Simultaneous Determination of Gold and Copper by Reversed-Phase High-Performance Liquid Chromatography Using Online Column Enrichment as Their *syn*-Phenyl-2-Pyridylketoximate Chelates

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Abstract

Gold and copper ions react with *syn*-phenyl-2-pyridylketoxime (PPKO) at 40°C to form neutral chelates. Metal–PPKO chelates subsequently become preconcentrated on a minicolumn packed with a divinylbenzene–methacrylate copolymer. By switching the valve, these chelates are separated on the silica-based phenyl column and detected with a photometric detector. These processes occur automatically except for chelation. The results of the chelation, preconcentration, and separation conditions studies are presented. Calibration curves for Au and Cu ions are linear from nanograms per milliliter (parts per billion) to micrograms per milliliter (parts per million) in the original solution. The precision for 0.5-ppm standards of Au and Cu is 2.1 and 0.9 relative standard deviation (%), respectively. The accuracy of the present method is verified for Au and Cu based on the analysis of a standard alloy of the National Institute of Standards and Technology (NIST). The limits of detection for Au and Cu are 16.7 and 0.6 ppb, respectively. The effects of foreign ions on the determination of Au and Cu are discussed.

Introduction

Molecular absorption spectroscopy, atomic absorption spectrophotometry (AAS), inductively coupled plasma (ICP)-atomic emission spectrometry (AES), and ICP-mass spectrometry (MS) are routinely used for the analysis of metal ions. Although multielement simultaneous analysis by molecular absorption spectroscopy and AAS is generally difficult, ICP-AES and ICP-MS make possible the simultaneous determination of multielements (maximum of 40 elements). But with ICP-AES and ICP-MS, the equipment and running cost are quite high. High-performance liquid chromatography (HPLC) is more economical, thus simultaneous determination methods for various metals by HPLC have been reported (1–3). In order for the determination of metal ions quantitatively by HPLC, the metal ions are separated as metal complexes with an appropriate chelating reagent. For chelation, precolumn, on-column, and postcolumn derivatization may be used. Preconcentration is easiest by precolumn derivatization, which includes solvent extraction and column concentration. We have developed multielement simultaneous determination methods by reversed-phase HPLC with photometric detection using dithiocarbamates (4–6) and β-diketones (7,8) as precolumn chelating reagents. The detection sensitivity of AAS and ICP-AES differs considerably according to the metal. In the case of the photometric detection of a metal chelate, detection sensitivity is proportional to the molar absorptivity of the chelate. A more sensitive quantitative analysis is possible by combining precolumn derivatization HPLC with an appropriate preconcentration. Many HPLC methods using manifold preconcentration have been reported, with solvent extraction being used the most. Although solvent extraction is convenient, the complexity of the operation and restrictions on the injection volume and concentration factors is a problem. HPLC equipment is complicated, but by using online column concentration with column switching, the injection of large sample volumes and automation readily becomes possible. Conventional solvent extraction requires the use of hazardous organic solvents such as chloroform and carbon tetrachloride as extractants in contrast with precolumn concentration. *Syn*-phenyl-2-pyridylketoxime (CAS RN:1826-28-4) was used in this study as the chelating reagent. Several metal ions react with this reagent to form metal complexes. Fe, Cu, Au, and Pd were determined quantitatively as PPKO chelates by molecular absorption spectroscopy (9–13), and Au, Co, Cu, Fe, Pd, and V

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ions were noted to form the PPKO chelates that could be extracted with chloroform for preconcentration. The simultaneous determination of these metal ions as PPKO chelates by automatic reversed-phase HPLC using online column concentration was thus conducted.

Experimental

Reagents

All reagents used were of analytical-reagent grade unless otherwise stated. Milli-Q water was used for aqueous solution preparation. The chelating reagent (PPKO) was obtained from Dojindo Laboratories (Kumamoto, Japan) for use as a methanol solution. Metal standard solutions of 1000 µg/mL (parts per million) for AAS were obtained from Wako Pure Chemical Industries (Osaka, Japan). Metal solutions of Au were prepared with $HAuCl_4$ and 1-mol/L HCl and of Cu with $Cu(NO_3)_2$ and 0.1-mol/L HNO_3. Other metal standard solutions were obtained by the dilution of these solutions (1000 ppm) with Milli-Q water and adjusted to pH 1 with hydrochloric acid. Methanol was distilled and filtered through a membrane filter (0.45-µm pore size). All eluents were run at 1.0 mL/min. Eluent compositions were expressed as volume ratios. The acetate buffer solution was prepared with 2-mol/L acetic acid and 2-mol/L sodium acetate solution. A standard reference material Au–Cu wire set (NIST SRM-482) was used to assess the accuracy of the proposed method.

Apparatus

The HPLC system consisted of a Shimadzu (Kyoto, Japan) LC-6A pump (pump A), Shimadzu LC-10A*i* pump (titanium alloy, pump B), Shimadzu SCL-10A system controller, Shimadzu SIL-10A auto injector, Shimadzu SPD-10AV *VP* photometric detector, Shimadzu PCV-12AH six-port valve, Supelguard TPR-100 column packed with a divinylbenzene–methacrylate copolymer (enrichment column, 20- × 4.6-mm i.d.) (Sigma-Aldrich Japan Co., Tokyo, Japan), Cosmosil 5 Ph column (analytical column, 250- × 4.6-mm i.d., stainless steel) (Nacalai Tesque, Kyoto, Japan), Shimadzu C-R4A data processor, and a constant temperature water bath (40°C), as shown in Figure 1. A Plasma-Spec I ICP-AES (Leeman Labs Inc., MA) was provided, as was also a JMS-SX 102A mass spectrometer (Japan Electron Optics Laboratory Co., Ltd., Tokyo Japan).

Separation conditions

Cosmosil 5 C_{18} and Cosmosil 5 Ph were examined as stationary phases for the analytical column (250- × 4.6-mm i.d., stainless steel). Mixtures of methanol, water, buffer solution, and PPKO solution were examined as analytical eluents (eluent A) for use in the separation of metal–PPKO chelates.

The effects of PPKO concentration and acetate buffer solution pH on peak areas of Au and Cu were examined. The eluents were essentially the same as those recommended for HPLC, except for the eluent containing 1% 40-mmol/L PPKO and pH of the buffer solution.

The detection wavelength for PPKO chelates was based on the visible spectra of the chelates.

Enrichment conditions

A buffer solution and then PPKO solution were added to a sample solution containing Au and Cu ions to produce a solution containing metal–PPKO chelates as well as excess PPKO. The PPKO chelates were concentrated in the enrichment column prior to separation by the analytical column. Prior to enrichment, the enrichment column was preconditioned with an enrichment eluent (eluent B). The flow direction of eluent A was also determined.

After conditioning the enrichment column, a portion of the pretreated sample solution (1.5 mL) was injected into the column along with eluent B. Injection of the solution (1.5 mL) required only 1.5 min, but the eluent had to run for several minutes (rinse time) in order to remove excess PPKO and buffer solution. The optimum rinse time as well as conditioning time was found, and the methanol content, PPKO concentration, and eluent pH were determined.

A mixed-metal standard solution of Au and Cu was pretreated by a recommended chelation procedure and injected into the enrichment column. The eluate from the end of the column was collected, and the concentration of metal in the solution was measured by ICP-AES to confirm that the PPKO chelates were completely retained in the column. A blank solution was also used.

Chelation conditions

Metal ions that formed PPKO chelates were screened at various pH levels. Preliminary experiments indicated that Au, Co, Cu, Fe, Pd, and V were extracted into the chloroform phase as colored metal chelates. Extraction of the metal ions was exam-
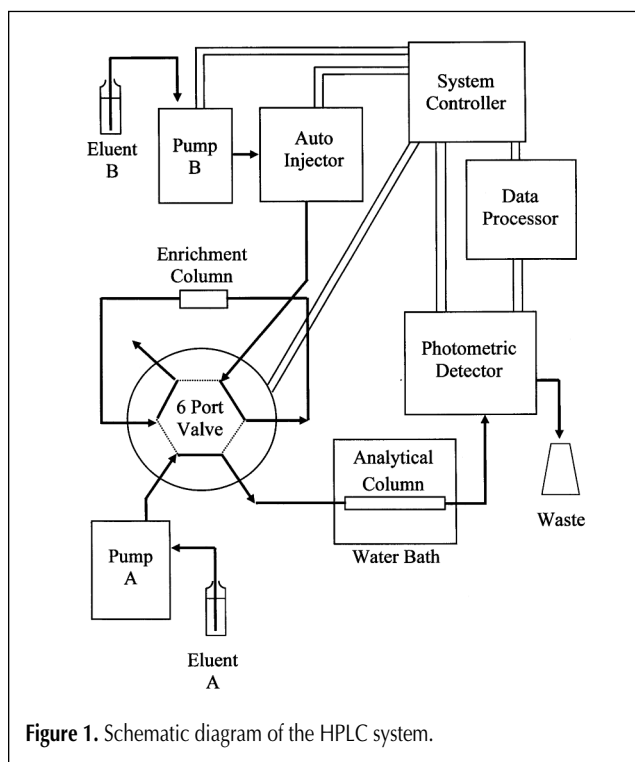

Figure 1. Schematic diagram of the HPLC system.

ined from pH 2.5 to 6.5. The effects of pH on PPKO chelate formation were examined for Au, Co, Cu, Fe, Pd, and V. A 0.5-mL buffer solution at various pH levels and 0.5 mL of a 40-mmol/L PPKO methanol solution was added to 2 mL of a 1-ppm metal solution, and the mixed solution was left overnight. PPKO chelates in solution were separated by recommended HPLC, except the eluent contained 1% 40-mmol/L PPKO, and the peak areas were plotted against pH.

For quantitative chelation, the optimum concentration of PPKO solution was found. A 0.5-mL buffer solution (pH 4) and 0.5-mL PPKO solution was added to a 2-mL standard solution of Au and Cu. PPKO was varied from 0.1 to 100 mmol/L. After standing overnight, peak areas of metal–PPKO chelates were measured. The reaction time for quantitative chelation was determined at room temperature and 40°C.

Chelate compositions of Au and Cu

These parameters were found by the continuous variation method (Job's method). Metal (0.1 mmol/L) was reacted with PPKO in the presence of the acetate buffer (pH 4), and the quantities of the chelates were found by molecular absorption spectroscopy.

For MS analysis, an Au-PPKO chelate was prepared by reacting 1.25 mmol Au (25 mL of a 50-mmol/L NaAuCl$_4$ aqueous solution) with 2.5 mmol PPKO (25 mL of a 100-mmol/L PPKO methanol solution) at pH 4 (10 mL of a 2-mol/L acetate buffer solution). The chelate (precipitate) thus obtained was washed with hot water and 50% methanol, dried, and its melting point and mass spectrum were measured by fast atom bombardment (FAB) (the matrix being nitrobenzyl alcohol). No Cu chelate was precipitated by a similar procedure.

Recommended method for chelation

A sample or standard solution (2 mL) was placed in a 4-mL sample vial. To the sample vial, 0.5 mL of a 2-mol/L acetate buffer solution (pH 4) was added. After mixing the solution, 0.5 mL of a 40-mmol/L PPKO solution was added. The solution was then heated for 40 min at 40°C. After cooling to room temperature over 40 min, the vial was placed in the auto injector.

Recommended HPLC conditions

After conditioning the enrichment column for 3 min with eluent B (methanol–water–acetate buffer solution, pH 4, 40-mmol/L PPKO, 25:65:5:5) (Figure 1), 1.5 mL of a pretreated metal solution was injected onto the enrichment column. After 7 min, the valve was switched and the concentrated chelates flushed from the enrichment column to the analytical column with eluent A (methanol–water–2-mol/L acetic acid–2-mol/L acetate buffer solution, pH 4, 40-mmol/L PPKO, 54:36:2.5:2.5:5). Data collection began at this time. The valve was switched again 3 min before the end of the run in order to allow eluent B to condition the enrichment column for the next run.

Calibration curves and precision

Optimum analytical conditions were based on the results previously mentioned. The peak area of each metal chelate was measured and plotted against metal ion concentration.

The straight-line range and correlation coefficient of the calibration curves were computed.

Eight standard solutions of 0.5-ppm Au and Cu were analyzed by automatic HPLC after chelation at pH 4, and mean peak areas, standard deviation, and relative standard deviation (RSD) were calculated.

Detection limit

The detection limit of Cu was obtained as the concentration corresponding with 3 times the standard deviation of the blank peak area. The detection limit of Au was found as the concentration corresponding with 3 times the peak height of the noise near the retention time of the Au chelate peak.

Effects of foreign ions on Au and Cu determination

Foreign ions were added to standard solutions containing 50-ppb Au and Cu. Peak areas with and without this addition were compared. Interference appeared to occur when the peak area of the Au or Cu chelate changed more than 5%.

Accuracy of Au and Cu determination

A standard alloy of Au–Cu wire (NIST SRM-482) was pretreated with chloroform to remove the vinyl coating, and a few milligrams were accurately weighed after drying and dissolved in 200 µL aqua regia. The solution was diluted to an appropriate concentration, and 1.5 mL of it was analyzed by the present method. The results were compared with those by the National Institute of Standards and Technology (NIST).

Results and Discussion

Column for metal–PPKO chelate separation

Cosmosil 5 C$_{18}$ and Cosmosil 5 Ph columns were examined for their capability to separate metal–PPKO chelates. PPKO chelates of Au, Co, Cu, Pd, and V were not retained significantly in the Cosmosil 5 C$_{18}$ column, but were moderately in the Cosmosil 5 Ph column (Figure 2), which was thus used in subsequent experiments. The reason why Co gave two peaks was not clarified.

Chelation conditions

Optimal reaction pH was found using various buffer solutions in a manner similar to that recommended for chelation and HPLC. Peak areas of Au and Cu chelates were constant at a pH of 3.4 to 5.1, thus a pH of 4 was used for the simultaneous determinations of Au and Cu ions. The effects of PPKO concentration on chelation were studied. The peak areas of Au and Cu were constant at 10 to 60 mmol/L, and consequently, 40 mmol/L was taken as the optimal PPKO concentration for chelation. The time for chelation was determined. Three hours were necessary to attain constant peak areas of metal chelates at room temperature, but this was possible by reaction for 20 to 60 min at 40°C. Therefore, 40 min and 40°C served as the optimal reaction time and temperature, respectively.

Separation of Au– and Cu–PPKO chelates

Without the addition of PPKO to both eluents, the Cu chelate peak disappeared from the chromatogram, indicating the eluents required PPKO to prevent on-column dissociation
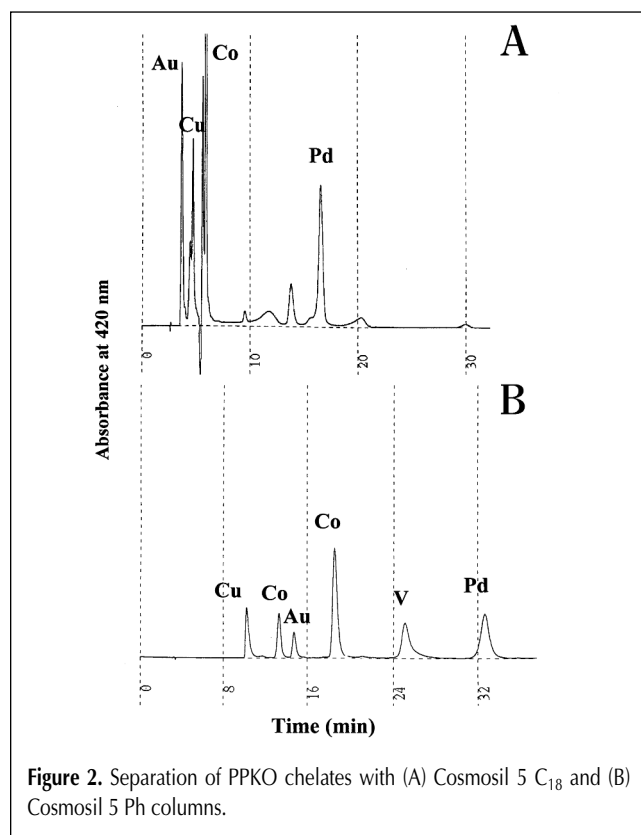

Figure 2. Separation of PPKO chelates with (A) Cosmosil 5 C_{18} and (B) Cosmosil 5 Ph columns.
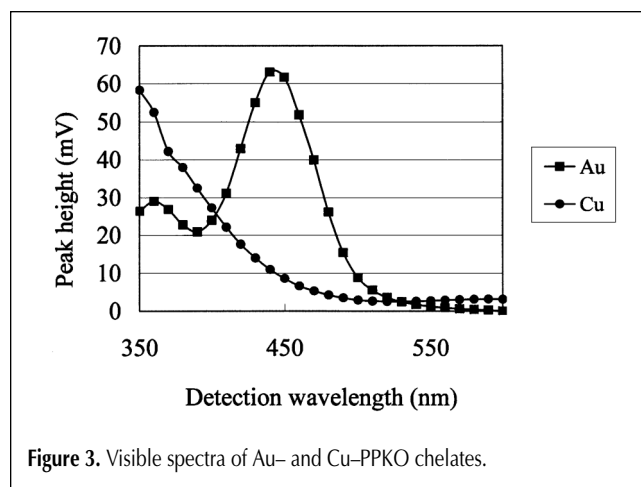

Figure 3. Visible spectra of Au– and Cu–PPKO chelates.

Table I. Calibration Curves, Detection Limit, Lower Determination Limit, and Reproducibility for Au and Cu

Metal ion	Linear range*	Detection limit[†]	Lower determination limit[†]	RSD (%)[‡]
Au	20 ppb–10 ppm	16.7 ppb (100 ppb)	20 ppb (100 ppb)	2.1
Cu	2 ppb–10 ppm	0.6 ppb (2 ppb)	2 ppb (2 ppb)	0.9

* The correlation coefficient was more than 0.999.
[†] The value in the parenthesis was obtained by ICP.
[‡] The concentration of metal was 0.5 ppm ($n = 8$).

of the PPKO chelates. The effects of PPKO concentration on peak areas of Au and Cu chelates were examined at 0.01 to 5 mmol/L. The peak area in either case was found to be constant at 0.1 to 5 mmol/L. This concentration was thus made to be 2 mmol/L in all subsequent experiments.

The effects of eluent pH on Au and Cu chelate peak areas were examined with 2-mol/L acetic acid, acetate buffer solutions with pH 3 to 6, and mixtures. When the enrichment eluent contained a buffer solution of pH 4 (actual eluent pH was 4.5), a constant peak area was obtained at an actual analytical eluent of pH 3.6 to 7.0 (measured by a pH meter). An acetate buffer solution of pH 4 was thus used as a component of the enrichment eluent, and a 2-mol/L acetic acid and acetate buffer mixture with pH 4 (1+1) was used as the analytical eluent (actual pH was 4.8).

The detection wavelength used was 420 nm (shown in Figure 3).

Enrichment

The flow direction of the analytical eluent in the enrichment column was the reverse of that of the enrichment eluent, because this resulted in sharper peaks for PPKO chelates.

The eluent containing 25% to 45% methanol gave the maximum and constant peak areas for the Au and Cu chelates. The optimal methanol content in the enrichment eluent was thus set at 30%.

Conditioning and rinse times were 3 min and 7 min, respectively, in consideration of the constant and maximum peak areas of Au and Cu chelates.

No metal could be found by ICP-AES in the effluents from the enrichment column compared with the blank solution, indicating that metal ions were completely retained in the enrichment column as PPKO chelates.

Compositions of Au and Cu chelates

Job's method indicated PPKO mole ratios of 1:2 for Au–PPKO and 1:1 for Cu–PPKO. It is well-known that FAB–MS with nitrobenzyl alcohol gives an $(M+H)^+$ ion and sometimes an M^+ ion by the type of the sample. The mass spectrum of the Au–PPKO chelate (mp 209°C) gave low fragment peaks of m/z 591, 592, and 593, but there was no clear molecular ion peak. The molecular weight of PPKO is 198.2 and the mass of Au 197.0. If the Au(III) ion is chelated with two deprotonated PPKOs and one other anion, a fragment peak of Au(PPKO)$_2^+$($C_{24}H_{18}O_2N_4Au$) will appear. The peak height ratio of 591, 592, and 593 was found to be approximately 10:4:1, respectively. The peaks of 591, 592, and 593 can be explained as $M^+(^{12}C)$; the total of $M^+(^{13}C)$ and $(M(^{12}C)+H)^+$; and the total of $M(^{13}C\times2)^+$, $(M(^{13}C)+H)^+$, and $M^+(^{18}O)$, respectively. If the ratio of M^+ and $(M+H)^+$ is 5:1, then the peak ratio of 591, 592, and 593 is calculated at 10:4:1. Therefore, the mass spectrum supported the fragment consisting of Au and two deprotonated PPKOs.

There were no charges in the chelates on the HPLC column because the chelates were completely retained in the di-

vinylbenzene– methacrylate copolymer column. Charges on the Au and Cu ions were +3 and +2, respectively. A binding of
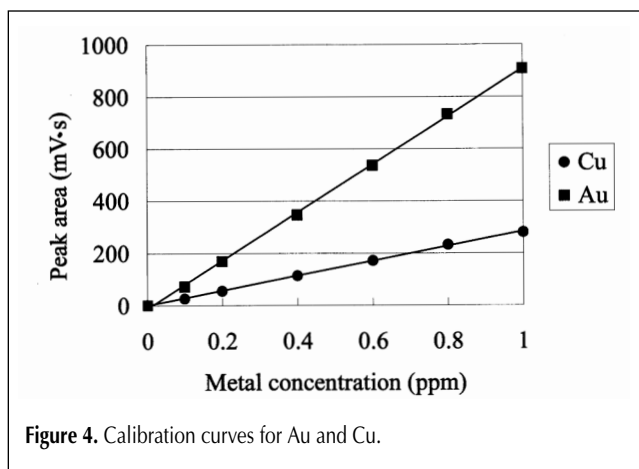

Figure 4. Calibration curves for Au and Cu.

Table II. Effect of Foreign Ions on the Determination of Gold and Copper*

Tolerance limit	Metal ion or sodium chloride
1 ppb	Co (added as $CoCl_2$)
20 ppb	Fe (added as $Fe(NO_3)_3$), Ru (added as $RuCl_3$)
50 ppb	Hg (added as $HgCl_2$)
0.1 ppm	Cd (added as $CdCl_2$), V (added as NH_4VO_3)
0.5 ppm	Ni (added as $Ni(NO_3)_2$)
1 ppm	Rh (added as $Rh(NO_3)_3$), Sn (added as $SnCl_2$), W (added as $Na_2WO_4 \cdot 2H_2O$)
2 ppm	Pt (added as H_2PtCl_6), Zr (added as $ZrO(NO_3)_2 \cdot 2H_2O$)
5 ppm	As (added as As_2O_3), Ba (added as $BaCl_2$), Cr (added as $K_2Cr_2O_7$), Ga (added as $Ga(NO_3)_3$), Ge (added as GeO_2), Pd (added as $PdCl_2$), Sc (added as $Sc(NO_3)_3$), Sr (added as $Sr(NO_3)_2$), Ti (added as $Ti(SO_4)_2$), Tl (added as $TlNO_3$), Zn (added as $Zn(NO_3)_2$)
10 ppm	Nd (added as $Nd(NO_3)_3$), Pb (added as $Pb(NO_3)_2$), Se (added as SeO_2), Te (added as $TeCl_4$)
20 ppm	Be (added as $Be(NO_3)_2$), Bi (added as $Bi(NO_3)_3$), K (added as KCl), Mn (added as $MnCl_2$), Nb (added as NbF_5), Ta (added as TaF_5)
50 ppm	Al (added as $Al(NO_3)_3$), Ca (added as $CaCl_2$), Dy (added as $Dy(NO_3)_3$), Er (added as $Er(NO_3)_3$), Eu (added as $Eu(NO_3)_3$), Gd (added as $Gd(NO_3)_3$), Ho (added as $Ho(NO_3)_3$), In (added as In^{3+}), La (added as $La(NO_3)_3$), Lu (added as $Lu(NO_3)_3$), Mg (added as $Mg(NO_3)_2$), Mo (added as $(NH_4)_6Mo_7O_{24}$), Pr (added as $Pr(NO_3)_3$), Sm (added as $Sm(NO_3)_3$), Tb (added as $Tb(NO_3)_3$), Tm (added as $Tm(NO_3)_3$), Y (added as $Y(NO_3)_3$), Yb (added as $Yb(NO_3)_3$)
6%	NaCl

* 50 ppb.

the deprotonated ligands and the stoichiometries of the complex will result in $Au(PPKO)_2^+$ and $Cu(PPKO)^+$.

When Au and Cu were chelated with PPKO without an acetate buffer solution, chelate peaks appeared on the chromatogram with the enrichment and analytical eluents containing an acetate buffer solution. These peaks disappeared when the eluents without acetate buffer solutions were used. Eluents containing 10-mmol/L NaCl instead of acetate buffer also gave Au and Cu chelate peaks in the absence of acetate buffer during chelation. For the eluent containing NaCl, retention times of Au and Cu chelates were longer than with the acetate buffer. Thus, acetate and chloride ions possibly eliminate charges in $Au(PPKO)_2^+$ and $Cu(PPKO)^+$.

Three Cu chelate solutions were prepared with 4 mL of a 15-ppm Cu solution and 1 mL of a 40-mmol/L PPKO in the presence of 1 mL of 2-mol/L sodium chloride, 1 mL of 2-mol/L acetate buffer (pH 4), and 1 mL of Milli-Q water. After 4 and 24 h, visible spectra of the solutions were measured. The Cu solution contained only 0.5-μmol/L NO_3^- ion; therefore, three different spectra could be expected: $Cu(PPKO)CH_3COO$, $Cu(PPKO)Cl$, and $Cu(PPKO)^+$. However, in actuality they were the same. From the spectra and HPLC results, the Cu–PPKO chelate in the HPLC column does not appear to be a coordination compound but an ion pair consisting of $Cu(PPKO)^+$ and CH_3COO^-. The chelate compositions of Au and Cu chelates could not be clarified.

Calibration curves

Calibration curves for Au and Cu were prepared by HPLC after chelation at pH 4. Calibration curves of peak areas versus the metal concentration (in original solution before chelation) were linear in all cases over a wide concentration range (as shown in Table I). Those for Au and Cu at 0.1 to 1 ppm are shown in Figure 4.

Detection limits and precision

The detection limits of Au and Cu ions and lower determination limits are presented in Table I along with those for ICP-

Table III. Analytical Results of Gold and Copper in Au–Cu Alloys*

Sample	Metal	Found ± %SD[†] (w/w)	Certified value (w/w) (%)
C (yellow)	Au	60.0 ± 0.2[‡] (61.1 ± 0.6)[§]	60.41
	Cu	39.0 ± 0.2[‡] (39.8 ± 0.3)[§]	39.62
D (blue)	Au	39.8 ± 0.3[‡] (40.3 ± 0.3)[§]	40.11
	Cu	59.4 ± 1.6[‡] (59.7 ± 0.1)[§]	59.97

* NIST SRM 482.
[†] SD, standard deviation.
[‡] Average and standard deviation were based on six replicate determinations.
[§] Analytical results obtained in the different day.

AES. Eight standard solutions of 0.5-ppm Au and Cu showed RSD values of 2.1% and 0.9%, respectively.

Effects of foreign ions on Au and Cu determination

These effects are summarized in Table II. The left column shows tolerance limits of metal ions or sodium chloride. The peak area of the Au chelate increased with the addition of certain metal ions such as Fe(III), V(VO^{3+}), and Hg(II) ions. The Ru(III) ion gave several peaks on the chromatogram and increased Au and Cu chelate peak areas. Ni(II) ions decreased that of the Au chelate, and Cd(II) ions decreased those of both. Other ions in Table II, as well as sodium chloride, had no significant effect on the determination of Au and Cu.

Accuracy of method

The determination of Au and Cu in standard Au–Cu wire is listed in Table III. The results on two different days were in close agreement with certified NIST values, thus demonstrating the high accuracy of the present method.

Conclusion

The present HPLC method has wide calibration ranges for Au and Cu. The detection limits, lower determination limits, and reproducibility of metals were excellent compared with ICP-AES. The analysis of an NIST standard sample clearly demonstrated the accuracy of this method.

References

1. G. Nickless. Trace metal determination by chromatography. *J. Chromatogr.* **313:** 129–59 (1985).
2. A.R. Timerbaev, O.M. Petrukhin, and Y.A. Zolotov. Analytical application of liquid chromatography of metal chelates. *Fresenius Z. Anal. Chem.* **327:** 87–101 (1987).
3. P. Wang and H.K. Lee. Recent application of high-performance liquid chromatography to the analysis of metal complexes. *J. Chromatogr. A* **789:** 437–51 (1997).
4. S. Ichinoki, T. Morita, and M. Yamazaki. Simultaneous determination of heavy metals in bovine liver and oyster tissue by solvent extraction reversed-phase high-performance liquid chromatography. *J. Liq. Chromatogr.* **7:** 2467–82 (1984).
5. S. Ichinoki and M. Yamazaki. Simultaneous determination of nickel, lead, zinc, and copper in citrus leaves and rice flour by liquid chromatography with hexamethylenedithiocarbamates extraction. *Anal. Chem.* **57:** 2219–22 (1985).
6. S. Ichinoki and M. Yamazaki. Multielement trace analysis by HPLC followed by on-line minicolumn enrichment as hexamethylenedithiocarbamate chelates. *J. Chromatogr. Sci.* **29:** 184–89 (1991).
7. S. Ichinoki and M. Yamazaki. Simultaneous determination of copper and iron in bovine liver and tomato leaves by reversed-phase high performance liquid chromatography. *Anal. Sci.* **5:** 465–69 (1989).
8. S. Ichinoki and M. Yamazaki. Evaluation of benzoylacetone as a chelating agent for multielement analysis by reversed-phase HPLC with photometric detection. *J. Chromatogr. Sci.* **28:** 258–62 (1990).
9. F. Trusell and H. Diehl. Phenyl-2-pyridyl ketoxime, a reagent for iron in strong alkalies. A method for determining oxidized iron in the presence of metallic iron. *Anal. Chem.* **31:** 1978–80 (1959).
10. C.K. Bhaskare and S.G. Kawatkar. Extractive photometric simultaneous determination of iron(II) and copper(II) with *syn*-phenyl-α-pyridyl ketoxime and analysis of ferrites. *Talanta* **22:** 189–93 (1975).
11. D. Reiner and D.P. Poe. Removal of iron, copper, cadmium, cobalt, and nickel from sodium hydroxide by precipitation and extraction with phyney-2-pyridyl ketoxime. *Anal. Chem.* **49:** 889–91 (1977).
12. B. Sen. Spectrophotometric determination of gold with phenyl-α-pyridyl ketoxime. *Anal. Chim. Acta* **21:** 35–40 (1959).
13. B. Sen. A new spot test for palladium. *Anal. Chim. Acta* **22:** 22–24 (1960).