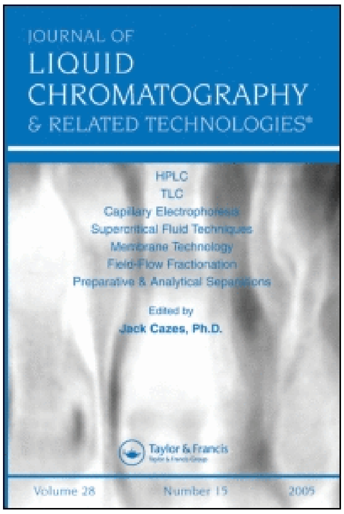

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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Susumu Ichinoki[a]; Akiko Ishizawa[a]; Mayumi Asano[a]; Youichi Fujii[a]
[a] Faculty of Pharmaceutical Sciences, Hokuriku University, Kanazawa, Japan

To cite this Article Ichinoki, Susumu , Ishizawa, Akiko , Asano, Mayumi and Fujii, Youichi(2010) 'DETERMINATION OF MERCURY ION IN RIVER WATER BY SOLVENT EXTRACTION WITH 5-METHYL-1,3,4-THIODIAZOLE-2-THIOL FOLLOWED BY REVERSED PHASE HPLC', Journal of Liquid Chromatography & Related Technologies, 33: 6, 793 — 801
To link to this Article: DOI: 10.1080/10826071003684265
URL: http://dx.doi.org/10.1080/10826071003684265

Journal of Liquid Chromatography & Related Technologies, 33:793–801, 2010

ISSN: 1082-6076 print/1520-572X online
DOI: 10.1080/10826071003684265

DETERMINATION OF MERCURY ION IN RIVER WATER BY SOLVENT EXTRACTION WITH 5-METHYL-1,3,4-THIODIAZOLE-2-THIOL FOLLOWED BY REVERSED PHASE HPLC

Susumu Ichinoki, Akiko Ishizawa, Mayumi Asano, and Youichi Fujii
Faculty of Pharmaceutical Sciences, Hokuriku University, Kanazawa, Japan

☐ *A determination method for mercury (Hg) ion in river water has been developed by reversed phase HPLC. The Hg(II) ion was quantitatively extracted into benzene from an acidic solution as 5-methyl-1,3,4-thiodiazole-2-thiol (MTT) chelate. The extracted Hg-MTT chelate was then separated on a C_{18} column with an eluent of methanol/water/0.01 mol/L MTT (72:26:2, v/v) and detected at 270 nm. The correlation coefficients of the calibration curves obtained with 5 mL Hg standards were more than 0.999 in the range of 0.1 to 10 μg/mL (ppm). The detection limit of the Hg ion in 5 mL of water was 0.05 ppm by a signal to noise ratio of 3. Relative standard deviations of peak areas (N= 6) for 5 and 0.5 ppm Hg standards were less than 1%. Effects of foreign ions on the determination of 0.4 ppm Hg were investigated with 57 metal ions. Almost none of the ions interfered except for Au(III), Cu(II), Pt(IV), Ag(I), Cr(VI), and Pd(II) ions. The recoveries with a spiked river water sample for 5 and 0.5 ppm Hg ion (N= 6) were 100.0 ± 0.8% and 99.3 ± 0.9%, respectively.*

Keywords 5-methyl-1,3-4-thiodiazole-2-thiol, mercury ion, river water, solvent extraction

INTRODUCTION

For mercury analysis, atomic absorption spectrometry combined with a reducing vaporization technique, inductively coupled plasma-atomic emission spectroscopy (ICP-AES), and inductively coupled plasma-mass spectrometry are routinely used. 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.[1–4] HPLC is a very popular and inexpensive apparatus, while the running cost is very low. In addition, 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

Address correspondence to Susumu Ichinoki, Faculty of Pharmaceutical Sciences, Hokuriku University, Ho 3, Kanagawa-machi, Kanazawa 920-1181, Japan. E-mail: s-ichinoki@hokuriku-u.ac.jp

as metal chelates.[5–9] In a previous paper,[9] we have reported 5-methyl-1,3,4-thiodiazole-2-thiol (MTT) as the precolumn chelating reagent for Pd(II) ion.

Further experiments indicated that MTT reacted with the Hg(II) ion, and the Hg-MTT chelate was extracted into benzene from a weak acidic solution. The Hg chelate was stable on a C_{18} column when a certain amount of MTT was added into an eluent. Thus, the MTT was investigated as a precolumn reagent for Hg analysis by HPLC.

In this paper, extraction and HPLC conditions were studied for determination of the Hg ion in river water by reversed phase HPLC. Effects of the foreign ions on the determination of the Hg ion were investigated with 57 metal ions. Hg ions in a spiked river water sample were determined by the HPLC method.

EXPERIMENTAL

Instrumentation

The HPLC system consisted of a Jasco PU-1580*i* inert pump (Japan Spectroscopic Co. Ltd, Tokyo, Japan), a Rheodyne 9725*i* injector (Cotati, CA) equipped with a 200 µL sample loop of polyether etherketone, an SPD-10AV*vp* photometric detector (Shimadzu Co. Kyoto, Japan), a Shimadzu Chromatopac C-R8A integrator, a Cosmosil 5 C_{18}-MS-II stainless steel column (250 × 4.6 mm ID, Nacalai Tesque, Kyoto, Japan), and a Thermo Minder SX-10R thermostat water bath (Taitec Co., Koshigaya, Japan). A Taitec SR-2 DS auto shaker was used for solvent extraction. An MS-E10R microsyringe (10 µL) with Ni-Ti alloy plunger was used for sample injection (Ito Co. Ltd., Fuji, Japan). Micropipettes were used for solutions of 2.5 mL or less.

Reagents

All reagents used were of analytical reagent grade unless otherwise stated. Milli-Q water was used for aqueous solution preparation and the extraction procedure. The chelating reagent MTT ($C_3H_4N_2S_2 = 132.21$, CAS No. 29490-19-5) was obtained from Tokyo Kasei Kogyou Co. Ltd. (Tokyo, Japan). Further details are shown in a previous paper.[9] A 0.01 mol/L (M) MTT solution was prepared as follows: MTT of 0.132 g was dissolved in 20 mL of 2 M ammonia-2 M ammonium chloride buffer solution (pH 9.0), and diluted to 100 mL with water. The 0.01 M MTT was prepared fleshly each week and preserved in a refrigerator (about 4°C). All metal standard solutions of 1000 µg/mL (ppm) for atomic absorption spectrometry were obtained from Kanto Chemical Co. Inc. (Tokyo, Japan). The Hg standard of 1000 ppm contained 0.1 M HNO_3. The other Hg solutions were prepared by dilution of the above 1000 ppm solution with 0.1 M HNO_3. Methanol was distilled and filtered through a membrane filter (pore size, 0.45 μm). A hydrochloric acid-sodium acetate solution of pH 2.0 was prepared with 1 M hydrochloric acid and 1 M sodium acetate. Acetate buffer solutions (pH 3.0 to 6.0) were prepared with 2 M acetic acid and 2 M sodium acetate. Ammonia-ammonium chloride (NH_3-NH_4Cl) buffer solutions (pH 8.0–11.0) were prepared with 2 M ammonia and 2 M ammonium chloride solutions. River water was collected from the Asano River (Kanazawa, Japan). The river water was filtered through a membrane filter (0.45 μm pore size) and used as the river water sample for HPLC analysis.

Recommended Extraction Procedure and HPLC Conditions

Transfer 4 mL of sample solution and 1 mL of 0.1 M HNO_3 into a 10 mL centrifuge tube with a glass stopper. For calibration curves, transfer an Hg standard solution (0.1 M HNO_3) and 0.1 M HNO_3 (total volume 1 mL) and 4 mL of water into a centrifuge tube. Add 2 mL of 2 M acetate buffer (pH 4.5). After mixing by hand, add 200 μL of 0.01 M MTT and 500 μL of benzene into the tube. Shake the contents for 5 min by an auto shaker. After standing for 5 min, collect the organic layer. Determine the Hg concentration as Hg-MTT chelate in the extract under the following HPLC conditions.

Column: Cosmosil 5 C_{18}-MS-II (250 × 4.6 mm ID), column temp.: 40°C, eluent: methanol/water/0.01 M MTT (72:26:2, v/v), flow rate: 1.0 mL/min, injection volume of organic layer: 5 μL, detection wavelength: 270 nm.

Extraction Conditions

In order to extract Hg ion as MTT chelate quantitatively, extraction conditions such us volume of 0.01 M MTT, extraction pH, and shaking time were investigated with 5 ppm Hg standard (5 mL).

The effect of the volume of 0.01 M MTT on extraction of the Hg-MTT chelate was examined over the range of 10 to 1000 μL; pH of buffer solution, 5.0; shaking time, 20 minutes.

Extraction pH was investigated with various acidic buffer solutions according to the recommended procedure; 0.01 M MTT, 200 μL; shaking time, 20 minutes.

Shaking time was also investigated over the range of 1 to 60 minutes; 0.01 M MTT, 200 μL; pH of buffer solution, 4.5.

Effects of Foreign Ions

The effects of foreign ions on the determination of the 0.4 ppm Hg ion were tested with 57 metal ions. Each foreign ion of *x* μL, (600-*x*) μL of 0.1 M HNO_3, and 400 μL of 5 ppm Hg standard, and 4 mL of water were placed into a centrifuge tube; Hg concentration: 0.4 ppm. For 200 ppm foreign ion, 1 mL of 1000 ppm foreign ion, 400 μL of 5 ppm Hg standard, and 3.6 mL of water were placed. The concentration of the Hg ion in the 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 (0.4 ppm) containing no foreign metal ions. The tolerance limit 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 Tests with a River Water Sample

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

RESULTS AND DISCUSSION

Extraction Conditions

The effect of the volume of 0.01 M MTT on extraction of the Hg-MTT chelate is shown in Figure 1. Because peak areas of the Hg chelates were

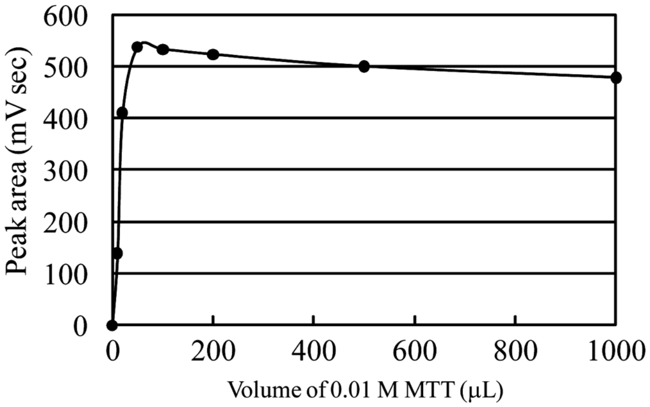

FIGURE 1 Effect of 0.01 M MTT volume on extraction of Hg-MTT chelate.

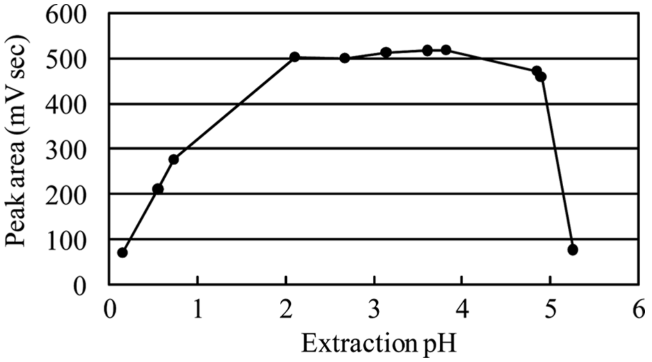

FIGURE 2 Effect of pH on peak area of Hg-MTT chelate.

approximately constant in the range of 50 to 1000 μL, the volume of 0.01 M MTT was set at 200 μL.

The effect of pH on extraction of the Hg chelate is shown in Figure 2. Maximum and constant peak areas were obtained over the pH range of 2.1 to 3.8 (buffer solution pH: 3.0 to 5.0). Thus, 2 M acetate buffer solution of pH 4.5 was employed. When the buffer solution of pH 4.5 was used, pH of the aqueous layer was 3.6.

Shaking time was investigated according to the recommended procedure; 0.01 M MTT, 200 μL; pH of buffer solution, 4.5. The Hg ion was quantitatively extracted by shaking for 2–60 minutes. Subsequently, 5 min was selected as the shaking time.

HPLC Conditions

The effect of MTT concentration in eluent on the peak area of the Hg-MTT chelate was investigated, and the result is shown in Figure 3. Because maximum peak area was obtained at 200 μM MTT, a mixture of methanol/water/0.01 M MTT (72:26:2, v/v) was employed as the eluent.

Figure 4 shows the effect of detection wavelength on peak area of the Hg-MTT chelate. Since maximum peak area was obtained at 270 nm, detection wavelength was set at 270 nm for sensitive detection of Hg chelate.

Typical chromatograms of the Hg-MTT chelates are shown in Figure 5. The retention time of Hg chelate was 3.4 min, while a blank peak appeared at 6.3 min. Therefore, 7 or 8 min was required for the HPLC analysis.

Calibration Curves, Repeatability, and Detection Limit

Calibration curves for the Hg(II) ions were prepared with Hg standards of varying concentrations by the recommended procedure. The correlation

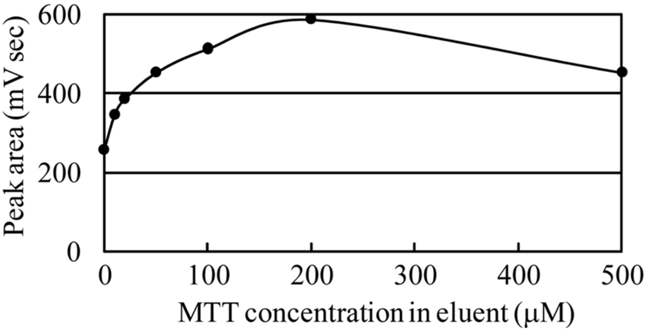

FIGURE 3 Effect of MTT concentration in eluent on peak area of Hg-MTT chelate. Concentration of Hg ion in 5 mL solution was 5 ppm. Eluent: methanol/water/0.01 M MTT (72:28-*x*:*x*, v/v). Where *x* were 0, 0.1, 0.2, 0.5, 1, 2, and 5. The concentrations of MTT in eluents were 0, 10, 20, 50, 100, 200, and 500 μM, respectively.

coefficients of the calibration curves obtained with 5 mL Hg standards were more than 0.999 in the range of 0.1 ppm to 10 ppm. Repeatabilities of the peak areas for 5 and 0.5 ppm Hg standards were also investigated. Relative standard deviations of the peak areas (N = 6) were less than 1%. More details are shown in Table 1. The detection limit of the Hg ion in 5 mL solution was 0.05 ppm by a signal to noise ratio of 3.

Effects of Foreign Ions

The effects of 57 foreign ions on the determination of the 0.4 ppm Hg(II) ion (5 mL) were investigated. It is found that 40 metal ions did not interfere at 500 times (200 ppm) or more the concentration of the Hg ion. The Ag(I) and Cr(VI) ion of 10 ppm, 0.1 ppm of Au(III), Cu(II), and Pt(IV) interfered with the determination of the 0.4 ppm Hg ion. More details are shown in Table 2.

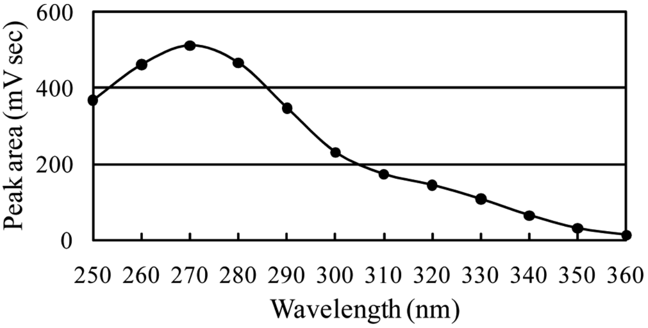

FIGURE 4 Effect of detection wavelength on peak area of Hg-MTT chelate.

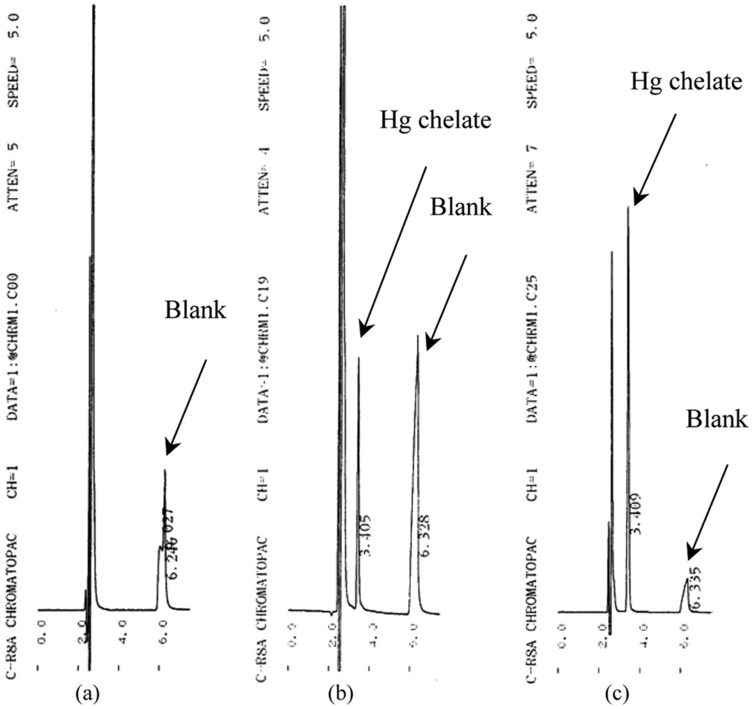

FIGURE 5 Typical chromatograms of Hg-MTT chelates. Concentration of Hg in 5 mL solution: (a) 0 ppm; (b) 0.5 ppm; (c) 5 ppm. Attenuation of integrator: (a) 5: (b) 4: (c) 7.

TABLE 1 Calibration Curves for Hg(II) Ion and Repeatability of Peak Areas

Concentration range	Equation of line	Correlation coefficient	Measuring point (ppm)
1–10 ppm	$y = 121.52x - 0.644$	0.9999	0, 1, 2, 4, 6, 8, 10
0.1–1 ppm	$y = 111.1x - 3.7321$	0.9996	0.1, 0.2, 0.4, 0.6, 0.8, 1.0

5 ppm		0.5 ppm	
No. of run	Peak area	No. of run	Peak area
1	605.994	1	49.872
2	599.856	2	49.926
3	605.747	3	49.499
4	603.773	4	48.908
5	605.099	5	49.486
6	600.243	6	48.935
Average	603.452	Average	49.413
SD[a]	2.749	SD[a]	0.401
RSD[b] (%)	0.5	RSD[b] (%)	0.8

y: peak area (mV sec), x: concentration of Hg ion (ppm).

[a] Standard deviation.

[b] Relative standard deviation.

TABLE 2 Effects of Foreign Metal Ions on Determination of 0.4 ppm Hg(II) Ion

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

Note: The tolerance limit of the foreign ion concentration was taken as the value that caused an error of less than 10% in the recovery of Hg(II) ion (0.4 ppm).

Recovery Tests of Hg Ion with River Water Sample

The results of recovery tests for 5 and 0.5 ppm Hg ions are summarized in Table 3. The recoveries of 5 and 0.5 ppm Hg ions were 100.0 ± 0.8% and 99.3 ± 0.9%, respectively. The correlation coefficients of calibration curves were more than 0.999. More details are shown in Table 3. Recoveries obtained by the HPLC method on other days were 99.8 ± 0.5% for 5 ppm

TABLE 3 Recovery Tests for Hg Ion with a River Water Sample

No. of run	Added (ppm)	Found (ppm)	Recovery (%)	Equation of calibration curve	Correlation coefficient
5 ppm					
1	5.00	5.03	100.5	$y = 111.16x - 8.4324$	0.9999
2	5.00	5.06	101.2		
3	5.00	5.00	100.0		
4	5.00	4.94	98.7		
5	5.00	5.00	100.0		
6	5.00	4.99	99.7		
Av.		5.00	100.0		
SD		0.04	0.8		
RSD		0.8	0.8		
0.5 ppm					
1	0.500	0.503	100.7	$y = 108.46x - 6.0149$	0.9996
2	0.500	0.492	98.3		
3	0.500	0.496	99.3		
4	0.500	0.493	98.7		
5	0.500	0.495	99.1		
6	0.500	0.500	100.1		
Av.		0.497	99.3		
SD		0.004	0.9		
RSD		0.9	0.9		

Hg and 99.4 ± 1.3% for 0.5 ppm Hg (N = 6). The high recoveries indicated that the ions in river water did not interfere with the proposed HPLC method for the Hg ion of ppm level.

CONCLUSION

The MTT was found to be a chelating reagent for ppm levels of Hg(II) ion. The proposed extraction and HPLC procedure was simple and easy, and the HPLC apparatus used was the most popular HPLC apparatus equipped with a photometric detector. The HPLC method was applied to the determination of the ppm levels of Hg ion in spiked river water sample with precise results.

REFERENCES

1. Cassidy, R.M. The separation and determination of metal species by modern liquid chromatography. Trace Anal. **1981**, *1*, 122–192.
2. Timerbaev, A.R.; Petrukhin, O.M.; Zolotov, Y.A. Analytical application of liquid chromatography of metal chelates. Fresenius Z. Anal. Chem. **1987**, *327*, 87–101.
3. Sarzanini, C. High performance liquid chromatography: trace metal determination and speciation. Adv. Chromatogr. **2001**, *41*, 249–310.
4. Cecchi, T. Application of ion pairing chromatography to the analysis of inorganic analytes: review. J. Liq. Chromatogr. & Rel. Technol. **2007**, *30*, 1205–1225.
5. Ichinoki, S.; Yamazaki, M. Simultaneous determination of nickel, lead, zinc, and copper in citrus leaves and rice flour by liquid chromatography with hexamethylenedithiocarbamates extraction. Anal. Chem. **1985**, *57*, 2219–2222.
6. Ichinoki, S.; Hongo, N.; Yamazaki, M. Multielement analysis by high-performance liquid chromatography following solvent extraction with acetylacetone. Anal. Chem. **1988**, *60*, 2099–2104.
7. Ichinoki, S.; Iwase, H.; Arakawa, F.; Hirano, K.; Fujii, Y. Selective determination of tin(II) ion in water by solvent extraction with salicylideneamino-2-thiophenol followed by reversed-phase high-performance liquid chromatography with photometric detection. J. Liq. Chromatogr. & Rel. Technol. **2003**, *26*, 3129–3139.
8. Ichinoki, S.; Otani, S.; Fujii, Y. Selective determination of palladium ion in river water by solvent extraction with 5-chloro-2-mercaptobenzothiazole followed by reversed-phase HPLC. J. Liq. Chromatogr. & Rel. Technol. **2006**, *29*, 2457–2469.
9. Ichinoki, S.; Mikami, T.; Fujii, Y. Determination of palladium ion in river water by solvent extraction with 5-methyl-1,3,4-thiadiazole-2-thiol followed by reversed phase HPLC. J. Liq. Chromatogr. & Rel. Technol. **2008**, *31*, 2988–3000.