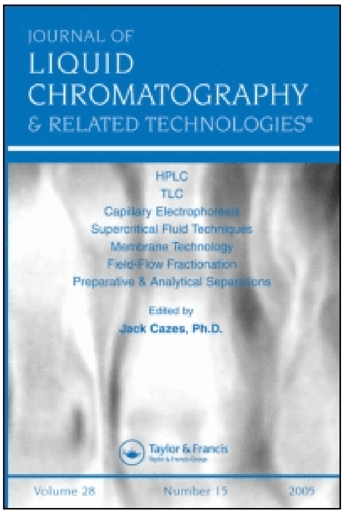

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# Determination of Copper Ion in River Water by Solvent Extraction with 2-Mercaptobenzothiazole Followed by Reversed Phase HPLC

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**Abstract:** A determination method for Copper (Cu) ion in river water has been developed by reversed phase HPLC combined with solvent extraction. The Cu(II) ion was quantitatively extracted into benzene from a weak acidic solution as 2-mercaptobenzothiazole (MBT) chelate. The extracted Cu-MBT chelate was then separated on a $C_{18}$ column with an eluent of methanol/water (88:12, v/v) and detected at 275 nm. The correlation coefficients of the calibration curves obtained with 5 mL Cu standards were more than 0.999 over the range of 0.01 to 10 μg/mL (ppm). The detection limit of the Cu ion in 5 mL water was 3 ng/mL, which corresponded to 3 times the standard deviation ($N = 8$) of the blank peak area. Effects of foreign ions on the method were investigated with 0.4 ppm Cu standard and 57 metal ions. Almost none of the ions interfered except for Au(III), Pt(IV), V(V), and Zr(IV) ions. Recoveries with a spiked river water sample for 5 and 0.5 ppm Cu ion were $99.9 \pm 0.5\%$ and $99.8 \pm 0.8\%$, respectively ($N = 6$).

**Keywords:** 2-Mercaptobenzothiazole, Copper (Cu) ion, High performance liquid chromatography (HPLC), Photometric detection, River water, Solvent extraction

## INTRODUCTION

Atomic absorption spectrometry (AAS) and inductively coupled plasma-atomic emission spectrometry (ICP-AES) are routinely used for metal

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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. 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 a very popular and not as expensive 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 as metal chelates[6–10] combined with solvent extraction and spectrophotometric detection.

The MBT has been used as a chelating reagent. For example, inorganic mercury and organomercury were determined as MBT chelates by reversed phase HPLC with UV detection.[11] Li and Hu also used the MBT for determination of Zn, Cu, and Hg ions by normal phase HPLC.[12] They also reported the separation of Ag(Zn or Al), Cu, and Hg ions as MBT chelates by reversed phase HPLC.

We found that the MBT reacted with a Cu(II) ion, and the Cu-MBT chelate was extracted into benzene from a weak acidic solution. In addition, the Cu chelate was stable in benzene and an eluent of methanol/water in the absence of MBT.

In this paper, extraction and HPLC conditions have been optimized for sensitive determination of the Cu ion. In addition, the linearity of calibration curves and the detection limit of the Cu ion were investigated. Effects of foreign ions on the determination of the Cu ion were also investigated with 57 metal ions. The HPLC method was applied to determination of the Cu ion in a river water sample.

## EXPERIMENTAL

### Instrumentation

The HPLC system consisted of a Jasco PU-2080*i* 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 UV-2070 Plus photometric detector, a Cosmosil 5 $C_{18}$-MS-II stainless steel column (100 × 4.6 mm ID, Nacalai Tesque, Kyoto, Japan), a Shimadzu Chromatopac C-R6A integrator (Shimadzu Co., Kyoto, Japan). A longer Cosmosil 5 $C_{18}$-MS-II column (150 × 4.6 mm ID) was used for a screening test. An MS-E10 R microsyringe (10 μL) with Ni-Ti alloy plunger was used for sample injection (Ito. Co. Ltd., Fuji, Japan). A SR-2 DS auto-shaker (Taitec Co., Koshigaya, Japan) was used for solvent extraction. 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 MBT ($C_7H_5NS_2 = 167.26$) was obtained from Tokyo Kasei Kogyou Co., Ltd. (Tokyo, Japan). Further details are shown in Figure 1. The 0.01 mol/L (M) MBT solution was prepared as follows: MBT of 0.167 g was dissolved in 20 mL of 2 M ammonia–ammonium chloride buffer solution (pH 10.0) and diluted to 100 mL with water. The 0.01 M MBT was prepared freshly every week and preserved in a refrigerator (about 4°C). All metal standard solutions of 1000 ppm were obtained from Kanto Chemical Co., Inc. (Tokyo, Japan). The Cu standard of 1000 ppm contained 0.1 M $HNO_3$. The other Cu solutions were prepared by dilution of the above solution with 0.1 M $HNO_3$. Methanol was distilled and filtered through a membrane filter (pore size, 0.45 μm). Acetate buffer solutions (pH 3.0–6.0) were prepared with 2 M acetic acid and 2 M sodium acetate. A hydrochloric acid-sodium acetate buffer solution of pH 2.0 was prepared with 1 M hydrochloric acid and 1 M sodium acetate. River water was collected from the Tomuro River (a branch of 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 a Cu 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

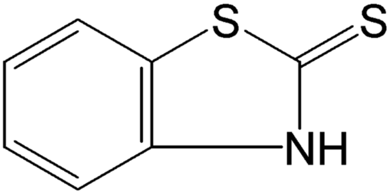

***Figure 1.*** 2-mercaptobenzothiazole (MBT). $C_7H_5NS_2 = 167.26$, CAS No. 149-30-4.

of pH 5.0. After mixing by hand, add 300 μL of 0.01 M MBT and 500 μL of benzene into the tube. Shake the contents for 10 min by an auto shaker. After standing for 10 min, collect the organic layer. Determine the Cu concentration as Cu-MBT chelate in the extract under the following HPLC conditions.

Column: Cosmosil 5$C_{18}$-MS-II (100 × 4.6 mm ID), column temperature: ambient, eluent: methanol/water (88:12, v/v), flow rate: 1.0 mL/min, injection volume of organic layer: 5 μL, detection wavelength: 275 nm.

## Screening Test for 58 Metal Ions

To a 10 mL centrifuge tube, 25 μL of each metal solution of 1000 ppm, 1 mL of 0.1 M $HNO_3$, 4 mL of water, 2 mL of 2 M acetate buffer (pH 4.5), 500 μL of 0.01 M MBT, and 500 μL of benzene were added. After shaking for 20 min, the organic layer was separated and used for HPLC analysis. The HPLC conditions used were as follows: column, Cosmosil 5 $C_{18}$-MS-II (150 × 4.6 mm ID); eluent, methanol/water/0.01 M MBT (80:18:2, v/v); detection, 270 nm. The other conditions were the same as the recommended HPLC conditions. A blank test was also conducted, and the chromatographic peaks were compared.

## Effects of Foreign Ions

The effects of foreign ions on the determination of the Cu(II) ion were tested with 57 metal ions. To a centrifuge tube, $x$ μL of each foreign ion, $(600-x)$ μL of 0.1 M $HNO_3$, and 400 μL of 5 ppm Cu standard, and 4 mL of water were added. Thus, the Cu concentration in the 5 mL solution was 0.4 ppm. For 200 ppm foreign ion, 1 mL of 1000 ppm solution and 400 μL of 5 ppm Cu standard were added, and diluted to 5 mL with water. The concentration of the Cu ion in the solution was determined by the recommended procedure. The recovery percentage was calculated from the peak area of the Cu chelate and that of the Cu 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 Cu ion.

## Recovery Test with a River Water Sample

Concentration of the Cu ion in collected river water was lower than the detection limit of the presented method. Thus, the Cu ions were added to the river water, and recovery tests were carried out. To a centrifuge tube, 4.0 mL of a river water sample, 500 µL of Cu standard (50 and 5 ppm), and 500 µL of 0.1 M $HNO_3$ were added. The Cu concentrations in these solutions (5 and 0.5 ppm) were determined according to the recommended procedure, and the recovery percentages were calculated.

## RESULTS AND DISCUSSION

### HPLC Conditions

The screening test for 58 metal ions indicated that Cu, gold (Au), mercury (Hg) and palladium (Pd) ions were extracted into benzene as MBT chelates from weak acidic solution. Though, the Pd ion gave yellow colored chelates, no chromatographic peak appeared under the conditions used. The Cu, Au, and Hg ions gave chromatographic peaks. The Hg chelate peak appeared as a tailing peak before those of Cu and Au. The peaks of Cu and Au chelates could not be separated. Because Cu ion is a popular metal ion, the determination method for the Cu ion was investigated.

MBT was initially added to the eluent of methanol/water as a stabilizer for the metal-MBT chelates. However, the Cu-MBT chelate was found stable in the absence of the MBT in the eluent. Considering the reasonable separation and retention time, the column length was changed to 10 cm, and the eluent of methanol/water (88:12, v/v) was employed.

Effect of the detection wavelength on the peak height of the Cu-MBT chelate is shown in Figure 2. Because maximum peak height was obtained, the detection wavelength was set at 275 nm.

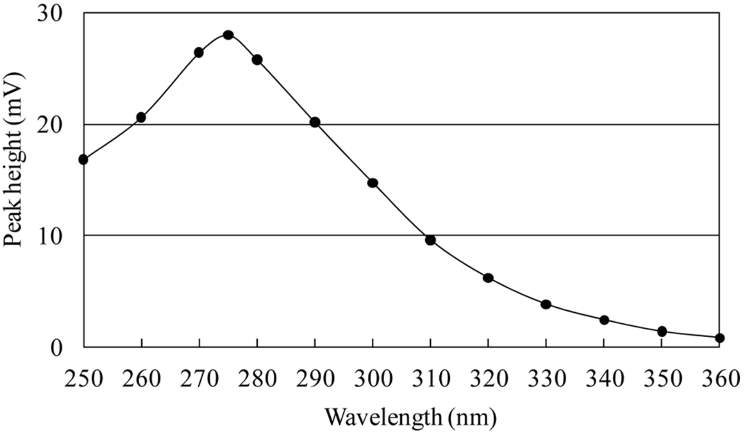

***Figure 2.*** Effect of detection wavelength on peak height of Cu-MBT chelate.

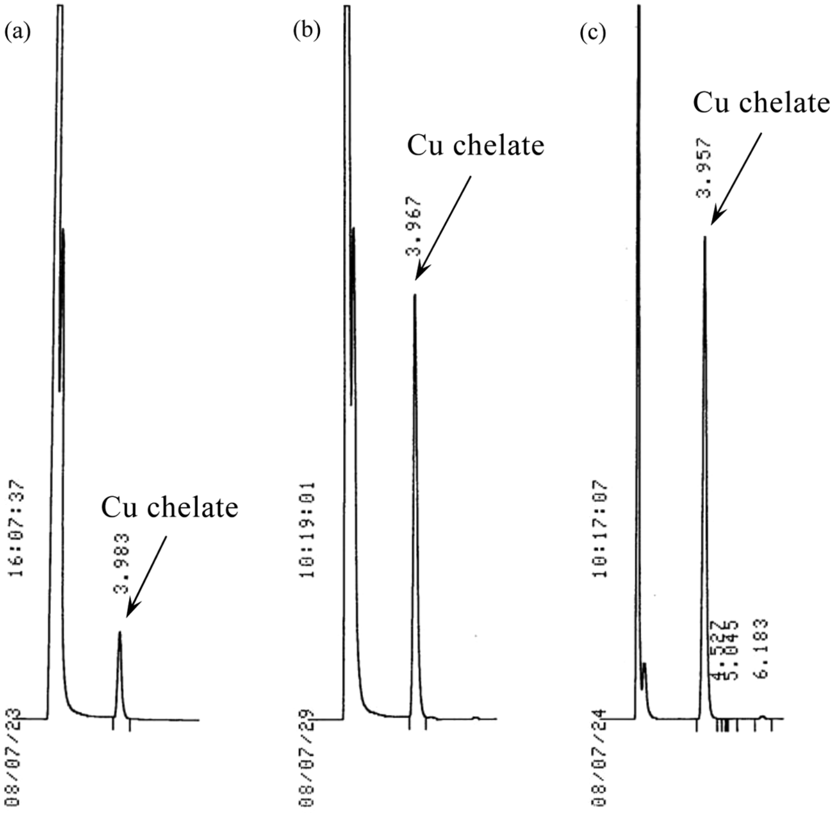

***Figure 3.*** Typical chromatograms of Cu-MBT chelates. Concentration of Cu ion in 5 mL solution: (a), 0.04 ppm; (b), 0.4 ppm; (c), 4 ppm. Attenuation of integrator: (a), 4; (b), 4; (c), 8.

The chromatograms of the Cu-MBT chelates obtained with various Cu concentrations are shown in Figure 3.

## Extraction Conditions

For quantitative extraction of the Cu(II) ion, the volume of 0.01 M MBT, extraction pH, and shaking time were investigated with 5 ppm Cu standard.

Effect of the volume of 0.01 M MBT on extraction of the Cu-MBT chelate was examined over the range of 10 to 1000 μL. Figure 4 shows that constant peak areas were obtained in the range of 200 to 1000 μL. On the other hand, a blank peak appeared at the same retention time

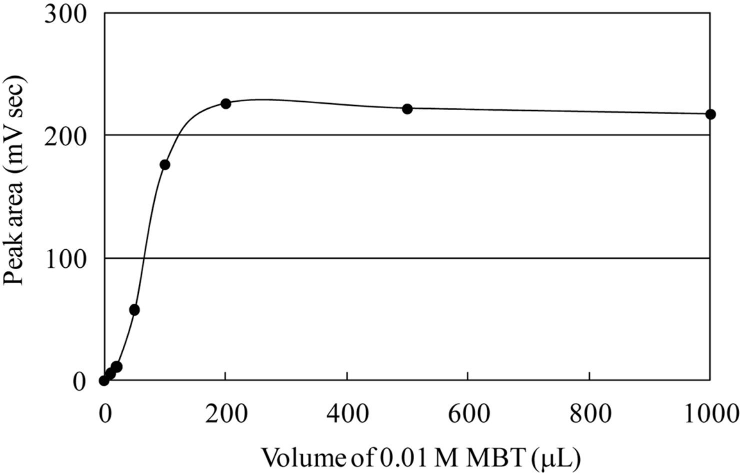

***Figure 4.*** Effect of 0.01 M MBT volume on peak area of Cu-MBT chelate.

of the Cu chelate, and the blank peak area increased with an increase in the volume of 0.01 M MBT. Considering the above results, the volume of 0.01 M MBT was set at 300 μL.

Extraction pH was investigated with acidic buffer solutions according to the recommended procedure. The effect of pH on extraction of the Cu-MBT chelate is shown in Figure 5. As constant peak areas were obtained over the pH range of 3.9 to 5.7, an acetate buffer solution of pH 5.0 was used in the recommended extraction procedure. When the buffer solution of pH 5.0 was used, the pH of the aqueous layer was 4.9.

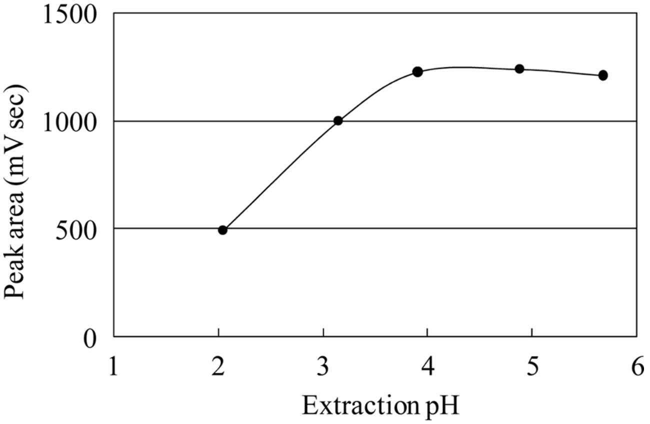

***Figure 5.*** Effect of extraction pH on peak area of Cu-MBT chelate.

Under the recommended extraction conditions, the Cu ion was quantitatively extracted by shaking for 2–60 minutes. Subsequently, 10 min was selected as the shaking time.

## Calibration Curves, Repeatability, and Detection Limit

Calibration curves for the Cu(II) ions were prepared with the Cu standards of varying concentrations by the recommended procedure. The correlation coefficients of the calibration curves obtained with 5 mL Cu standards were more than 0.999 over the range of 0.01 to 10 ppm. Repeatability of the peak areas for 5 and 0.5 ppm Cu 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 Cu ion in 5 mL water was 3 ng/mL (ppb), which corresponded to 3 times the standard deviation ($N = 8$) of the blank peak area.

***Table 1.*** Calibration curves for Cu(II) ion and repeatabilities of peak areas calibration curves for Cu ion

| Concentration range | Equation of line | Correlation coefficient | Measuring point (ppm) |
|---|---|---|---|
| 0.01–0.1 ppm | $y = 206.75x + 12.055$ | 0.9991 | 0, 0.01, 0.02, 0.04, 0.06, 0.08, 0.1 |
| 0.1–1 ppm | $y = 212.57x + 11.519$ | 0.9999 | 0, 0.1, 0.2, 0.4, 0.6, 0.8, 1 |
| 1–10 ppm | $y = 214.78x + 12.901$ | 0.9999 | 0, 1, 2, 4, 6, 8, 10 |

| 5 ppm Cu standard | | | 0.5 ppm Cu standard | | |
|---|---|---|---|---|---|
| No. of run | Peak area | Peak area[c] | No. of run | Peak area | Peak area[c] |
| 1 | 1107.452 | 1098.581 | 1 | 119.010 | 117.946 |
| 2 | 1115.056 | 1096.399 | 2 | 118.063 | 116.814 |
| 3 | 1094.580 | 1099.960 | 3 | 117.944 | 117.872 |
| 4 | 1104.775 | 1107.814 | 4 | 119.897 | 117.371 |
| 5 | 1101.763 | 1111.163 | 5 | 119.325 | 118.245 |
| 6 | 1112.364 | 1101.301 | 6 | 120.271 | 116.997 |
| Average | 1105.998 | 1102.536 | Average | 119.085 | 117.541 |
| SD[a] | 7.409 | 5.723 | SD[a] | 0.946 | 0.570 |
| RSD[b] (%) | 0.7 | 0.5 | RSD[b] (%) | 0.8 | 0.5 |

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

[a] Standard deviation.

[b] Relative standard deviation.

[c] The results were obtained on other days.

*Table 2.* Effects of foreign metal ions on determination of 0.4 ppm Cu(II) ion

| Tolerance limit | Metal ion |
|---|---|
| 200 ppm | Al(III), As(III), Ba(II), Be(II), Ca(II), Cd(II), Ce(III), Co(II), Cs(I), Dy(III), Er(III), Eu(III), Fe(III), Ga(III), Gd(III), Ge(IV), Ho(III), In(III), K(I), La(III), Lu(III), Mg(II), Mn(II), Mo(VI), Na(I), Nb(V), Nd(III), Ni(II), Pb(II), Pr(III), Rh(III), Sc(III), Se(IV), Si(IV), Sm(III), Sr(II), Ta(V), Tb(III), Te(IV), Tl(I), Tm(III), W(VI), Y(III), Yb(III), Zn(II) |
| 100 ppm | Sb(III), Bi(III), Hg(II) |
| 40 ppm | Sn(II) |
| 20 ppm | Ag(I), Pd(II) |
| 4 ppm | Cr(VI), Ti(IV) |
| 2 ppm | Zr(IV) |
| 1 ppm | V(V) |
| 0.4 ppm | Pt(IV) |
| 0.04 ppm | Au(III) |

*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 Cu ion (0.4 ppm).

## Effects of Foreign Ions

The effects of 57 foreign ions on the determination of 0.4 ppm Cu(II) ion (5 mL) were investigated. Table 2 shows that 45 metal ions did not interfere at 500 times (200 ppm), or more, with the concentration of the Cu ion. An Au(III) ion of 0.1 ppm, 1 ppm Pt(IV), and 2 ppm V(V) interfered with the determination of 0.4 ppm Cu ion. The Au ion was extracted into benzene as MBT chelate, and the peak overlapped with the Cu chelate peak. More details are shown in Table 2.

## Recovery Test of Cu Ion with River Water Sample

The results of the recovery tests for 5 and 0.5 ppm Cu ions are summarized in Table 3. The correlation coefficients of the calibration curves were 0.9999. The recoveries of 5 and 0.5 ppm Cu ions were 99.9 ± 0.5%, and 99.8 ± 0.8%, respectively. The recoveries obtained on other days were 98.6 ± 0.6% for 5 ppm and 99.9 ± 0.9% for 0.5 ppm Cu ions. The high recoveries indicated that the ions in river water did not interfere with the proposed HPLC method for the Cu ion of ppb to ppm level.

***Table 3.*** Recovery tests for Cu 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 | 5.01 | 100.2 | $y = 225.36x - 4.5117$ | 0.9999 |
| 2 | 5.00 | 4.97 | 99.3 | | |
| 3 | 5.00 | 4.98 | 99.5 | | |
| 4 | 5.00 | 4.97 | 99.4 | | |
| 5 | 5.00 | 5.03 | 100.5 | | |
| 6 | 5.00 | 5.02 | 100.4 | | |
| Av. | | 4.99 | 99.9 | | |
| SD | | 0.03 | 0.5 | | |
| RSD | | 0.5 | 0.5 | | |
| 0.5 ppm | | | | | |
| 1 | 0.500 | 0.498 | 99.5 | $y = 219.18x + 7.9698$ | 0.9999 |
| 2 | 0.500 | 0.492 | 98.5 | | |
| 3 | 0.500 | 0.502 | 100.5 | | |
| 4 | 0.500 | 0.498 | 99.7 | | |
| 5 | 0.500 | 0.501 | 100.3 | | |
| 6 | 0.500 | 0.503 | 100.5 | | |
| Av. | | 0.499 | 99.8 | | |
| SD | | 0.004 | 0.8 | | |
| RSD | | 0.8 | 0.8 | | |

## CONCLUSION

The MBT was applied to the HPLC determination of the Cu(II) ion in river water. The HPLC apparatus used is the most popular HPLC apparatus equipped with a $C_{18}$ column and a photometric detector. The proposed extraction and HPLC procedure is simple and easy. The extraction time and HPLC analysis time is 10 and 7 min, respectively. The MBT was found to be a chelating reagent for ppb to ppm levels of Cu(II) ion. The HPLC method was applied to the determination of the Cu ion in river water with precise results.

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