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Use of 2-quinolyazo compounds for the reversed-phase high-performance liquid chromatographic separation of metal ions

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Abstract

The performance of six 2-quinolyazo compounds (HL) as precolumn derivatizing reagents for reversed-phase high-performance liquid chromatographic separation of metal ions using an ODS column was studied. Four metal ions were detected as $[\text{Fe}^{\text{II}}\text{L}_2]$, $[\text{Co}^{\text{III}}\text{L}_2]^+$, $[\text{NiL}_2]$ and $[\text{Cu}^{\text{II}}\text{L}]^+$, respectively. Addition of thiocyanate ion to the mobile phase enhanced the retention of $[\text{Cu}^{\text{II}}\text{L}]^+$ to give a better separation from the solvent peak, compared with other anions. Phenol derivatives afforded a shorter analytical time than naphthol derivatives. Replacement of acetonitrile by acetone as organic modifier reduced the retention of Fe, Ni and Co more than that of a free ligand and Cu, and induced the change of elution order from Cu, Co, HL, Ni, Fe to Co, Cu, Ni, Fe, HL. © 1997 Elsevier Science B.V.

Keywords: Derivatization, LC; 2-Quinolyazo compounds; Metal ions

1. Introduction

1-(2-Pyridylazo)-2-naphthol [β -PAN] has proved to be a useful reagent for analysis of metal ions and a variety of *o*-hydroxy azo heterocyclic compounds have been prepared to modify the selectivity and to enhance the sensitivity [1]. These act as a tridentate ligand and form stable complexes with various metal ions except alkali and alkaline earth metal ions. The diversity in chemical properties (electric charge and composition) and inertness of the resulting complexes enable their use as a precolumn derivatizing reagent for simultaneous determination of metal ions using reversed-phase high-performance liquid chromatography (RP-HPLC). The separation and determination of vanadium, chromium, iron, cobalt, nickel, copper, palladium, rhodium and platinum have been reported [2–10].

Previously [11], we have studied the chromatographic behaviors of metal complexes with β -PAN (HL as a neutral form). Four metal ions could be separated on an ODS column using acetonitrile–water (70:30, v/v) as a mobile phase within an analysis time of 18 min. The elution order was $[\text{Cu}^{\text{II}}\text{L}]^+ < [\text{Co}^{\text{III}}\text{L}_2]^+ \ll \text{HL} < [\text{NiL}_2] < [\text{Fe}^{\text{II}}\text{L}_2]$. The slope of the plot of $\log k'$ vs. acetonitrile content depended on the number of the β -PAN molecules involved in the chemical species; $[\text{Cu}^{\text{II}}\text{L}]^+$, $\text{HL} < [\text{Co}^{\text{III}}\text{L}_2]^+$, $[\text{NiL}_2]$, $[\text{Fe}^{\text{II}}\text{L}_2]$. An increase in acetonitrile content shortened the analytical time but deteriorated the separation between $[\text{Cu}^{\text{II}}\text{L}]^+$ and $[\text{Co}^{\text{III}}\text{L}_2]^+$.

A literature survey on the chromatographic performance of related compounds suggested the changes in separation factor and even in elution order by the substitution of diazo and coupling components of β -PAN; the elution order for 1-(2-thiazolylazo)-2-naphthol was $\text{HL} < [\text{Fe}^{\text{II}}\text{L}_2] < [\text{NiL}_2]$ and that for 2-(2-thiazolylazo)-*p*-cresol was

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$[\text{Fe}^{\text{II}}\text{L}_2] < [\text{NiL}_2] < \text{HL}$ [8]. In this paper, we examine the performance of six 2-quinolyazo compounds as precolumn derivatizing reagents and the effect of the structures of diazo and coupling components on chromatographic behavior.

2. Experimental

2.1. Reagents and materials

The 2-quinolyazo dyes were dissolved in ethanol or acetone to give stock solutions. Standard stock solutions (0.01 M, pH 2) of iron(III), cobalt(II), nickel(II) and copper(II) were prepared by dissolving the analytical-reagent grade sulfates or nitrates. All the other chemicals including organic solvents were of the analytical-reagent grade and used without further purification. Water used in this study was purified by passage through a Model Toraypure LV-10 ultra pure water manufacturing device (Toray, Tokyo, Japan).

2.2. Apparatus

The HPLC system consisted of a Model PU-880 intelligent pump (Jasco, Tokyo, Japan), a Model Multi-330 multiwavelength UV-vis detector (200–800 nm, Jasco) and a Model SVM-6U7 ceramic sample-injection valve (Sanuki, Tokyo, Japan) with a 10- μl sample loop. The separation column used was a Kaseisorb LC ODS-300-5 (particle size 5 μm , 250 \times 4.6 mm I.D. stainless steel column, Tokyo Kasei, Tokyo, Japan). A Model CS-300C column oven (Chromato Science, Osaka, Japan) was used to control the column temperature. The spectra of the metal complexes in batchwise experiments were obtained with a Model UV-250 double monochrometer spectrophotometer (Shimadzu, Tokyo, Japan).

2.3. Chromatographic procedure

A 1-ml portion of an aqueous solution containing four metal ions ($1 \cdot 10^{-4}$ M) was added to 4 ml of a reagent solution ($2.5 \cdot 10^{-4}$ M). If necessary, the mixture was heated at 80°C for 5 min. An aliquot of the resulting solution was injected into the column

using a 10- μl loop. Elution was carried out at a flow-rate of 0.8 ml/min and at a column temperature of 25°C or 40°C and the absorbance at 400–800 nm was monitored. The retention time of sodium nitrite as a measure of a void volume (3.34 min) was used to calculate the capacity factors (k') of the complexes.

3. Results and discussion

3.1. Precolumn derivatization reactions

The molecular structures of six 2-quinolyazo compounds used in this study are shown in Fig. 1. Compounds 4 to 6 were prepared according to the literature [12,13], while 1 to 3 were newly synthesized. In the simplest compounds 1 and 2, the methyl or methoxy group at a position para to a phenolic OH group was essential for the selective coupling reaction at the ortho position. Compound 3 was also examined for comparison between 2 and 4.

The acid–base properties of compounds 2, 3 and 6 were spectrophotometrically studied in an aqueous dioxane or ethanol medium. Compound 1 was sufficiently pure for the chromatographic study but not for this evaluation. The logarithmic protonation constants obtained were 9.7 and 2.2 for 2 in 30% dioxane, 10.8 and 2.0 for 3 in 50% dioxane and 12.9 and 1.3 for 6 in 60% ethanol. Taking into account the corresponding constants for the other compounds in the literature [12–14], it is shown that the compounds 4 and 6 are more basic than 2, 3 and 5.

The reactions between four metal ions and six chelating reagents in aqueous ethanol or acetone were spectrophotometrically studied by batchwise experiments. Nickel(II) exclusively formed $[\text{NiL}_2]$,

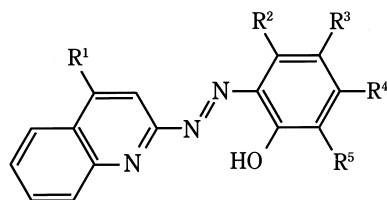


Fig. 1. Structural formulas of 2-quinolyazo compounds. 1, $\text{R}^1, \text{R}^2, \text{R}^3: \text{CH}_3$; 2, $\text{R}^3: \text{OCH}_3$; 3, $\text{R}^1: \text{CH}_3$, $\text{R}^2: \text{OCH}_3$; 4, $\text{R}^1: \text{CH}_3$, $\text{R}^4: \text{N}(\text{C}_2\text{H}_5)_2$; 5, $\text{R}^4, \text{R}^5: 1,3\text{-butadienyl}$; 6, $\text{R}^2, \text{R}^3: 1,3\text{-butadienyl}$.

while copper(II) formed both $[\text{CuL}]^+$ and $[\text{CuL}_2]$. Iron(III) formed $[\text{Fe}^{\text{II}}\text{L}_2]$ and $[\text{Fe}^{\text{III}}\text{L}_2]^+$; only $[\text{Fe}^{\text{II}}\text{L}_2]$ was formed in the presence of a reducing reagent such as ascorbic acid. Cobalt(II) first formed $[\text{Co}^{\text{II}}\text{L}_2]$, which was oxidized by dissolved oxygen to give $[\text{Co}^{\text{III}}\text{L}_2]^+$. The oxidation was slow compared with that of β -PAN complex. Even the oxidation using periodate was slow for 2, 3 and 5. The correlation between the oxidation rate and the protonation constant of a phenolic OH group has been suggested [15]. The mixture of Fe(III) and Co(II) at a comparable ratio, on the other hand, yielded appreciable amounts of $[\text{Fe}^{\text{II}}\text{L}_2]$ and $[\text{Co}^{\text{III}}\text{L}_2]^+$ without any oxidizing or reducing reagent.

When a sample solution containing complexes of these four metal ions was injected in a chromatographic system, only one peak was obtained for each metal ion. From the spectroscopic and retention behaviors, these were assigned as HL, $[\text{Fe}^{\text{II}}\text{L}_2]$, $[\text{Co}^{\text{III}}\text{L}_2]^+$, $[\text{NiL}_2]$, and $[\text{CuL}]^+$; no peaks were obtained for $[\text{Fe}^{\text{III}}\text{L}_2]^+$, $[\text{Co}^{\text{II}}\text{L}_2]$ and $[\text{CuL}_2]$.

3.2. Effects of additives

Among these four metal complexes, two cationic ones, $[\text{Co}^{\text{III}}\text{L}_2]^+$ and $[\text{CuL}]^+$ interact with residual silanol groups in the column and are poorly eluted using a simple mixture of water and an organic solvent as a mobile phase. Addition of a salt helped the elution; the cation competitively interacts with silanol groups and the anion neutralizes the cationic complexes. A recent study demonstrated that the ammonium ion showed the best performance among alkali and substituted ammonium cations in elution of these species [11]. In the present work, the effects of four anions (Cl^- , Br^- , I^- , SCN^-) on the chromatographic behavior of cationic complexes with 6 was studied using $\text{CH}_3\text{CN}-\text{H}_2\text{O}$ (60:40, v/v) as a mobile phase (Fig. 2).

The k' values of a free ligand as well as neutral complexes (not given) were independent of the addition of a salt, while those of $[\text{Co}^{\text{III}}\text{L}_2]^+$ and $[\text{CuL}]^+$ decreased with an increase in concentration of the salt and approached constant values. The limiting k' value for $[\text{Co}^{\text{III}}\text{L}_2]^+$ using I^- or SCN^- was appreciably larger than that using Cl^- or Br^- . This is explained by the distribution of an ion-pair of

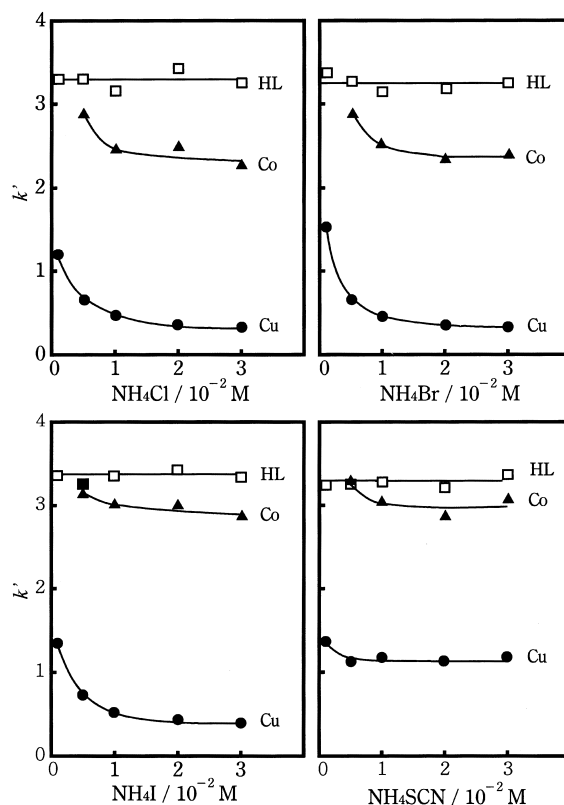


Fig. 2. Effects of additives on capacity factors of ligand and metal complexes for compound 6. Eluent: acetonitrile–water (60:40, v/v); column: Kaseisorb LC ODS-300-5; column temperature: 25°C.

the coordination-saturated Co(III) complex with an anion into the stationary phase. The limiting k' value for $[\text{CuL}]^+$ using SCN^- was much larger than that using Cl^- , Br^- or I^- . The limiting value was obtained at a lower concentration for NH_4SCN than for the other salts. This indicates the stronger interaction of SCN^- with Cu(II), giving a mixed-ligand complex.

A better separation of $[\text{Cu}^{\text{II}}\text{L}]^+$ from the solvent peak is achieved by using NH_4SCN in the mobile phase.

3.3. Comparison of the retention

Fig. 3 shows the comparison of the k' values of metal complexes at a fixed concentration (60:40 for 1 to 4 and 70:30 for 5 and 6) of organic modifiers

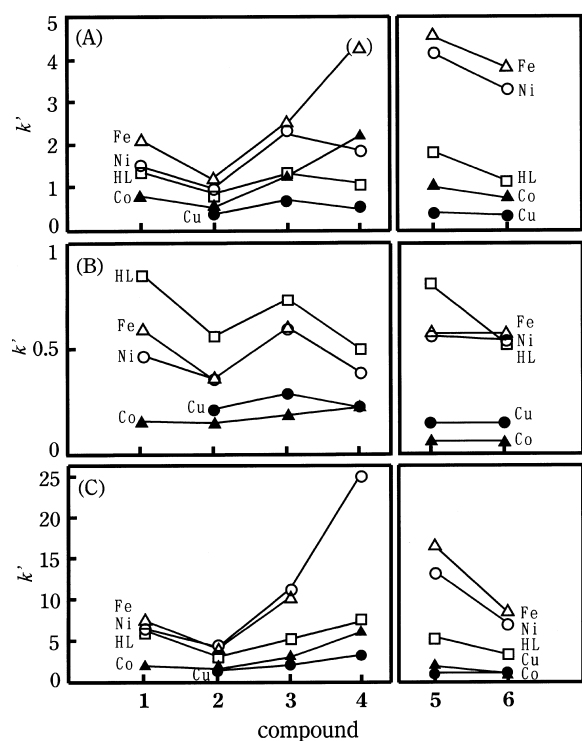


Fig. 3. Comparison of the retention of metal complexes with compounds 1 to 6. Organic modifier: (A) acetonitrile (B) acetone (C) methanol. Organic content: 60:40 for compounds 1 to 4; 70:30 for compounds 5 and 6. Column temperature: 40°C.

(CH_3CN , CH_3OH , $(\text{CH}_3)_2\text{CO}$) and at 0.01 M NH_4SCN . Copper was not detected with the compound 1, nor iron with 4.

3.3.1. Acetonitrile–water

Compound 1 showed comparable retention to that of β -PAN, which was expected from the similarity in lipophilicity of the ligand. The separation between $[\text{Fe}^{\text{II}}\text{L}_2]$ and $[\text{NiL}_2]$ for 1 was improved, compared with β -PAN. The substitution of a methyl by a methoxy group reduced the retention due to its polarity (2 compared with 1). The introduction of a methyl group onto a quinoline moiety appreciably enhanced the retention, especially for $[\text{Co}^{\text{III}}\text{L}_2]^+$, but the separation between $[\text{Fe}^{\text{II}}\text{L}_2]$ and $[\text{NiL}_2]$ was not satisfactory (3 compared with 2). The introduction of a 5-diethylamino group (4) selectively enhanced the retention of $[\text{Co}^{\text{III}}\text{L}_2]^+$, reversing the elution order

between $[\text{Co}^{\text{III}}\text{L}_2]^+$ and $[\text{NiL}_2]$. In the absence of NH_4SCN , a small peak was found for $[\text{Fe}^{\text{II}}\text{L}_2]$. The retention, shown in the brackets, was also enhanced. The substitution of a phenol ring by a naphthol enhanced the retention irrespective of metal ions (5 and 6 compared with 1 to 4; the retention was shown at different organic contents for these two compound groups), but the mutual separation was not improved, compared with β -PAN.

3.3.2. Acetone–water and methanol–water

Replacement of CH_3CN by $(\text{CH}_3)_2\text{CO}$ reduced the retention. The reduction was more remarkable for $[\text{Fe}^{\text{II}}\text{L}_2]$, $[\text{NiL}_2]$ and $[\text{Co}^{\text{III}}\text{L}_2]^+$ than for HL and $[\text{CuL}]^+$. As a result, the reversion in elution order between HL and $[\text{Fe}^{\text{II}}\text{L}_2]$ or $[\text{NiL}_2]$ was found except for compound 6, and that between $[\text{Co}^{\text{III}}\text{L}_2]^+$ and $[\text{CuL}]^+$ except for 4. Replacement of CH_3CN by CH_3OH , on the other hand, enhanced the retention. Along with this change, the separation between $[\text{Co}^{\text{III}}\text{L}_2]^+$ and $[\text{CuL}]^+$ deteriorated for all compounds.

The separation between $[\text{Fe}^{\text{II}}\text{L}_2]$ and $[\text{NiL}_2]$ for 1 was also satisfactory in the other solvent systems, while that for 2 and 3 became worse in $(\text{CH}_3)_2\text{CO}-\text{H}_2\text{O}$ and a reversed elution order was found in $\text{CH}_3\text{OH}-\text{H}_2\text{O}$.

3.4. Effect of organic modifier on elution order

The effects of the concentration of organic modifiers on the retention were systematically studied for all the combinations of three solvent systems and six compounds. Fig. 4 shows the plots of the $\log k'$ vs. the volume percentage of an organic solvent for several combinations. The reduction in retention with an increased amount of an organic modifier has two general features in any system, as was found for β -PAN [11]. First, the slope of the plot is greater for the species involving two reagents than for those involving one: $[\text{Co}^{\text{III}}\text{L}_2]^+$, $[\text{Fe}^{\text{II}}\text{L}_2]$, $[\text{NiL}_2] > [\text{CuL}]^+$, HL. Second, the intercept of the plot is greater for the neutral species than for the cationic ones: $[\text{Fe}^{\text{II}}\text{L}_2]$, $[\text{NiL}_2]$, HL $>$ $[\text{Co}^{\text{III}}\text{L}_2]^+$, $[\text{CuL}]^+$. These results imply that a reversion in elution order by the change in composition of the mobile phase may occur between the following pairs, ($[\text{Co}^{\text{III}}\text{L}_2]^+$

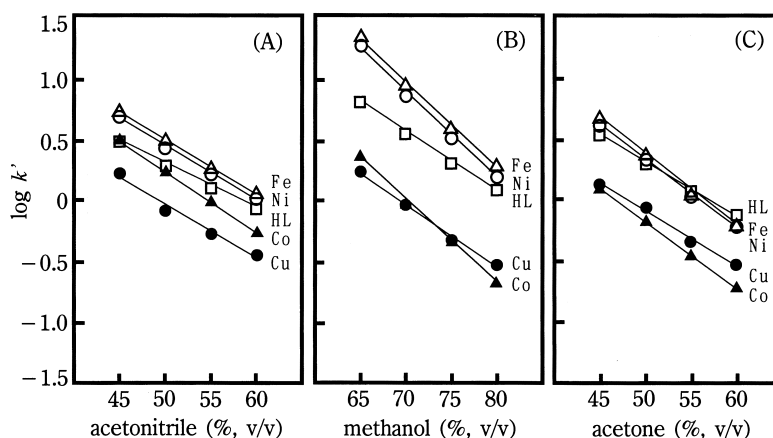


Fig. 4. Plot of $\log k'$ vs. volume percentage of organic modifiers in mobile phases. Derivatizing reagent: (A) compound 2 (B) compound 6 (C) compound 3. Mobile phase: (A) acetonitrile–water (B) methanol–water (C) acetone–water; each contained $1.0 \times 10^{-2} M$ NH_4SCN . Column temperature: 40°C .

and HL), $([\text{Co}^{\text{III}}\text{L}_2]^+ \text{ and } [\text{CuL}]^+)$, $([\text{NiL}_2] \text{ or } [\text{Fe}^{\text{II}}\text{L}_2] \text{ and HL})$ and $([\text{NiL}_2] \text{ or } [\text{Fe}^{\text{II}}\text{L}_2] \text{ and } [\text{CuL}]^+)$; no inversion is expected between $([\text{CuL}]^+ \text{ and HL})$ and $([\text{Co}^{\text{III}}\text{L}_2]^+ \text{ and } [\text{Fe}^{\text{II}}\text{L}_2] \text{ or } [\text{NiL}_2])$. Such inversion was not observed for β -PAN; the elution order found is shown in Fig. 4.

The concentration ranges of organic modifiers studied and the elution orders are summarized in Table 1; the retention of $[\text{NiL}_2]$ was selected as a representative of the neutral bis complexes for simplicity. Since copper was not detected with 1, two probable types are not distinguishable for the elution order of the residual three metal ions and both are given for this compound. Three types of elution orders (b, c and d) abound in this table, whereas a appears only for 3 in $\text{CH}_3\text{CN}-\text{H}_2\text{O}$, c' for 4 in $(\text{CH}_3)_2\text{CO}-\text{H}_2\text{O}$ and z for 4 in $\text{CH}_3\text{CN}-\text{H}_2\text{O}$. The

increase in concentration of an organic modifier induced the change of the elution order in several systems. The change (a \rightarrow b) corresponds to the inversion between $([\text{Co}^{\text{III}}\text{L}_2]^+ \text{ and HL})$, those of (b \rightarrow c) and (c' \rightarrow d) to the inversion between $([\text{Co}^{\text{III}}\text{L}_2]^+ \text{ and } [\text{CuL}]^+)$, and those of (b \rightarrow c') and (c \rightarrow d) to the inversion between $([\text{NiL}_2] \text{ and HL})$; the inversion between $([\text{NiL}_2] \text{ and } [\text{CuL}]^+)$ was possible but not observed within the solvent composition studied.

Typical chromatograms affording good separation

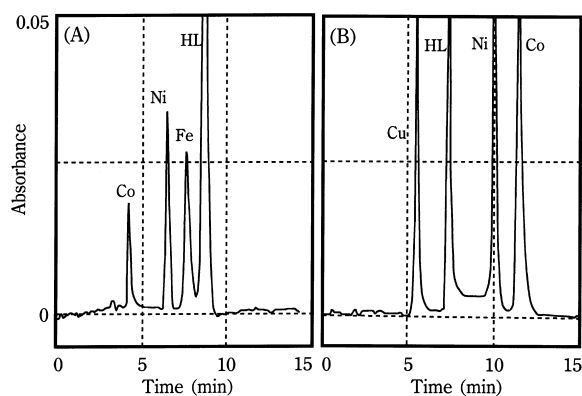


Fig. 5. Typical chromatograms. Derivatizing reagent: (A) compound 1 (B) compound 4. Mobile phase: (A) acetone–water (55:45, v/v); (B) acetonitrile–water (60:40, v/v); both contained $1.0 \times 10^{-2} M$ NH_4SCN . Column temperature: 40°C . Maximum absorbance at 400–800 nm.

Table 1
Composition of mobile phase and elution order

Compound	CH_3CN	CH_3OH	$(\text{CH}_3)_2\text{CO}$
1	(50–70) b or c	(60–75) c' or d	(45–60) c' or d
2	(45–60) b	(60–70) b	(40–60) c \rightarrow d
3	(45–60) a \rightarrow b	(60–70) b	(45–60) c \rightarrow d
4	(50–70) z	(60–75) b	(40–60) b \rightarrow c'
5	(55–75) b	(70–80) b	(50–70) c \rightarrow d
6	(55–75) b	(65–80) b \rightarrow c	(50–70) c

Numbers in parentheses represent the range of the volume percentage of organic modifiers studied.

Elution order, a: Cu, HL, Co, Ni; b: Cu, Co, HL, Ni; c: Co, Cu, HL, Ni; c': Cu, Co, Ni, HL; d: Co, Cu, Ni, HL; z: Cu, HL, Ni, Co.

of metal ions are shown in Fig. 5. Using compound 1 and $(\text{CH}_3)_2\text{CO}-\text{H}_2\text{O}$, Co, Ni, Fe and HL were eluted within 10 min. The presence of a methyl group may guarantee good separation for other types of heterocyclic azo compounds. Using compound 4 and $\text{CH}_3\text{CN}-\text{H}_2\text{O}$, Cu, HL, Ni and Co were eluted within 15 min. This system has another merit that iron does not interfere with the separation and determination of other metal ions.

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