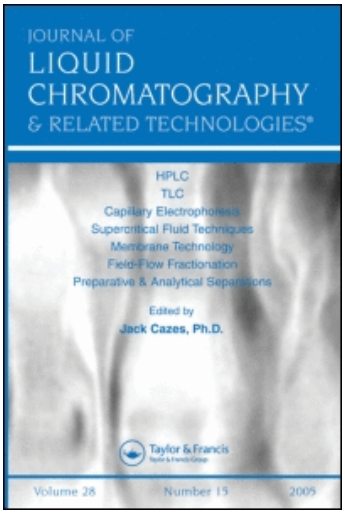

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Selective Determination of Mercury(II) Ion by Solvent Extraction with *N*-(Dithiocarboxy)sarcosine, Diammonium Salt Followed by Ligand Exchange and Reversed-Phase HPLC with Photometric Detection

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ABSTRACT

Mercury(II) ion was reacted with *N*-(dithiocarboxy)sarcosine (DTCS), diammonium salt and then extracted into 1-hexanol as Hg-DTCS chelate at pH 1. The organic layers of 15 and 60 μL of 10-mmol/L hexamethyleneammonium hexamethylenedithiocarbamate (HMA-HMDC) were injected into a C_{18} column. The Hg-DTCS chelate was converted to Hg-HMDC chelate on the column and detected at 280 nm. The

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correlation coefficient of the calibration curves obtained with 5 mL of mercury standard was more than 0.999 over the range of 10 ng/mL (ppb) to 10 μg/mL (ppm). The detection limit of Hg ion in 5 mL solution was 1.1 ppb, which corresponded to three times the standard deviation of the blank peak area. Reproducibilities for 5, 0.5, and 0.05 ppm were 1.7%, 1.6%, and 3.3%, respectively ($N = 5$). Recovery tests were carried out by the HPLC method presented and ICP-AES with spiked river water samples. The recoveries by the HPLC method for 5 ppm, 0.5 ppm, and 0.05 ppm Hg were 99%, 98%, and 95%, respectively. Effects of foreign ions on the method were investigated with 54 metal ions. Almost all ions did not interfere except for Co(II), Cu(II), Te(IV), and V(V).

Key Words: Mercury(II); Solvent extraction; *N*-(Dithiocarboxy)-sarcosine; Ligand exchange; RP-HPLC.

INTRODUCTION

For metal analysis, atomic absorption spectrometry (AAS), ICP-AES, and inductively coupled plasma-mass spectrometry (ICP-MS) are routinely used. However, ICP-AES and ICP-MS require expensive apparatuses and the detection sensitivity of AAS and ICP-AES differs considerably according to the metal. On the other hand, the application of high-performance liquid chromatography (HPLC) in the separation and determination of metal ions has increased in recent years.[1–5] The authors reported multi-element simultaneous determination methods by reversed-phase HPLC with photometric detection, using dithiocarbamate[6,7] and β-diketones[8,9] as pre-column chelating reagents. In the case of photometric detection of a metal chelate, detection sensitivity is influenced by the molar absorptivity and retention time of the chelate. A more sensitive quantitative analysis is possible by combining pre-column derivatization HPLC with appropriate pre-concentration.

N-(Dithiocarboxy)sarcosine (DTCS) has been used for determination of copper by molar absorption spectroscopy.[10,11] We found that Cu and Hg reacted with DTCS and formed neutral chelates and the chelates could be extracted into some organic solvents. However, the DTCS metal chelates of copper and mercury gave no chromatographic peak on a C_{18} column, because the DDTS metal chelates were unstable in the column. In a previous work,[12] *o*-salilicylideneaminophenol (SAPH) has been used for selective determination of Ni ion. Ni ion was extracted into organic solvent as SAPH chelate and then determined as HMDC chelate by ligand exchange reaction on a C_{18} analytical column.

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In this paper, DTCS was used as a chelating reagent for solvent extraction of Hg(II) ion, and the extracted Hg-DTCS chelate was then converted to stable Hg-HMDC chelate on a C_{18} column and detected at maximum absorption of the chelate. The presented method does not use chlorinated solvents for extraction or HPLC separation. Analytical conditions such as extraction solvent, extraction pH, shaking time, ligand exchange conditions, etc., were studied for selective determination of Hg ion. The detection limits, reproducibility, and recovery were investigated.

EXPERIMENTAL

Instrumentation

The HPLC system consisted of a Jasco PU-2080i inert pump (Japan Spectroscopic Co., Ltd, Tokyo, Japan), Rheodyne 7125 injector (Cotati, CA), Jasco UV-970M photometric detector, Capcellpac C_{18} SG-120 stainless steel column (250 × 4.6 mm i.d., Shiseido, Tokyo, Japan), Shimadzu Chromatopac C-R6A data processor (Shimadzu Co., Kyoto, Japan), and thermostat water bath (45°C). A Yamato SA-31 auto-shaker (Yamato Scientific Co., Ltd., Tokyo, Japan) was used for solvent extraction. A Plasma-Spec I ICP-AES (Leeman Labs Inc., MA) was also used for Hg analysis. Micropipettes were used for 1 mL or less volume of solution.

Reagents

All reagents used were of analytical-reagent grade, unless otherwise stated. Milli-Q water was used for aqueous solution preparation. The chelating reagent DTCS was obtained from Dojindo Laboratories (Kumamoto, Japan) for use as aqueous solution (50 mmol/L, 9.965 g/L). Hexamethyleneammonium hexamethylenedithiocarbamate (HMA-HMDC) was synthesized according to the previous work,[6] and used as 10-mmol/L (2.745 g/L) aqueous solution. *N*-(Dithiocarboxy)sarcosine (DTCS) and HMA-HMDC were prepared fleshly every 2 weeks. Additional information for DTCS and HMA-HMDC such as structure, molecular weight, and pK_a value[13] was shown in Fig. 1. The mercury(II) standard solution ($HgCl_2$ in 0.02-mol/L HCl) of 1000 ppm for AAS was obtained from Wako Pure Chemical Industries (Osaka, Japan). The other Hg solutions were obtained by dilution of the above solution (1000 ppm) with 0.1-mol/L HCl. Methanol was distilled and filtered through a membrane filter (pore size, 0.45 μm). A buffer solution of pH 1.15 was prepared with 1-mol/L HCl and 1-mol/L sodium acetate solution. River water

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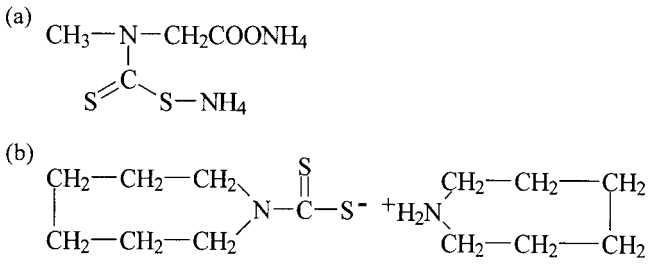

Figure 1. Structures of DTCS (a) and HMA-HMDC (b). Molecular weight: DTCS: $C_4H_5NO_2S_2 2NH_4 = 199.30$ (CAS No. 29664-09-3). HMA-HMDC: $C_{13}H_{26}N_2S_2 = 274.48$ (CAS No. 2608-11-9). The ionization constant (pK_a) of HMA-HMDC was reported to be 3.28 (Ref.[7]). The pK_a of DTCS is unknown.

was collected at the Kakehashi River (Komatsu, Japan). Concentrated hydrochloric acid was added to the river water immediately to adjust it to pH 1. The solution was then filtered through a membrane filter (0.45 μm pore size) and used as the river water samples for HPLC and ICP-AES analysis. Methanol–water eluent (82 : 18, v/v) was used for elution of the Hg-HMDC chelate.

Recommended Procedure for Hg Ion Determination

Transfer a sample or Hg standard solution (5.0 mL) into a 10 mL centrifuge tube with a stopper. Adjust pH of the solution to 1, if necessary. Add 1000 μL of buffer solution (pH 1.15), 1000 μL of 50-mmol/L DTCS aqueous solution, and 300 μL of 1-hexanol, in that order. Shake the contents for 10 min. After standing for 5 min, inject 15 μL of the organic layer and 60 μL of 10-mmol/L HMA-HMDC into a C_{18} analytical column. Detect Hg-HMDC chelate at 280 nm.

Effects of Foreign Ions

The effects of foreign ions on the determination of Hg ion were tested with 54 metal ions. Each foreign ion and 1000 μL of 0.5 ppm Hg standard were placed into a centrifuge tube, and diluted to 5 mL with 1-mol/L HCl. Concentrations of Hg ion in both solutions was determined by the recommended procedure. The recovery percentage was calculated from the peak area and that of the Hg standard containing no foreign metal ions. The limiting value of the foreign ion concentration was taken

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to be the value, which caused an error of more than 10% in the recovery of Hg ion.

Recovery Test with River Water Sample

Four milliliters of river water sample (pH 1) and 1000 μL of 0.1-mol/L HCl were taken into a centrifuge tube. To another centrifuge tube, 4 mL of river water sample and 1000 μL of Hg standard were added. The concentrations of the Hg standards were 25, 2.5, and 0.25 ppm. Hg concentrations in these solutions were determined according to the recommended procedure, and the recovery percent was calculated by the results. A similar experiment was carried out by ICP-AES (Ar plasma, 40 MHz) with 253.65 nm for the detection of Hg.

RESULTS AND DISCUSSION

HPLC Conditions

In order to convert Hg-DTCS chelate to Hg-HMDC, HMA-HMDC is required in the column. The eluent containing HMA-HMDC needed to cool in an ice bath because decomposition of the HMA-HMDC caused a baseline drift. Thus, HMA-HMDC was injected with an organic layer containing Hg-DTCS chelate. The Hg ion (1 ppm) was extracted as DTCS chelate according to the recommended extraction procedure, except for the pH (pH 3). The effects of 10-mmol/L HMA-HMDC amounts on ligand exchange of Hg-DTCS chelate to Hg-HMDC chelates were examined over the range from 1 to 100 μL. At first, an organic layer (containing Hg-DTCS chelate) must be injected into the C_{18} column, and then HMA-HMDC solution should be injected immediately for effective ligand exchange. Thus, HMA-HMDC should be injected into the Rheodyne 7125 injector at first, and the organic layer subsequently, because the two solutions in the sample loop go back into the C_{18} column. Constant peak areas were obtained by injection of 40–100 μL of 10-mmol/L. Though a larger volume of 10-mmol/L HMA-HMDC is advantageous for effective ligand exchange, base line drifts became larger after the HMA-HMDC peak. Therefore, an injection volume of 60 μL was selected.

Eluent flow rate was investigated in the range of 0.8–1.6 mL/min and set at 1.4 mL/min because of the good peak shape and appropriate separation time.

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Injection volume of the organic layer was also investigated over the range from 3 to 20 μL. Considering the higher peak shape of Hg chelate and separation from HMA-HMDC peak, an injection volume of 15 μL was employed. A typical chromatogram is shown in Fig. 2 with HPLC conditions.

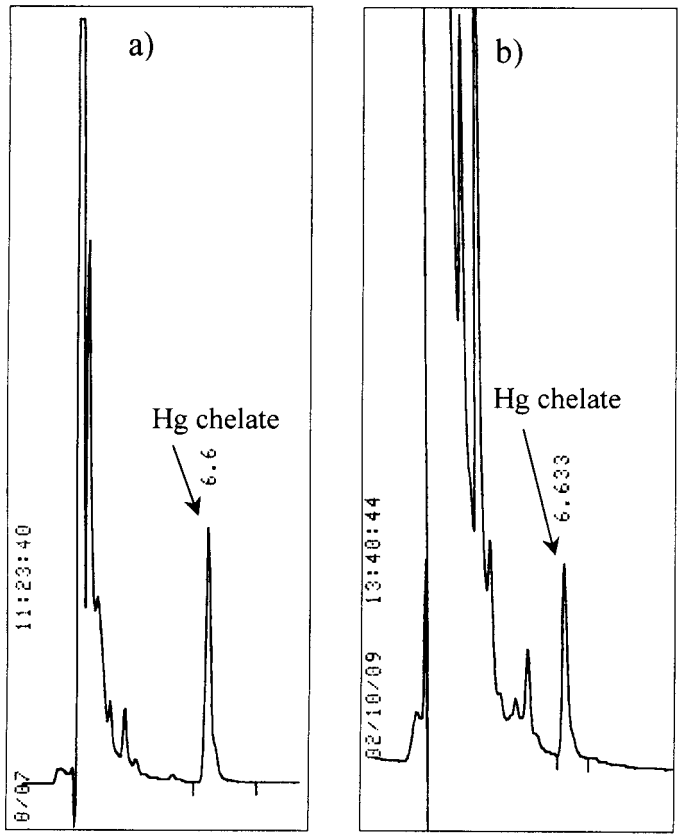

Figure 2. Typical chromatogram of Hg chelate. Column: Capcellpac C_{18} SG-120 (250 × 4.6 mm i.d., particle size 5 μm). Column temperature: 45°C. Eluent: methanol/water (82 : 18, v/v). Flow rate: 1.4 mL/min. Detection wavelength: 280 nm. Detection sensitivity: (a) 0.04 AUFS and (b) 0.01 AUFS. Hg concentration in 5 mL solution (a) 0.4 ppm and (b) 0.04 ppm. Injection volume of sample: 15 μL, Injection volume of 10 mmol/L HMA-HMDC: 60 μL. Extraction conditions are the same as in the recommended procedure.

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Stability of *N*-(Dithiocarboxy)sarcosine Chelate in 1-Hexanol

Hg ion in 5 mL of 5 ppm standard was extracted into 1-hexanol as DTCS chelate and the organic layer was put into a test tube with a stopper. If the Hg-DTCS chelate is unstable in the organic layer, the HPLC analysis must be done immediately after extraction. Thus, the effect of standing time on the peak area of Hg chelate was investigated for 0–295 min with nine replicate determinations including extraction and HPLC steps at varying standing times. The results indicated that the Hg-DTCS chelate in 1-hexanol is stable for at least 295 min and immediate injection is not required.

Extraction Conditions

A preliminary experiment indicated that Hg and Cu in acidic solution were extracted into some alcohols. Because Hg is the one of most toxic metals, we studied the selective determination method for Hg ion. As non-chlorinated extraction solvents, 1-hexanol, 1-octanol, 2-octanol, and 1-decanol were examined at pH 1 to 5 according to the recommended procedure. When 1-hexanol was used as the extraction solvent, Hg ion was extracted successfully in the pH range from 0.8 to 1.8, as shown in Fig. 3.

The effect of shaking time on extraction of Hg ion was also investigated at pH 1.15 with 5 mL of 1 ppm Hg standard and 1000 μL of 50-mmol/L DTCS solution. Constant peak areas were obtained at a shaking time of 5–30 min. Thus, 10 min was used for the remainder of the work. The smaller the volume of 50-mmol/L DTCS solution, the longer the shaking time was required.

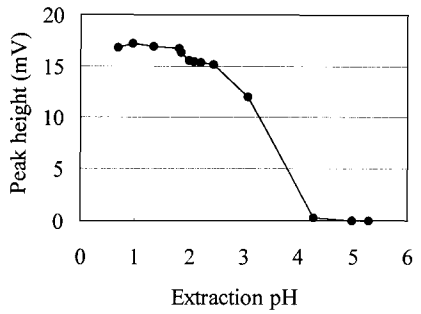

Figure 3. Effect of pH on extraction of Hg ion. Concentration of Hg ion in 5 mL solution was 0.5 ppm. The other extraction conditions are the same as the recommended procedure, except for extraction pH.

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Calibration Curves, Detection Limits, and Reproducibilities

To investigate the linear range of the calibration curve for Hg ion, calibration curves were prepared with Hg standard of varying concentrations by the recommended procedure. The calibration curves have good linearity in a wide concentration range (10 ppb–10 ppm), as shown in Table 1. Equations and correlation coefficients of the calibration curves obtained in different days were almost equal to the above results. A calibration curve of 1–10 ppm showed s minus intercept. The main reason of the minus intercept may be an increase of retention time. Since the retention time of Hg-HMDC chelate increased slightly with Hg concentration, the peak area increased with Hg concentration. The intercept of the calibration curve calculated by peak height was better than that by peak area. It is thought that the ligand exchange rate of higher Hg concentration is larger than that of lower concentration, and caused a long retention time. This phenomenon was not found at the Hg concentration of less than 0.1 ppm.

The detection limit of Hg ion in 5 mL solution was 1.1 ppb, which corresponded to three times the standard deviation of the blank peak area.

Reproducibilities ($N = 5$) of Hg chelate peak areas obtained with 5 ppm, 0.5 ppm, and 0.05 ppm Hg standard were 1.7%, 1.6%, and 3.3%, respectively.

Effects of Foreign Ions

The effects of 54 foreign ions on the determination of Hg ions were investigated. Alkali and alkaline earth metal ions did not interfere with the Hg determination at a thousand times the concentration of Hg ion. The determination of 0.1 ppm Hg ion was interfered by 0.5 ppm Co(II), Cu(II), Te(IV), and V(V). In these ions, Co and Cu ions caused lower Hg chelate

Table 1. Calibration curves for Hg ion.

Concentration range (ppm)	Equation of line	Correlation coefficient	Measuring point (ppm)
1–10	$y = 996.15x - 120.54$	0.9997	0, 1, 2, 4, 6, 8, 10
0.1–1	$y = 774.77x - 2.3036$	0.9993	0, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0
0.01–0.1	$y = 625.32x + 0.585$	0.9995	0, 0.01, 0.02, 0.04, 0.06, 0.08, 0.10

y, peak area (mV s); x, concentration of Hg (ppm).

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peaks, while Te and V ions caused higher peaks. The other metal ions could coexist at five times or more the concentration of Hg ion, as shown in Table 2.

Recovery Tests with a River Water Sample by HPLC and ICP-AES

Because the concentration of Hg ion in the collected river water sample was less than the determination limits (10 ppb) of the HPLC method, a recovery test was carried out with the sample. Hg standard was added to the river water sample at 5, 0.5, and 0.05 ppm. Then the solutions were analysed by the proposed HPLC method and ICP-AES. The results were summarized in Table 3. Both correlation coefficients of the calibration curves obtained by HPLC and ICP-AES were more than 0.999.

CONCLUSION

The presented method for mercury analysis does not use chlorinated solvents for extraction or HPLC separation. This method required a conventional

Table 2. Effects of foreign ions on determination of 0.1 ppm Hg ion.

Tolerance limit (ppm)	Metal ion
100	As(III), Al(III), Ba(II), Be(II), Ca(II), Cd(II), Ce(III), Cs(I), Dy(III), Eu(III), Er(III), Gd(III), Ge(IV), Ho(III), K(I), La(III), Lu(III), Mg(II), Mn(II), Na(I), Ni(II), Nd(III), Pr(III), Rh(III), Sb(III), Si(IV), Sm(III), Sn(II), Sr(II), W(IV), Y(III), Yb(III), Zr(IV)
40	Ga(III), Se(IV), Tb(III), Ti(IV), Tl(I), Zn(II)
20	Bi(III), In(III), Pb(II)
10	Sc(III)
5	Au(III), Pt(IV)
2	Pd(II)
0.5	Ag(I)[a], Cr(VI), Fe(III), Mo(VI)
0.2	Co(II), Cu(II), Te(IV), V(V)

Note: The tolerance limit value of the foreign ion concentration was taken as the value, which caused an error of less than 10% in the recovery of Hg ion. Large amounts of Cu, Pd, V, and Au ions also produced precipitation.
[a]Ag(I) ion of 0.5 ppm or more formed white precipitation (probably AgCl).

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Table 3. Recovery tests with a river water sample.

Concentration of Hg spiked (ppm)	Sample (ppm)	Added (ppm)	Found ± SD[a] (ppm)	Recovery ± SD[a] (%)	Equation of calibration curve	Correlation coefficient
			HPLC			
5.00	0.00	5.00	4.97 ± 0.04	99.4 ± 0.8	$y = 993.04x - 399.89$	0.9999
0.500	0.00	0.500	0.491 ± 0.004	98.2 ± 0.9	$y = 797.81x - 8.0484$	0.9999
0.050	0.00	0.050	0.046 ± 0.002	94.6 ± 2.2	$y = 678.54x + 0.737$	0.9997
			ICP-AES			
5.00	0.00	5.00	4.87 ± 0.05[b]	97.3 ± 1.0[b]	$y = 4693.5x + 1486.3$	0.9996

Note: Recoveries obtained by different days were 99.2% for 5 ppm, 100.2% for 0.5 ppm, and 93.1% for 0.05 ppm. Recovery tests by ICP-AES for 0.5 ppm and 0.05 ppm Hg ion were not carried out, since Hg ion could not detected by the ICP-AES used in this work.
[a]Number of run was 5.
[b]Number of run was 4.

HPLC apparatus equipped with a photometric detector. The extraction procedure is simple and required no extraordinary skill. Reproducibility of the simple and easy HPLC method was comparable to that of ICP-AES. The detection limit of Hg(II) ion was 1.1 ppb, and the lower determination limit was 10 ppb.

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