JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Cloud Point Extraction Preconcentration Prior to High-Performance Liquid Chromatography Coupled with Cold Vapor Generation Atomic Fluorescence Spectrometry for Speciation Analysis of Mercury in Fish Samples

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A cloud point extraction methodology was developed for simultaneous preconcentration of Hg(II), methylmercury (MeHg), ethylmercury (EtHg), and phenylmercury (PhHg) prior to reversed-phase high-performance liquid chromatography (HPLC) on-line coupled with cold vapor atomic fluorescence spectrometry for speciation analysis of mercury in fish. The four mercury species were taken into complexes with ammonium pyrrolidine dithiocarbamate (APDC) in aqueous nonionic surfactant Triton X-114 medium and concentrated in the surfactant-rich phase by bringing the solution to the temperature of 40 °C. Baseline separation of the enriched complexes was achieved on an RP-C_{18} column with a mixture of methanol, acetonitrile, and water (65:15:20, v/v) containing 200 mmol L^{-1} HAc (pH 3.5) as the mobile phase. An on-line postcolumn oxidation of the effluent from HPLC, in the presence of $K_2S_2O_8$ in HCl, was applied in the system followed by an optimal cold vapor generation of mercury species. The variables affecting the complexation and extraction steps were examined. The preconcentration of 10 mL of solution with 0.08% w/v Triton X-114 and 0.04% w/v APDC at pH 3.5 gave enrichment factors of 29, 43, 80, and 98 for MeHg, EtHg, PhHg, and Hg(II), respectively. Low detection limits ($S/N = 3$) were obtained, ranging from 2 to 9 ng L^{-1} (as Hg) for all species. The developed method was successfully applied to the speciation of mercury in real fish samples.

KEYWORDS: Mercury speciation; fish; cloud point preconcentration; high-performance liquid chromatography; atomic fluorescence spectrometry

INTRODUCTION

It is well-known that the toxicity of mercury is highly dependent on its chemical form. Methylmercury, the most toxic species of mercury normally found in environmental and biological material, is of particular concern because of its accumulation as it passes through the food chain (*1*). Accordingly, the rapid and sensitive determination of mercury species in addition to total mercury is highly essential for the interpretation of their biochemical behavior or assessment of the potential danger.

Conventionally, speciation and determination of mercury in biological and environmental samples have been performed by gas chromatography (GC) or high-performance liquid chromatography (HPLC) coupled with a mercury-specific detector. In comparison with GC, the use of HPLC for mercury speciation has the advantage of simplified sample preparation. The detection methods coupled with HPLC for mercury speciation include atomic absorption spectrometry (AAS) (*2*−*5*), atomic fluorescence spectrometry (AFS) (*6*, *7*), inductively coupled plasma mass spectrometry (ICP-MS) (*8*, *9*), inductively coupled plasma atomic emission spectrometry (ICP-AES) (*10*), and microwave-induced plasma atomic emission spectrometry (MIP-AES) (*11*). Nevertheless, the limited sample amount introduced into liquid chromatography makes these detection techniques unable to provide low enough detection limits, and in some cases, they are unsuitable for analysis of "real" environmental samples (*12*). Preconcentration methods based on solid phase extraction (*2*−*4*, *8*) have been employed prior to HPLC separation to achieve a final concentration level matching the detection limits accessible with the detector selected.

The cloud point extraction (CPE) methodology based on the property of nonionic and zwitterionic surfactant of separating into two liquid phases when their aqueous solutions are heated above a given temperature has been used to separate and preconcentrate metal ions (*13*, *14*) and organic compounds (*15*, *16*). Any hydrophobic species (hydrophobic organic compounds or metal ions after reaction with a suitable hydrophobicchelating agent) in solution are able to react with and bind to micelles and become concentrated in a small volume of the surfactant-rich phase. CPE as a preconcentration method offers many distinct advantages, such as low cost, safety, and a high capacity to concentrate analytes with high recoveries and high concentration factors (*13*). CPE has been shown to be an effective sample

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10.1021/jf051777k CCC: $30.25
Published on Web 11/18/2005

preconcentration technique for improving the sensitivity and selectivity prior to flame AAS (*17*, *18*), HPLC (*15*, *19*−*21*), capillary electrophoresis (*22*, *23*), and flow injection analysis (*24*−*26*).

The small volume of the surfactant-rich phase obtained from CPE is compatible with the hydroorganic phases usually employed in the reversed phase chromatographic mode. A variety of analytes of widely varying nature with high concentration factors have been exploited in the past few years for the CPE preconcentration of organic compounds prior to their liquid chromatographic separation and determination (*18*). The nonionic surfactants (such as Triton X series) are more frequently employed for the formation of the surfactant-rich phase due to its commercial availability and lower toxicity as well as its low cloud point temperature and high density of the surfactant-rich phase, which facilitates phase separation by centrifugation (*17*, *18*, *22*, *24*).

The common disadvantage of liquid chromatographic techniques is the lack of sensitive element selective detectors. Furthermore, when CPE is used as a prior preconcentration step before chromatographic separation, the nonionic surfactants present another important drawback: a high background absorbance in the ultraviolet region and high fluorescence signals due to the presence of an aromatic moiety in their structure. One possible way of overcoming this pitfall is to use surfactants that do not absorb at the working wavelengths normally used in chromatography (*27*, *28*). Another way is to use electrochemical detection (*29*, *30*), which has the limitation that many of the preconcentrated analytes may not be electroactive.

The aim of this work was to employ CPE as a preconcentration step prior to HPLC-CVAFS for speciation of mercury. It not only can overcome the above disadvantages but also offers a simple, sensitive, and inexpensive alternative to other preconcentration techniques prior to HPLC for mercury speciation. CPE preconcentration of MeHg, EtHg, PhHg, and Hg(II) in aqueous solution, using ammonium pyrrolidine dithiocarbamate (APDC) as the complexing agent and Triton X-114 as the surfactant, was studied. The parameters affecting the complexation and CPE preconcentration steps of four mercury species were examined.

INSTRUMENTATION AND REAGENTS

Instrumentation. The HPLC system consisted of a Varian 5000 solvent delivery system equipped with a 100 μL injection loop. The separation of the four mercury species studied took place on an analytical reversed phase column (Maxsil ODS, 25 cm i.d. × 4.6 mm length, 5 μm, Hertz Biotech Co. Ltd., Zibo, China) at room temperature. The outlet of the HPLC column was connected to an on-line continuous flow cold vapor generation system of AFS.

A model AFS-820 nondispersive atomic fluorescence spectrometer (Beijing Titan Instruments Co. Ltd., Beijing, China) was employed throughout. A mercury hollow cathode lamp (Beijing Institute of Vacuum Electronics, Beijing, China) was used as the radiation source. The atomizer was an electrically heated quartz tube (6 mm i.d. × 65 cm length), into which the volatile species evolved from the reactor were swept by an argon carrier gas. The gas−liquid separator was a standard component supplied with the AFS instrument. The AFS system was controlled through a separate computer with its manufacturer's software.

Reagents. All reagents were at least of analytical grade. Doubly deionized water (DDW, 18 MΩ cm^{-1}) obtained from a WaterPro water system (Labconco Corporation, Kansas City, MO) was used throughout. Triton X-114 was obtained from Sigma and was used without further purification. The complexing agent solution was prepared by dissolving ammonium pyrrolidine dithiocarbamate (APDC, Sigma) in DDW daily. An oxidant solution of 2% w/v $K_2S_2O_8$ (Tianjin Taixing Chemicals Co., Tianjin, China) solution was prepared in 10% v/v HCl (The Third Chemicals Co., Tianjin, China). A 0.5% w/v KBH_4 solution was prepared by dissolving KBH_4 (Tianjin Institute of Chemical Reagents, Tianjin, China) in 0.2% w/v KOH (Beijing Chemicals Co., Beijing, China).

An inorganic mercury stock solution of 1000 mg L^{-1} was prepared by dissolving the mercury chloride (The Second Chemical Co., Beijing, China) in DDW. The stock solutions of 1000 mg L^{-1} (as Hg) of methylmercury (MeHg), ethylmercury (EtHg), and phenylmercury (PhHg) were prepared by dissolving methylmercury, ethylmercury, and phenylmercury chloride (Alfa Aesar) in methanol, respectively. Working solutions were prepared from the stock solutions by stepwise dilution just before use.

Mobile phase for the separation of mercury species was obtained with a mixture of methanol (Concord Technology Co. Ltd., Tianjin, China), acetonitrile (Concord Technology Co. Ltd.) and water-containing HAc (Tianjin Taxing Chemicals Co.). The mobile phase was filtered through a 0.45 μm filter (Automatic Science Instrument Co., Ltd., Tianjin, China) prior to use.

EXPERIMENTAL PROCEDURES

CPE Preconcentration. Aliquots of 10 mL of the solutions containing the analytes, 0.08% w/v Triton X-114, 0.04% w/v APDC, at pH 3.5, maintained by 1 mol L^{-1} HCl, were held for 10 min in a thermostatic bath at 40 °C, and the two phases were separated by centrifugation for 10 min at 3500 rpm. On cooling in an ice bath, the surfactant-rich phase became viscous. The supernatant aqueous phase was carefully removed with a pipet. The final volume of the surfactant-rich phase was usually 50 μL.

HPLC-CVAFS Analysis. Mercury species separation and determination were performed utilizing the HPLC-CVAFS system. A 50 μL amount of methanol was added to the surfactant-rich phase obtained from CPE just before HPLC separation, and a final volume of 100 μL was injected into the HPLC system. The best chromatographic resolution for the separation of mercury species was obtained with a mixture of methanol, acetonitrile, and water (65:15:20) containing 200 mmol L^{-1} HAc at a pH 3.5 at a flow rate of 1.0 mL min^{-1}.

A postcolumn oxidation of the effluent from HPLC, in the presence of $K_2S_2O_8$ in HCl, was applied. A 200 cm knotted reactor was used in order to achieve an efficient mixing of the effluent from the HPLC with the oxidant solution. The oxidant solution and $NaBH_4$ solutions were continuously added to the column effluent using a peristaltic pump via a T-piece assuming the reduction of organomercury species to Hg(II) and the generation of mercury vapor. The mercury vapor was purged from the gas−liquid separator by an argon flux and then passed into the quartz cell where atomic fluorescence was measured. The reagent concentration, reagent flow rate, carrier flow rate, and KR length had been optimized in a previous work (*31*). The optimized chromatographic and AFS operating conditions are summarized in **Table 1**.

Under these conditions, the four mercury species were eluted in the order MeHg, EtHg, PhHg, and Hg(II) with retention times of 7.1, 9.8, 11.7, and 15.2 min, respectively. A typical chromatogram for MeHg, EtHg, PhHg, and Hg(II) after CPE preconcentration followed by HPLC separation and CVAFS determination is shown in **Figure 1**. As can be seen, all species were fully resolved and the separation was completed within 18 min.

Extraction of Mercury Species. An acid leaching procedure applied to the materials was based on that described by Ortiz et al. (*32*). Briefly, 5 mL of 5 mol L^{-1} hydrochloric acid was added to 0.02 g of the certified reference material DORM-2 dogfish muscle (NRCC, 4.47 ± 0.32 μg g^{-1}), or 0.2 g of the homogenized fish tissue in a 10 mL centrifuge tube. The mixture was then placed in an ultrasonic bath and sonicated

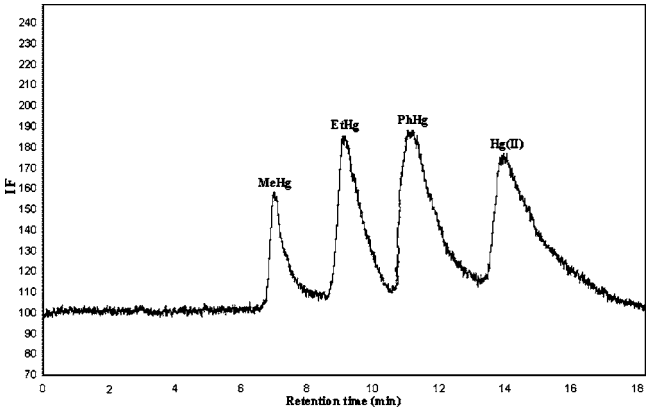

Figure 1. Typical chromatogram obtained by the HPLC-CVAFS for mercury-PDC complexes after a CPE preconcentration of a mixture standard solution of 0.5 μg L^{-1} MeHg, EtHg, and PhHg and 0.2 μg L^{-1}Hg (II). HPLC separation column: Maxsil ODS, 4.6 mm i.d. × 25 cm length, 5 μm; mobile phase: a mixture of methanol, acetonitrile, and water (65:15:20) containing 200 mmol L^{-1} HAc (pH 3.5); flow rate of the mobile phase: 1 mL min^{-1}. Other conditions as in **Tables 1** and **2**.

Table 1. Operational Parameters of the HPLC-CVAFS System

parameter	setting
HPLC	
column	Maxsil ODS, 25 cm i.d. × 4.6 mm length, 5 μm
oven temperature	room temperature
mobile phase	methanol, acetonitrile, and water (65:15:20, v/v) containing 200 mmol L^{-1} HAc (pH 3.5)
mobile phase flow rate	mL min^{-1}
sample loop	100 μL
postcolumn oxidation	
length of KR	200 cm
oxidant solution	2% w/v $K_2S_2O_8$ in 10% v/v HCl
oxidant solution flow rate	mL min^{-1}
cold vapor generation	
reductant solution	0.5% w/v KBH_4 in 0.2% w/v KOH
reductant solution flow rate	3.3 mL min^{-1}
AFS	
lamp	mercury
lamp current	30 mA
negative high voltage	290 V
carrier gas	argon
shield gas flow rate	1000 mL min^{-1}

for 10 min. After extraction, the suspension was centrifuged at 3500 rpm for 10 min and the supernatant was transferred to a 50 mL flask. The residue was extracted again as described above. The two supernatant portions were combined, neutralized with 5 mol L^{-1} NaOH, adjusted to pH 3.5, and diluted to volume with DDW for further analysis.

RESULTS AND DISCUSSION

The preconcentration efficiency can be optimized by modifying the concentration of the surfactant as well as the experimental conditions under which extraction and phase separation were carried out. Any parameters affecting the complex reactions and micelle formation were included in the optimization experimental design.

pH. The pH was the first critical parameter evaluated for its effect on the extraction of four mercury species, for the fast formation of strong and neutral complexes of APDC with mercury species is dependent on the pH. CPE for MeHg, EtHg, PhHg, and Hg(II) was performed in different pH buffer solutions. Mercury and organomercury complexes can easily form with APDC in a pH range from 0.5 to 12. Usually, the preservation of liquid samples and acid leaching of solid samples containing mercury lead to acidic sample solutions, so that the pH range from 0.75 to 7 was examined in detail. The results shown in **Figure 2** illustrate that the maximum extraction efficiency for the four mercury species could be achieved in the pH 3−4.5 range. According to Giokas et al. (*18*, *33*), using the same complex and surfactant, pH 4−6 favored the extraction of Fe(II) and Fe(III) and offered maximum extraction efficiency at pH 5. To avoid interference effects, a pH 3.5 was selected as the working value.

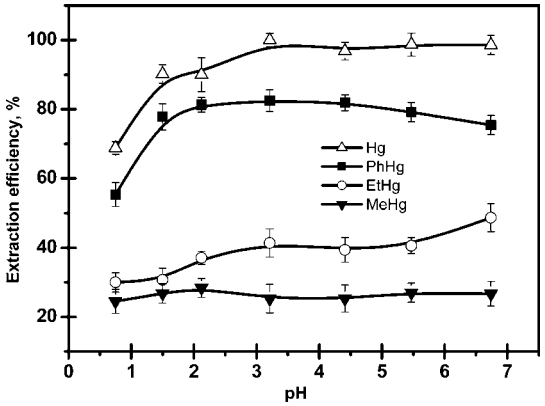

Figure 2. Effect of pH on the preconcentration performance of 0.5 μg L^{-1} MeHg, EtHg, and PhHg and 0.2 μg L^{-1} Hg (II). Conditions: 0.05% w/v APDC, 0.1% w/v Triton X-114, and 40 °C. Other conditions are as in **Tables 1** and **2**.

Concentration of APDC. The preconcentration of mercury species by the CPE method involves prior formation of a complex with sufficient hydrophobicity to be extracted into the small volume of surfactant-rich phase, thus obtaining the desired preconcentration. The nature of the complexing agent and its concentration are important factors considered.

Prior to complex formation, MeHg, EtHg, PhHg, and Hg(II) are polar and exhibit some solubility in water and so do not completely partition to the micellar phase. However, the APDC complexes of the four mercury species have high hydrophobicity and their structures are as shown in references *2* and *8*. The CPE efficiency increased rapidly as the concentration of APDC increased from 0.005 to 0.02% w/v and then reached a plateau between 0.02 and 0.1% w/v, resulting in complexes formation and quantitative extraction. When real samples are treated, the concentration of the chelating agent has to compensate sufficiently for any consumption of the reagent by other metals (*18*). Therefore, the APDC concentration of 0.04% w/v was employed for further experiments.

Concentration of Triton X-114. Triton X-114 is a nonionic surfactant. The variation of extraction efficiency upon the surfactant concentration was examined within the range of 0.025−0.15% w/v. At lower concentrations, the extraction efficiency of mercury complexes was low. Quantitative extraction was observed for a surfactant concentration higher than 0.08% w/v, as shown in **Figure 3**.

An increase in the concentration of Triton X-114 leads to a slight increase in the volume. The theoretical preconcentration factor (maximum attainable value for 100% recovery) was calculated as the ratio of the volume of solution used to surfactant-rich phase volume. A successful CPE should maximize the extraction efficiency by minimizing the phase volume ratio, thus, improving its concentrating ability. In addition, at higher concentrations, the viscosity of the surfactant-rich phase

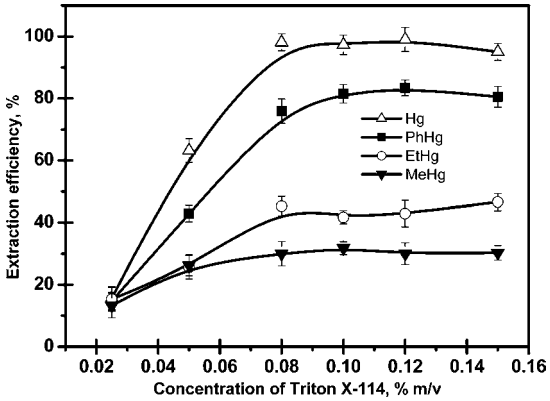

Figure 3. Effect of Triton X-114 concentration on the preconcentration performance of 0.5 μg L^{-1} MeHg, EtHg, and PhHg and 0.2 μg L^{-1} Hg (II). Conditions: 0.04% w/v APDC, pH 3.5, and 40 °C. Other conditions are as in **Tables 1** and **2**.

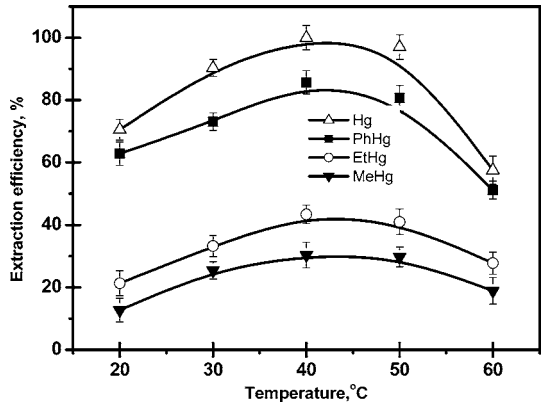

Figure 4. Effect of temperature on the preconcentration performance of 0.5 μg L^{-1} MeHg, EtHg, and PhHg and 0.2 μg L^{-1} Hg (II). Conditions: 0.04% w/v APDC, 0.08% w/v Triton X-114, and pH 3.5. Other conditions are as in **Tables 1** and **2**.

would lead to long retention times in HPLC. This may result in overlap of the chromatographic peaks for the surfactant and those for the more polar analytes, which calls for a cleanup operation. To achieve a good preconcentration factor and rapid analysis, a high Triton X-114 concentration should be avoided, and so 0.08% w/v was chosen as optimal.

Equilibration Temperature and Incubation Time. Optimal equilibration temperatures and incubation times are necessary to complete reactions and to achieve easy phase separation and efficient preconcentrations as possible. It was desirable to employ the shortest incubation time and the lowest possible equilibration temperature, which compromised completion of the reaction and efficient separation of phases.

Triton X-114 can form normal micelles in aqueous solution, and its cloud point temperature (CPT) in pure water is about 20 °C. To obtain a more favorable preconcentration factor, CPE should be carried out at a temperature higher than the cloud point temperature and should be maintained for a given time. The effect of equilibration temperature is shown in **Figure 4**. Mild temperatures of 20−40 °C increase extraction performance at pH 3.5 with a Triton X-114 concentration of 0.08% w/v. Higher temperatures lead to a decrease in the signal. Thus, a temperature of 40 °C was maintained during the experiments.

The dependence of extraction efficiency upon equilibration time was studied within a range of 5−30 min. An equilibration time of 10 min was chosen as the best time to achieve quantitative extraction and experimental convenience.

Table 2. Optimum Parameters for the CPE Preconcentration of Mercury Species

parameter	selected value
pH	3.5
APDC concentration	0.04% w/v
Triton X-114 concentration	0.08% w/v
temperature	40 °C
incubation time	10 min
centrifugation rate	3500 rpm
centrifugation time	10 min

Table 3. Effect of Interfering Ions on the Detection of 0.5 μg L^{-1} MeHg under the Conditions Given in **Tables 1** and **2**

foreign ion	concn (mg L^{-1})	recovery (mean $\pm \sigma$, $n = 3$) (%)
Fe(III)	2	96 ± 1
	5	83 ± 2
	8	74 ± 2
Co(II)	2	100 ± 1
	10	86 ± 1
	12	76 ± 2
Ni(II)	4	99 ± 1
	12	87 ± 1
	15	79 ± 2
Cu(II)	5	98 ± 1
	8	87 ± 1
	12	72 ± 2
As(III)	4	97 ± 1
	8	86 ± 1
	10	79 ± 2
Se(IV)	4	95 ± 1
	8	84 ± 2
	10	77 ± 1

Ionic Strength. The CPT of Triton X-114 can be altered by additives such as acids, salts, or alcohols. The influence of ionic strength was examined by observing the temperature when the aqueous mixture became turbid. It was proved that the overall process in the temperature of 40 °C is not affected seriously by the NaCl concentration in the range of 0−2 mol L^{-1}. In agreement with previous researchers (*34*), it was also demonstrated that an increase in the hydrochloric acid up to 2 mol L^{-1} in the micelle-mediated extraction system did not seriously alter the efficiency of extraction. Then, the present technique can be applied to real samples with high ionic strength.

Other parameters such as a centrifugation time of 10 min and a centrifugation rate of 3500 rpm were selected for the entire procedure, since no appreciable improvements were obtained when these values increased. All selected values for CPE preconcentration are summarized in **Table 2**.

Efficiency of CPE. The efficiency of CPE mainly depends on the hydrophobicity of the ligand and the complex formed, the apparent equilibrium constants in the micellar medium, the kinetics of the complex formation, and the transference between the phases (*16*). Hydrophobic mercury complexes of MeHg, EtHg, PhHg, and Hg(II) with APDC formed rapidly. The high hydrophobicity of the complexes in water is necessary for preconcentration by CPE. Under the optimal conditions, the highest extraction efficiency was obtained. The CPE efficiency increased with the hydrophobicity of the complexes and in the order of 29, 43, 80, and 98% for MeHg, EtHg, PhHg, and Hg(II), respectively. For the most hydrophobic Hg(II)−PDC complex, nearly 100% extraction efficiency is observed because the complex completely partitions and binds to the micellar phase. Thus, the enhancement factor, which is defined as the concentration ratio of the analyte in the final diluted surfactant-

Table 4. Characteristic Data of CPE Preconcentration Prior to HPLC-CVAFS for Speciation Analysis of Mercury under the Optimal Conditions

	MeHg	EtHg	PhHg	Hg(II)
enhancement factor[a]	29	43	80	98
detection limits ($S/N = 3$) (ng L^{-1})	9	4	4	2
RSD of retention time ($n = 7$) (%)[b]	0.8	1.6	1.3	2.3
RSD of peak area ($n = 7$) (%)[b]	3.4	1.0	1.2	2.7
linear range (μg L^{-1})	0.009–10	0.004–10	0.004–10	0.002–4
calibration curve (X, μg L^{-1})	$Y = 2 \times 10^4X - 119$	$Y = 4 \times 10^4X - 148$	$Y = 4 \times 10^4X - 305$	$Y = 2 \times 10^5X - 187$
relation coefficient	0.997	0.998	0.995	0.998

[a] As compared with 100 μL of 10 μg L^{-1} direct injection. [b] Using a synthetic sample mixture of 0.5 μg L^{-1} MeHg, EtHg, and PhHg and 0.2 μg L^{-1} Hg(II).

Table 5. Comparison of the Detection Limits of Mercury Species by Different Preconcentration Techniques Prior to HPLC

			detection limits				
method	preconcentration technique	sample consumed (mL)	MeHg	EtHg	PhHg	Hg(II)	ref
HPLC-CVAAS	RP-C_{18} column	100	0.5 μg L^{-1}	0.09 μg L^{-1}	0.5 μg L^{-1}	0.15 μg L^{-1}	*2*
HPLC-UV-PCO-CVAAS	RP-C_{18} column	300	0.5 ng L^{-1}	0.5 ng L^{-1}	0.6 ng L^{-1}	0.5 ng L^{-1}	*3*
HPLC-ICPMS	RP-C_{18} column	5	15 pg	20 pg	20 pg	10 pg	*8*
HPLC-CVAAS	SPE[a]	58.5	9.3 ng L^{-1}	5.5 ng L^{-1}	10.4 ng L^{-1}	7.6 ng L^{-1}	*4*
HPLC-UV	SPE[a]	500	0.14 μg L^{-1}		0.16 μg L^{-1}	0.14 μg L^{-1}	*36*
HPLC-UV-PCO-CVAFS	RP-C_{18} column	5	8 pg	10 pg	10 pg	10 pg	*37*
HPLC-PCO-CVAFS	CPE[b]	10	9 ng L^{-1}	4 ng L^{-1}	4 ng L^{-1}	2 ng L^{-1}	this work

[a] Solid phase extraction. [b] Cloud point extraction.

rich phase ready for HPLC separation and in the initial solution, is 29, 43, 80, and 98 for MeHg, EtHg, PhHg, and Hg(II), respectively.

Interferences. APDC is not selective and reacts with many metals ions. Commonly encountered matrix components such as alkali and alkaline earth elements generally do not form stable complexes with APDC. Ions of some heavy metals form complexes with APDC, which interfere with the UV detection after HPLC separation (*35*) but did not exhibit any interference with mercury detection by CVAFS in view of its high selectivity (*6*, *7*). The only interference effects related to preconcentration steps can be avoided to some extent by using excess APDC. Thus, heavy metal ions Fe(II), Co(II), Ni(II), Cu(II) and hydride-forming elements such as As(III) and Se(IV) could be tolerated up to 10 mg L^{-1}, and the results are shown in **Table 3**.

Analytical Figures of Merit. The analytical characteristic data of the present system for the four mercury species were summarized in **Table 4**. Calibration graphs obtained by preconcentrating 10 mL standard solutions were linear in the ranges of 0.002−4 μg L^{-1} for Hg, 0.009−10 μg L^{-1} for MeHg, and 0.004−10 μg L^{-1} EtHg and PhHg (as Hg). The enhancement factor achieved is 29, 43, 80, and 98 for MeHg, EtHg, PhHg, and Hg(II), respectively. The detection limits (defined as $S/N = 3$) ranged from 2 to 9 ng L^{-1} (as Hg). Those values were lower than most of the results reported using other preconcentration techniques prior to HPLC (see **Table 5**). The precision (RSD) of the peak area for seven successive injections of a mixture of 0.5 μg L^{-1} MeHg, EtHg, and PhHg and 0.2 μg L^{-1} Hg(II) (as Hg) were in the range of 1.2−3.4%. The RSDs of the retention time were in the range of 0.8−2.3%.

Analysis of Real Samples. To evaluate the usefulness of the developed method for the speciation analysis of mercury at the levels usually found in fish products, five real fish samples collected from local markets were analyzed. The analytical results obtained by the proposed method were given in **Table 6**. Methylmercury was detected in these samples. The concentration of methylmercury in a certified reference material DORM-2 (dogfish muscle) obtained by the developed method agreed well with the certified value. Chromatograms obtained for the injection of a surfactant-rich phase of (**A**) a fish sample and (**B**) a certified reference material DORM-2 are shown in **Figure 5**. According to the analytical results, the concentration of methylmercury in the fish samples ranged from 15 to 35 ng g^{-1} (as Hg). The mercury speciation in real samples was

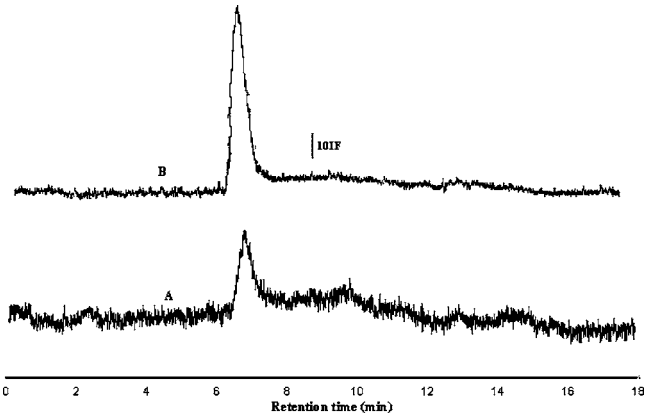

Figure 5. Chromatograms obtained for the injection of a surfactant-rich phase of (**A**) a fish sample and (**B**) a certified reference material DORM-2. All other conditions and peak assignments are as described in **Figure 1**.

Table 6. Analytical Results for the Speciation of Mercury in Fish Samples

	concentration determined (mean ± σ, $n = 3$)/ ng g^{-1} (as Hg)				recovery[a] (%)			
sample	MeHg	EtHg	PhHg	Hg(II)	MeHg	EtHg	PhHg	Hg(II)
fish sample 1	18 ± 3	ND[b]	ND	ND	90	96	91	104
fish sample 2	33 ± 5	ND	ND	ND	101	105	90	100
fish sample 3	15 ± 3	ND	ND	ND	95	96	88	101
fish sample 4	35 ± 4	ND	ND	ND	86	98	92	109
fish sample 5	28 ± 3	ND	ND	ND	92	96	99	103
CRM DORM-2	4427 ± 70	ND	ND	ND				

[a] For 0.5 μg L^{-1} MeHg, EtHg, and PhHg and 0.2 μg L^{-1} Hg(II), respectively, spiked in the extract. [b] Not detected.

performed after three procedures including acid leaching, CPE preconcentration, and HPLC-AFS analysis. Probably too many procedures resulted in high percent RSDs for real samples. The recoveries of mercury species spiked in the extracts of the studied samples ranged from 86 to 109%.

In conclusion, CPE with Triton X-114 is an easy and practical procedure for the preconcentration of mercury species in biological materials after their acid leaching. The method gives low detection limit, good precision, and solvent-free extraction of the element from its initial matrix following a single-step extraction procedure. The CPE technique described in the present work overcomes several problems and interferences encountered in the determination of these species by creating stable micelle and offers a simple, sensitive, and inexpensive alternative to other preconcentration techniques prior to HPLC hyphenated with element-specific detectors for mercury speciation. Further improvement is feasible by preconcentration of larger amounts of sample solution.

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Received for review July 23, 2005. Revised manuscript received October 23, 2005. Accepted October 24, 2005. This work was supported by the Tianjin University Foundation for Young Teacher.

JF051777K