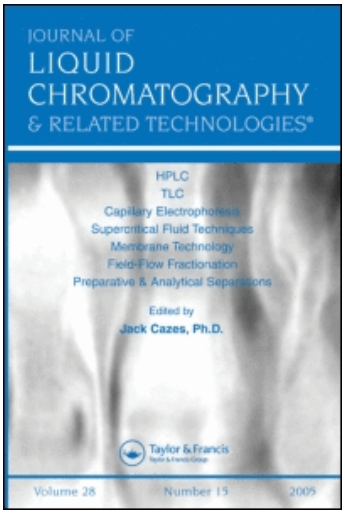

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Selective Determination of Mercury Ion in River Water by Solvent Extraction with 4,6-Dimethyl-2-Mercaptopyrimidine Followed by Reversed-Phase HPLC

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Abstract: A selective determination method for mercury (Hg) ion in river water has been developed by solvent extraction, followed by reversed-phase HPLC with photometric detection. The Hg(II) ion was quantitatively extracted into chloroform over the pH range of 2.9 to 5.4 as 4,6-dimethyl-2-mercaptopyrimidine (DMMP) chelate. Job's method indicated that the Hg-DMMP chelate composition was $Hg(DMMP)_2$. The extracted Hg-DMMP chelate was then separated on an ODS column with an eluent of methanol/water/0.1 M DMMP (60:40:0.25, v/v) and detected at 255 nm. The correlation coefficients of the calibration curves obtained with 5 mL Hg standards were more than 0.999 over the range of 0.2 μg/mL (ppm) to 10 ppm. The detection limit of the Hg ion in 5 mL water was estimated as 0.02 ppm, by a signal to noise ratio of 3. The recoveries with a spiked river water sample for 0.5 and 5 ppm Hg ion ($N = 5$) were $100.9 \pm 1.2\%$ and $100.1 \pm 0.8\%$, respectively. Effects of foreign ions on the determination of 0.5 ppm Hg ion were investigated with 57 metal ions. Almost none of the ions interfered except for Ag(I), Au(III), and Cu(II).

Keywords: Mercury ion, River water, Solvent extraction, RP-HPLC

INTRODUCTION

For metal analysis, atomic absorption spectrometry (AAS), and inductively coupled plasma atomic emission spectrometry (ICP-AES) are routinely used

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for metal analysis. Inductively coupled plasma mass spectrometry (ICP-MS) is also used for more sensitive metal analysis. However, ICP-AES and ICP-MS require expensive instrumentation, and the detection sensitivity of AAS and ICP-AES varies considerably according to the metal. On the other hand, the application of high performance liquid chromatography (HPLC) for the separation and determination of metal ions has increased in recent years.[1–5] HPLC is very popular and not as expensive an apparatus; the running cost is very low. Additionally, operation of the HPLC is easy, and a more sensitive quantitative analysis is possible by combining precolumn derivatization HPLC with a simple solvent extraction. We also determined various metal ions by HPLC as metal chelates[6–10] combined with solvent extraction and spectrophotometric detection.

We found that 4,6-dimethyl-2-mercaptopyrimidine (DMMP) reacted with Hg(II) ion, and the Hg-DMMP chelate was extracted into chloroform from an acidic solution. The Hg chelate was stable in chloroform. However, the analytical application of DMMP is not found. Thus, DMMP was tested as a chelating reagent for Hg analysis by reversed-phase (RP) HPLC.

In this paper, analytical conditions such as extraction pH, shaking time, and eluent composition, were studied for selective determination of the Hg ion by RP-HPLC combined with solvent extraction. The composition of Hg-DMMP chelate was also determined by Job's method. In addition, the linearity of calibration curves and the detection limit of the Hg ion were also investigated. Effects of foreign ions on the determination of the Hg ion were investigated with 57 metal ions. The HPLC method was applied to determination of the Hg ions in the spiked river water sample.

EXPERIMENTAL

Instrumentation

The HPLC system consisted of a Jasco PU-2080i inert pump (Japan Spectroscopic Co., Ltd, Tokyo, Japan), a Rheodyne 7125 injector (Cotati, CA) equipped with a 200 μL sample loop of polyether etherketone, a Jasco UVIDEC-100-VI photometric detector, a Cosmosil 5 C_{18} MS-II stainless steel column (250 × 4.6 mm ID, 5 μm particle, Nacalai Tesque, Kyoto, Japan), a Shimadzu Chromatopac C-R6A integrator (Shimadzu Co., Kyoto, Japan), and a thermostat water bath (Taitec Co., Koshigaya, Japan). A Yamato SA-31 auto shaker (Yamato Scientific Co., Ltd., Tokyo, Japan) was used for solvent extraction. Micropipettes were used for 1 mL or less volume of solutions.

Reagents

All reagents used were of analytical reagent grade unless otherwise stated. Milli-Q water was used for the aqueous solution preparation and the

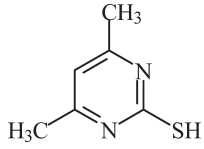

Figure 1. 4,6-Dimethyl-2-mercaptopyrimidine (DMMP). $C_6H_8N_2S = 140.21$, CAS No. 22325–27–5.

extraction procedure. The chelating reagent DMMP was obtained from Tokyo Kasei Kogyou Co., Ltd. (Tokyo, Japan). Further details are shown in Figure 1. The DMMP was dissolved in Milli-Q water in a concentration of 0.1 mol/L (M). The 0.1 M DMMP was preserved in a refrigerator (about 4°C). The 58 metal standard solutions of 1000 μg/mL (ppm) used were obtained from Kanto Chemical Co., Inc. (Tokyo, Japan). The Hg standard of 1000 ppm consisted of $HgCl_2$ and 0.1 M HNO_3. The other Hg solutions were prepared by dilution of the above solution (1000 ppm) with 0.1 M HNO_3. Methanol was distilled and filtered through a membrane filter (pore size, 0.45 μm). Ammonia-ammonium chloride buffer solutions (pH 8.0–11.0) were prepared with 2 M ammonia and 2 M ammonium chloride solutions. Acetate buffer solutions (pH 4.0–7.0) were prepared with 2 M acetic acid and 2 M sodium acetate solutions. Hydrochloric acid-acetic acid solutions (pH 1.5–2.5) were prepared with 1 M hydrochloric acid and 1 M acetic acid. River water was collected from the Kakehashi River (Komatsu, Japan). Concentrated nitric acid was added to the river water immediately to adjust the pH to 1. The river water was filtered through a membrane filter (0.45 μm pore size), and the filtrate was used as the river water sample.

Recommended Extraction Procedure and HPLC Conditions

Transfer 4 mL of sample solution and 1000 μL of 0.1 M HNO_3 into a 10 mL centrifuge tube with a stopper. For calibration curves, transfer an Hg standard solution (0.1 M HNO_3) and 0.1 M HNO_3 (total volume 1000 μL) along with 4 mL of water into a centrifuge tube. Add 2 mL of 2 M acetic acid-2 M sodium acetate buffer solution (pH 4.5) to the centrifuge tube. After mixing the contents, add 500 μL of 0.1 M DMMP solution and 500 μL of chloroform into the tube. Shake the contents for 10 min. After standing for 10 min, collect the organic layer. Determine the Hg concentration in the extract as Hg-DMMP chelate under the following HPLC conditions. Column: Cosmosil 5 C_{18} MS-II (250 × 4.6 mm ID, particle size 5 μm), column temp.: 40°C, eluent: methanol/water/0.1 M DMMP (60:40:0.25, v/v), flow rate: 1.0 mL/min, injection volume of organic layer: 5 μL, detection wavelength: 255 nm.

Screening Test for 58 Metal Ions

To a 10 mL centrifuge tube, 20 μL of each metal solution of 1,000 ppm, 5 mL of water, 2 mL of 2 M acetate buffer solution (pH 5.5), 500 μL of 0.1 M DMMP, and 500 μL of 1-octanol were added. After shaking for 20 min, the organic layer was separated and used for HPLC analysis. The HPLC conditions used were as follows: eluent, methanol/water/0.1 M DMMP (75:25:0.1, v/v); detection, 254 nm. The other conditions are the same as the recommended HPLC conditions. A blank test was also conducted, and the chromatographic peaks were compared.

Chelate Composition of Hg-DMMP Chelate

To a 10 mL centrifuge tube, (1000-x) μL of 0.1 M HNO_3, x μL of 2.49×10^{-2} M (50.0 ppm) Hg standard solution, (5000-y) μL of water, y μL of 2.49×10^{-2} M DMMP, 2000 μL of 2 M acetate buffer solution (pH 4.5), and 500 μL of chloroform were added. After shaking for 20 min, each organic layer was chromatographed, and the peak area of the Hg-DMMP chelate was measured according to the recommended conditions. Where, (x, y) were (0, 1000), (100, 900), (200, 800), (300, 700), (333, 667), (400, 600), (500, 500), (600, 400), (700, 300), (800, 200), (900, 100), and (1000, 0); the mole fractions of [Hg]/([Hg] + [DMMP]) were 0, 0.1, 0.2, 0.3, 0.333, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1.0, respectively.

Effect of Foreign Ions

The effect of foreign ions on the determination of the Hg ion was tested with 57 metal ions. Each foreign ion, 500 μL of 5 ppm Hg standard, and 500 μL of 0.1 M HNO_3 were placed into a centrifuge tube, and diluted to 5 mL with water (Hg concentration: 0.5 ppm). The concentration of the Hg ion in the 5 mL solution was determined by the recommended procedure. The recovery percentage was calculated from the peak area of the Hg chelate and that of the Hg standard (0.5 ppm) containing no foreign metal ions. The tolerance limit value of the foreign ion concentration was taken as the value that caused an error of less than 10% in the recovery of the Hg(II) ion.

Recovery Test with a River Water Sample

As no Hg ion in the river water sample was detected by the HPLC method, the Hg ions were added to the river water sample. A 4.0 mL of a river water sample, 500 μL of Hg standard (50 or 5 ppm), and 500 μL of 0.1 M HNO_3 were added to a centrifuge tube. The Hg concentrations in these solutions (0.5 or 5 ppm) were determined according to the recommended procedure, and the recovery percentages were calculated.

RESULTS AND DISCUSSION

Extraction Conditions

The screening test for 58 metal ions indicated that Hg, copper (Cu), palladium (Pd), and platinum (Pt) ions gave chromatographic peaks of the DMMP chelates. The Cu and Pt formed yellow color chelates at pH 5.5. The Cu and Pt chelates were found unstable in the 1-octanol because the color of the organic layer (yellow) decreased with time. On the other hand, the Pd ion produced two chromatographic peaks. Among the metals, only the Hg-DMMP chelate gave one symmetrical peak and was stable in the 1-octanol. These results indicated that the DMMP was suitable as the chelating reagent for the Hg ion. However, 1-octanol was not sufficiently soluble in the water rich eluent (methanol: water = 60:40), and the baseline of the chromatogram and peak shapes of some DMMP chelates were not very good. Therefore, 1-hexanol, 1-octanol, 1-decanol, 4-methyl-2-pentanone, and chloroform were investigated as extraction solvents. Among the solvents, chloroform was the best solvent with respect to the base line and peak shape. Thus, chloroform was employed as the extraction solvent.

Extraction pH was investigated with various buffer solutions according to the recommended procedure. After extraction, the pH of each aqueous layer was measured. The peak area of the Hg-DMMP chelate was plotted against the measured pH. The effect of pH on extraction of the Hg-DMMP chelate is shown in Figure 2. As constant peak areas were obtained over the pH range of 2.9 to 5.4, 2 mL of 2 M acetate buffer solution of pH 4.5 was used in the recommended extraction procedure. When the buffer solution of pH 4.5 was used, the Hg(II) ion was quantitatively extracted into the chloroform by shaking for 3–60 minutes. Subsequently, 10 min was selected as the optimum shaking time.

Effect of the volume of 0.1 M DMMP on extraction of the Hg-DMMP chelate was also investigated. Because the peak areas were constant in the

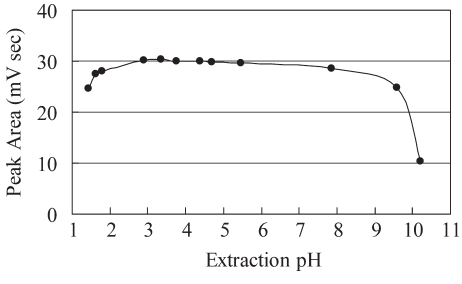

Figure 2. Effect of pH on extraction of Hg-DMMP chelate. Buffer solutions used: 1 M HCl-1 M CH_3COOH (pH 1.5, 2.0, 2.5), 2 M acetate buffer (pH 3.0, 3.5, 4.0, 4.5, 5.0, 6.0), 2 M NH_3-NH_4Cl (pH 8.0, 10.0, 11.0).

range of 10 to 1000 μL, 500 μL of 0.1 M DMMP was used in the recommended extraction procedure.

The extracted Hg-DMMP chelate was found stable in chloroform for at least 7 hours, because the peak areas of the Hg-DMMP chelate were almost constant. Thus, immediate injection of the organic layer was not required.

HPLC Conditions

The DMMP was required in the eluent to prevent the dissociation of the Hg-DMMP chelate in an ODS column. Consequently, the effect of the DMMP concentration on the peak area of Hg-DMMP chelate was investigated with 5 mL of 1 ppm Hg standard. Constant peak areas (about 30 mV sec) were obtained in the range of 50 to 400 μM in eluent, while small peak areas (about 11 mV sec) without DMMP. Considering the above results, the eluent of methanol/water/0.1 M DMMP (60:40:0.1, v/v) was employed: the concentration of DMMP in eluent is 100 μM.

The peak height of the Hg-DMMP chelate was measured at 220–500 nm. Detection wavelength was set at 255 nm, which gave the maximum peak height of the Hg-DMMP chelate.

Hg-DMMP Chelate Composition

The composition of the Hg-DMMP chelate was investigated by Job's method. The peak areas were plotted against the mole fractions of [Hg]/([Hg] + [DMMP]), as shown in Figure 3. The maximum peak area was obtained at a mole fraction of 0.33 (that is [Hg]:[DMMP] = 1:2). The results indicated that the DMMP ionized to H^+ and $DMMP^-$, then reacted with the Hg^{2+} ion to form $Hg(DMMP)_2$ chelate.

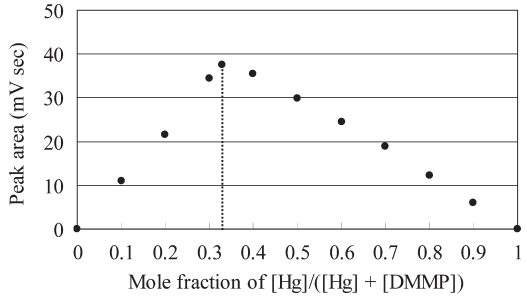

Figure 3. Determination of Hg-DMMP chelate composition by Job's method. Experimental conditions are in the text.

Table 1. Calibration curves for Hg(II) ion

Concentration range	Equation of line	Correlation coefficient	Measuring point (ppm)
2–10 ppm	$y = 12.763x - 2.6399^a$	0.9997	2, 4, 6, 8, 10
0.2–1 ppm	$y = 113.23x - 4.6509^b$	0.9991	0.2, 0.4, 0.6, 0.8, 1.0

y: peak area (mV sec), x: concentration of Hg(II) ion (ppm).
Detector range (AUFS): [a]0.32, [b]0.04.

Calibration Curves and Detection Limit

Calibration curves for the Hg(II) ions were prepared with the Hg standards of varying concentrations by the recommended procedure. The correlation coefficients of the calibration curves obtained with 5 mL Hg standards were more than 0.999 over the range of 0.2 to 10 ppm as shown in Table 1. The y intercept of each calibration curve for Hg was a minus value. The result suggested that the Hg-DMMP chelate is slightly unstable in the ODS column and the eluent used. However, more concentration of DMMP in the eluent caused larger baseline noise. Thus, the calibration curve for the lower concentration range (0.01–0.1 pm) could not be prepared.

The detection limit of the Hg ion in 5 mL water was estimated as 0.02 ppm, by a signal to noise ratio of 3.

Effect of Foreign Ions

The effect of 57 foreign ions on the determination of 0.5 ppm Hg(II) ion (5 mL) are summarized in Table 2. Table 2 shows that 49 metal ions did not interfere, at 200 times or more, the concentration of Hg ion. An Ag(I) ion of 5 ppm, 5 ppm Au(III), and 2 ppm Cu(II) interfered with the

Table 2. Effects of foreign metal ions on determination of 0.5 ppm Hg(II) ion

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

Note: The tolerance limit value of the foreign metal ion concentration was taken as the value that caused an error of less than 10% in the recovery of Hg(II) ion (0.5 ppm).

determination of 0.5 ppm Hg ion. The Cu(II) ion also extracted into the chloroform as Cu-DMMP chelate, and the chelate eluted near the Hg-DMMP chelate peak with the HPLC conditions used. The Ag ion reacted with the chloride ion of $HgCl_2$ (Hg standard) and formed precipitation of AgCl. More details are shown in Table 2.

Recovery Test of Hg Ion with a River Water Sample

Typical chromatograms of the Hg-DMMP chelates are shown in Figure 4. The results of recovery tests for 0.5 and 5 ppm Hg ions are summarized in Table 3. The recoveries of 0.5 and 5 ppm Hg(II) ions were $100.1 \pm 0.8\%$ and $101.4 \pm 1.5\%$, respectively. Recoveries obtained by HPLC on other days were $100.7 \pm 1.5\%$ for 5 ppm Hg and $101.1 \pm 1.7\%$ for 0.5 ppm Hg ($N = 5$). The correlation coefficients of the calibration curves were more

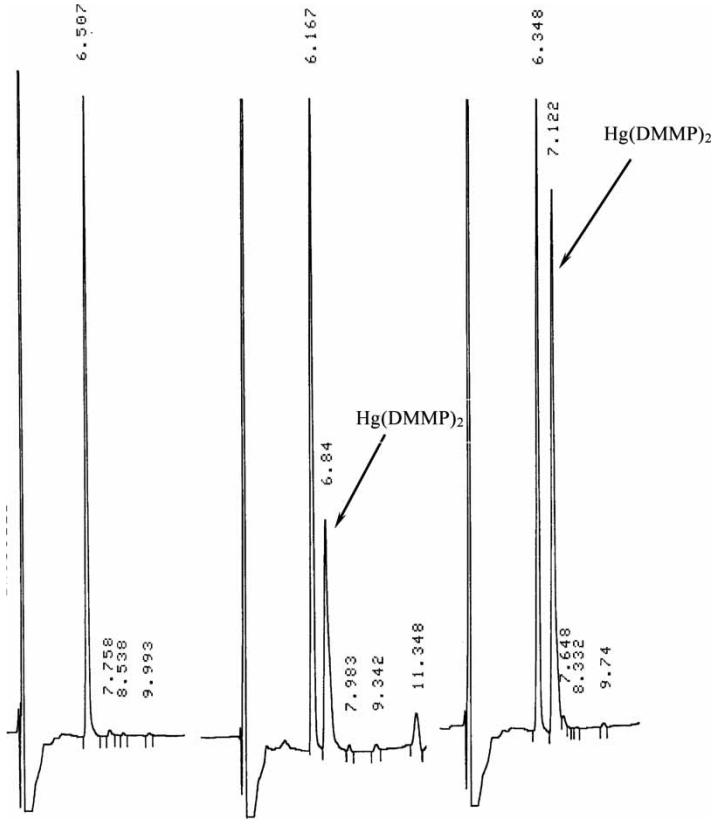

Figure 4. Chromatograms of blank and $Hg(DMMP)_2$ chelates. a) blank, b) 0.6 ppm Hg standard, c) 1.0 ppm Hg standard.

Table 3. Recovery tests for Hg(II) ion with a river water sample

No. of run	Sample (ppm)	Added (ppm)	Found (ppm)	Recovery (%)	Equation of calibration curve	Correlation coefficient
5 ppm						
1	0.00	5.00	5.00	100.0	$y = 12.355x - 3.6737$	0.9999
2	0.00	5.00	4.97	99.4		
3	0.00	5.00	4.97	99.4		
4	0.00	5.00	5.01	100.1		
5	0.00	5.00	5.07	101.4		
Av.			5.00	100.1		
SD			0.04	0.8		
RSD			0.8	0.8		
0.5 ppm						
1	0.000	0.500	0.506	101.2	$y = 81.336x - 3.0075$	0.9997
2	0.000	0.500	0.496	99.2		
3	0.000	0.500	0.508	101.6		
4	0.000	0.500	0.517	103.4		
5	0.000	0.500	0.508	101.7		
Av.			0.507	101.4		
SD			0.008	1.5		
RSD			1.5	1.5		

Detector response of the HPLC was 0.32 AUFS (5 ppm) and 0.04 AUFS (0.5 ppm).

Recoveries obtained on other days were $100.7 \pm 1.5\%$ for 5 ppm Hg and $101.1 \pm 1.7\%$ for 0.5 ppm Hg ($N = 5$).

than 0.999. The high recoveries indicated that the ions in river water did not interfere with the HPLC determination of the Hg ion.

CONCLUSION

The proposed extraction and HPLC procedure is simple and easy, and the HPLC apparatus used is the most popular HPLC apparatus equipped with a photometric detector. The extraction time and HPLC analysis time is 10 and 12 min, respectively. The DMMP was found to be a selective chelating reagent for ppm levels of Hg(II) ion. The HPLC method was applied to the determination of the Hg ion in river water with precise results.

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