption percentage of Hg^{2+} and $MeHg^{+}$ on YPA_4 resin. Conditions: Hg^{2+} and $MeHg^{+}$, 100 ng mL^{-1}; sample volume, 2 mL; sample flow rate, 2.2 mL min^{-1}.

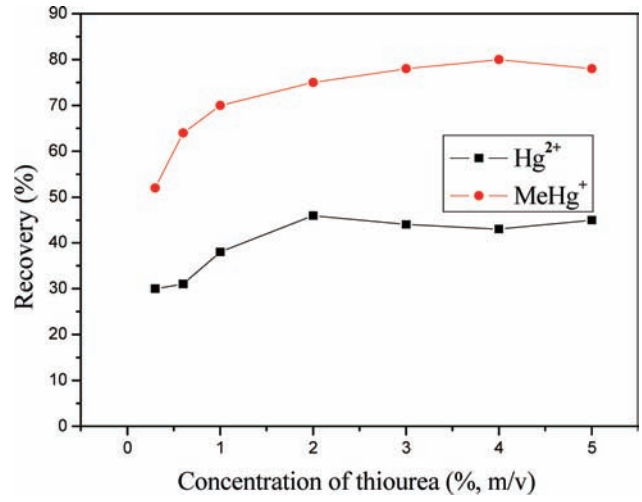

Figure 3. Effect of the concentration of thiourea in the eluent on the recovery of Hg^{2+} and $MeHg^{+}$ on YPA_4 resin. Conditions: Hg^{2+} and $MeHg^{+}$, 100 ng mL^{-1}; acidity, $MeHg^{+}$, pH 1.5, Hg^{2+}, 5 mol L^{-1} HCl; sample volume, 2 mL; elution volume, 2 mL; sample or elution flow rate, 2.2 mL min^{-1}; no HCl existing in eluent.

0.05 g of the certified reference material DORM-2 or 4 g of the homogenized fish tissue in a 15 mL centrifuge tube. The mixture was then placed in an ultrasonic bath for 10 min. After extraction, the suspension was centrifuged at 3500 rpm for 10 min, and the supernatant was taken to a flask. The residue was extracted again as described above. The two supernatant portions were combined and subjected directly to separation/preconcentration of Hg^{2+} from $MeHg^{+}$.

Procedure of Hg^{2+} Determination. Two milliliter aliquots of sample solution (about 5 mol L^{-1} HCl) containing the analytes of interest were pumped through the microcolumn by using a peristaltic pump at a flow rate of 2.2 mL min^{-1}, and the effluent was collected for $MeHg^{+}$ analysis. Then high-purity deionized water was passed through the microcolumn to wash off the residual sample matrix on the column. After the loading and washing time, the retained inorganic mercury was directly eluted with 0.15 mL of 1 mol L^{-1} HCl + 3% thiourea at a flow rate of 2.2 mL min^{-1} into the ICP-OES for Hg^{2+} determination.

Procedure of $MeHg^{+}$ Determination. Two milliliters of effluent (collected during the procedure of Hg^{2+} determination) was neutralized with 10 mol L^{-1} NaOH, adjusted to pH 1.5 with HCl−NaAc buffer, and diluted to 10 mL with high-purity deionized water for the determination of $MeHg^{+}$ in sample. The solution was then subjected to the above Hg^{2+} determination procedure.

RESULTS AND DISCUSSION

Acidity. The effect of acidity on the extraction efficiency of the adsorbent was the first critical parameter evaluated. Separation of Hg^{2+} and $MeHg^{+}$ solely depended on the selection of suitable acidic medium; therefore, the effect of sample acidity on the adsorption percentage of Hg^{2+} and $MeHg^{+}$ on YPA_4 resin was studied systematically, and the results are shown in **Figure 2**. It can be seen that, at pH 1.0−7.0, both Hg^{2+} and $MeHg^{+}$ could be adsorbed quantitatively; however, with the increase in sample acidity, the adsorption percentage of $MeHg^{+}$ on YPA_4 resin decreased, and almost no adsorption was found at acidity ≥2.0 mol L^{-1} HCl, whereas the adsorption percentage for inorganic mercury was unchanged even with the acidity increased to 6 mol L^{-1} HCl. Hence, Hg^{2+} and $MeHg^{+}$ could be separated by controlling the sample acidity to >2.0 mol L^{-1} HCl. In this paper, a strong acid (about 5 mol L^{-1} HCl), which could liberate mercury species from biological samples (*26*), was used to directly separate Hg^{2+} and $MeHg^{+}$.

Sample Flow Rate. The sample flow rate should be optimized to ensure quantitative adsorption and minimize the time required for sample processing. The relationship between adsorption percentages of analytes and the flow rate has been studied, and the results indicated that even if the flow rate was increased to the maximum (2.2 mL min^{-1}), Hg^{2+} and $MeHg^{+}$ were still quantitatively adsorbed by YPA_4 resin, which indicated a rapid reaction mechanism for the adsorption of mercury species on YPA_4 resin at desired acidity. Therefore, the sample flow rate of 2.2 mL min^{-1} was selected for subsequent experiments.

Concentration of Thiourea. It was found that Hg^{2+} and $MeHg^{+}$ could not be recovered even though 4 mol L^{-1} HNO_3 or HCl was used as elution solution. Because thiourea has strong ability to complex with Hg^{2+}, a mixed solution of HCl and thiourea was used as elution solution for Hg^{2+} (*24*). **Figure 3** shows the effect of thiourea concentration on the recovery of analytes. It can be seen that, when thiourea solution was used for elution only, the recovery of Hg^{2+} and $MeHg^{+}$ increased with the increase in concentration of thiourea and then remained constant with further increase when thiourea concentration was >2%. Therefore, 3% (m/v) thiourea was chosen in this work.

Concentration of HCl. By keeping the thiourea concentration at 3%, the effect of HCl concentration on the recovery of Hg^{2+} and $MeHg^{+}$ was explored. As can be seen in **Figure 4**, $MeHg^{+}$ could be eluted easily with the HCl concentration >0.001 mol L^{-1}. However, Hg^{2+} could be recovered quantitatively only when the HCl concentration was >0.1 mol L^{-1}. Therefore, a mixture of 3% (m/v) thiourea and 1 mol L^{-1} HCl was used for the elution of both mercury species.

Elution Volume. It is well-known that the less volume of eluent used, the higher the enrichment factor achieved. Therefore, three samples of 0.1 or 0.15 mL of elution solution (1 mol L^{-1} HCl + 3% thiourea) was continually pumped through the microcolumn one after the other and subsequently determined by ICP-OES. The results indicated that the first 0.15 mL of the eluent produced quantitative recovery of the analytes. Hence, 0.15 mL of elution solution was selected for future study.

Elution Flow Rate. The effect of elution flow rate on recovery of analytes was investigated by keeping the elution volume of 0.15 mL containing 1 mol L^{-1} HCl and 3% (m/v) thiourea constant. The experimental results indicated that analytes could be recovered quantitatively over the whole tested

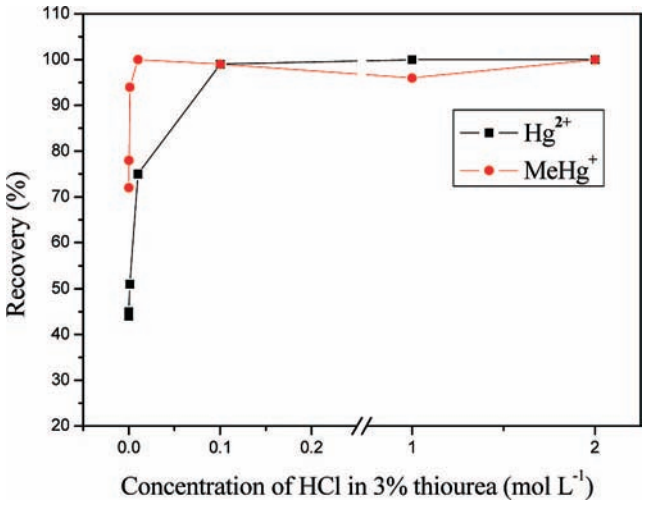

Figure 4. Effect of the concentration of HCl in the eluent on the recovery of Hg^{2+} and $MeHg^{+}$ on YPA_4 resin. Conditions: Hg^{2+} and $MeHg^{+}$, 100 ng mL^{-1}; acidity, $MeHg^{+}$, pH 1.5, Hg^{2+}, 5 mol L^{-1} HCl; sample volume, 2 mL; elution volume, 2 mL; sample or elution flow rate, 2.2 mL min^{-1}; thiourea concentration in elution, 3% (m/v).

flow rate range of 0.4−2.2 mL min^{-1}. In this study, the elution flow rate was chosen as 2.2 mL min^{-1} to accelerate the analysis speed.

Sample Volume. Solutions containing 100 ng Hg^{2+} and $MeHg^{+}$ with different volumes were pumped through the microcolumn according to the proposed procedures. The effect of sample volume on the recovery of analytes has been studied, and the results indicated that even though the volume of sample was increased to 50 mL, the recovery of mercury species was >90% and remained constant. As described in the previous section, 0.15 mL of 1 mol L^{-1} HCl and 3% thiourea could be used to recover the analytes completely; therefore, a theoretical enrichment factor of >330 could be obtained in this work. However, considering the analysis time and the concentration of real sample, a 2 mL sample volume was used to determine the analytes in real samples.

Adsorption Capacity. To evaluate the amount of YPA_4 required to quantitatively concentrate the target analytes from a given solution, it is important to determine the adsorption capacity. The adsorption capacities for Hg^{2+} on YPA_4 with different acidic media were studied by evaluating and comparing their breakthrough curves. The detailed procedure was as follows: sample solution containing 10 mg L^{-1} of analyte at desired acidity was passed through the column, and 10 mL of effluent was collected one after the other; the residual analyte in the effluent was determined by ICP-OES. The results indicated that the acidity of solution had a remarkable effect on the capacity values for Hg^{2+}. The capacity values decreased sharply with the increase in acidity, and a high capacity value was obtained at low acidic medium (pH 1.5 and 5.0). The possible reasons for this were that at low acidity (pH 1.5 and 5.0) two mechanisms contributed to the retention of Hg^{2+}. One was the chelating reaction between the −SH and −S− groups in the structure of YPA_4 and Hg^{2+}, and the other was ion exchange, which resulted from the functional groups of R′RHN and R″R′RN in the structure of YPA_4 with Hg^{2+} (as $[HgCl_4]^{2-}$). With increase in acidity, the functional groups −SH and −S− could not form complexes with Hg^{2+}, and the adsorption of Hg^{2+} on YPA_4 mainly depended on ion exchange reactions at high acidity.

Table 2. Comparison of the Adsorption Capacity of Hg^{2+} and $MeHg^{+}$ on YPA_4 Resin with Other Sorbents

sorbent	species[a]	capacity (mg g^{-1})
YPA_4 resin	$MeHg^{+}$	8.6[b]; 8.5[c]
	Hg^{2+}	298[b]; 271[c]; 40[d]; 4.4[e]
polyaniline (*12*)	$MeHg^{+}$	2.5[b]
	Hg^{2+}	100[b]
resin functionalized with a 1,2-bis(*o*-aminophenylthio)-ethane moiety (*14*)	$MeHg^{+}$	60[b]
	Hg^{2+}	76[b]
silica gel 2-mercaptobenzimidazol sorbent (*13*)	$MeHg^{+}$	0.30[f]
	Hg^{2+}	0.70[f]

[a] $MeHg^{+}$ as Hg. [b] pH 5.0. [c] pH 1.5. [d] 2 mol L^{-1} HCl. [e] 5 mol L^{-1} HCl. [f] pH 7.0.

Table 3. Tolerance Limits for Coexisting Ions[a]

coexisting ion	tolerance limit (mg L^{-1})
Na^{+}	50000[b]
K^{+}	10000[b]
Ca^{2+}, Mg^{2+}	2000[b]
Ba^{2+}	1000[b]
Al^{3+}, Fe^{3+}, Zn^{2+}, Cu^{2+}	250
Co^{2+}	100[b]
Mn^{2+}, Ni^{2+}	50
CH_3COO^{-}, $H_2PO_4^{-}$	3000
Cl^{-}	255000[b]
SO_4^{2-}	8000
$citrate^{-}$	1000

[a] Hg^{2+}, 10 ng mL^{-1}, 5 mol L^{-1} HCl; $MeHg^{+}$, 10 ng mL^{-1}, pH 1.5. [b] Not the highest tolerance limits of ions.

Typically expected ion exchange reactions between YPA_4 and Hg^{2+} at studied acidic medium are given below:

$$2[R'\,RH_2N]^{+} + [HgCl_4]^{2-} \rightarrow [R'\,RH_2N^{+}]_2{\cdot}[HgCl_4]^{2-}$$

$$2[R''\,R'\,RHN]^{+} + [HgCl_4]^{2-} \rightarrow [R''\,R'\,RHN^{+}]_2{\cdot}[HgCl_4]^{2-}$$

A comparison of adsorption capacities of this method with other literature values is given in **Table 2**.

Study of Residual of Online System. The element Hg could be easily adsorbed on the wall of the online system during sample preservation and transmission (*25*). Hence, to avoid possible adsorption of Hg on the wall of the online system, an appropriate volume of solution to wash and clean the online system is required. Various volumes of high-purity deionized water (0.2, 0.4, 0.6 mL) were passed through the online system after the analyte had been eluted from the microcolumn, followed by 0.15 mL of eluent. The eluted solutions (eluents) were then analyzed to check the Hg residual. The experimental results showed that 0.4 mL of high-purity deionized water could effectively reduce the blank values in the sample introduction and detection system; therefore, 0.4 mL of high-purity deionized water was used to eliminate the memory effect after each sampling and detection.

Interferences. Studies were conducted to determine whether other coexisting ions interfered with the separation of mercury species and their determination by ICP-OES, and the results are shown in **Table 3**. The results indicated that the method has a high tolerance limits for the interference ions.

Analytical Figures of Merit. According to the IUPAC definition, the detection limits (3σ) of the proposed method

Table 4. Analytical Figures of Merit of the Method[a]

element	calibration graph	linear range (ng mL^{-1})	R^2	EF	RSD (%)	LOD (ng L^{-1})	LOD in fresh tissue[b] (ng g^{-1})
Hg^{2+}	$Y = 14.83X + 0.0377$	0.5−100	0.9902	12.5	2.7	72	0.25[c]
$MeHg^+$	$Y = 24.37X + 0.0394$	0.1−100	0.9976	12.1	2.0	44	0.77[d]

[a] Hg^{2+}, 5 mol L^{-1} HCl; $MeHg^+$ (as Hg), pH 1.5; sample volume, 2 mL; elution volume, 0.15 mL; linear range, not the highest studied concentration; EF, enrichment factor obtained by comparing the slope of the calibration graph with/without preconcentration; RSD, relative standard deviation, $C = 5$ ng mL^{-1}, $n = 9$; LOD, limit of detection. [b] For 14 mL extract of 4 g of fresh tissue. [c] No dilution of extract direct for Hg^{2+}. [d] Five times dilution of the effluent from *c* for $MeHg^+$.

Table 5. Analytical Results (Mean ± SD, $n = 3$) of Hg^{2+} and $MeHg^+$ Contents in Seafood Muscle

	added		Hg^{2+}		$MeHg^+$	
sample	Hg^{2+} (ng g^{-1})	$MeHg^+$ (ng g^{-1})	found (ng g^{-1})	recovery (%)	found (ng g^{-1})	recovery (%)
shell 1	0	0	23.9 ± 2.2		nd[a]	
(snow clam)	50.0	60.0	69.8 ± 1.5	94.4	58.7 ± 2.7	97.9
shell 2	0	0	nd		nd	
(paphiaundulata)	50.0	60.0	48.8 ± 7.0	97.5	55.7 ± 4.7	92.7
shell 3	0	0	nd		11.9 ± 1.6	
(clam)	50.0	60.0	45.4 ± 3.5	90.7	72.9 ± 6.1	101.3
shell 4	0	0	nd		19.7 ± 2.7	
(razor clam)	50.0	60.0	43.5 ± 3.3	87.0	71.7 ± 4.4	89.9
shell 5	0	0	63.7 ± 2.7		24.8 ± 1.7	
(oyster)	50.0	60.0	109.4 ± 14.2	96.1	84.4 ± 6.9	99.6
shell 6	0	0	nd		10.6 ± 1.2	
(scallops)	50.0	60.0	50.8 ± 5.8	101.6	68.5 ± 9.1	97.0
fish 1	0	0	nd		nd	
(*Harpondon nehereus*)	50.0	60.0	48.1 ± 2.5	96.2	55.0 ± 1.6	91.7
fish 2	0	0	nd		38.1 ± 5.5	
(hairtail)	50.0	60.0	45.0 ± 2.0	90.0	93.7 ± 12.5	95.5
shrimp 1	0	0	nd		7.3 ± 0.9	
(shrimps)	50.0	60.0	52.3 ± 4.5	104.6	68.9 ± 4.4	102.4

[a] Not determined.

were 72 and 44 ng L^{-1} for Hg^{2+} and $MeHg^+$ (as Hg) with online concentration factors of 12.5 and 12.1 for a sample consumption of 2 mL, respectively. Considering a 5-fold dilution for $MeHg^+$ in real sample analysis, the real enrichment factor for $MeHg^+$ was 2.42. The relative standard deviations (RSDs) were 2.7 and 2.0% ($C = 5$ ng mL^{-1}, $n = 9$) for Hg^{2+} and $MeHg^+$, respectively. The analytical performance data are summarized in **Table 4**, and these results indicated that this method was capable of analysis of mercury species in seafood samples.

The regeneration is one of the key factors in evaluating the performance of the adsorption material. In this work, a microcolumn packed with 10 mg of YPA_4 was subjected to the proposed procedure again and again, and the study has shown that the column could be reused at least 100 times.

Analysis of Real Sample. Nine real seafood samples collected from local markets were analyzed, and the results are shown in **Table 5**. It can be seen that the recoveries of mercury species spiked in the seafood samples were in the range of 89.9−102.4% ($MeHg^+$) and 87.0−104.6% (Hg^{2+}), respectively. Inorganic mercury (Hg^{2+}) was found in only two shell samples; one was 23.9 ng g^{-1} and the other was 63.7 ng g^{-1} (wet mass), which were lower than the values reported in the literature (*16*). The concentrations of $MeHg^+$ were determined to be 7.3−38.1 ng g^{-1} (wet mass) in six out nine samples studied by this method; the detection values were similar with the results reported in ref *6*, but lower than other values in the literature (*5*, *16*). The concentration levels of mercury species in the seafood samples studied were generally below the maximum permissible mercury concentration in fish (usually in the range of 0.4−1.0 μg g^{-1}, wet mass) used for human consumption established by many countries (*16*).

Table 6. Analytical Results (Mean ± SD, $n = 3$) of Hg^{2+} and $MeHg^+$ Contents in the Certified Reference Material DORM-2 (Dogfish Muscle, NRCC)

certified value (μg g^{-1})		determined value (μg g^{-1})		
total Hg	$MeHg^+$	Hg^{2+}	$MeHg^+$	total Hg
4.64 ± 0.26	4.47 ± 0.32	0.18 ± 0.02	4.41 ± 0.65	4.59 ± 0.63

To evaluate the accuracy of the developed method, a certified reference material, DORM-2 (dogfish muscle, NRCC), was analyzed. The determined values (listed in **Table 6**) were in good agreement with the certified values.

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