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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF COPPER, COBALT, NICKEL, CHROMIUM AND VANADIUM CHELATES WITH 2-(5-BROMO-2-PYRIDYLAZO)-5-(N-PROPYL-N-SULPHOPROPYLAMINO)-PHENOL

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SUMMARY

The reversed-phase liquid chromatography of several metal chelates with 2-(5-bromo-2-pyridylazo)-5-(N-propyl-N-sulphopropylamino)phenol (5-Br-PAPS) on a C_{18}-bonded stationary phase using methanol–water as the mobile phase was investigated. The retention of the chelates depends on the central metal ions, the methanol–water ratio and the concentration of salt in the mobile phase. The chelates of Cu(II), Co(II), Ni(II), Cr(III) and V(V) at parts per billion levels were separated in about 15 min using a LiChrosorb RP-18 column (125 mm × 4 mm I.D.) with methanol–water (65:35) as the mobile phase containing 10^{-4} M 5-Br-PAPS, 0.1 M lithium chloride and 0.01 M acetate buffer (pH 4).

INTRODUCTION

High-performance liquid chromatography (HPLC), initially employed almost exclusively for the separation of organic compounds, is now being increasingly applied to the separation of metallic compounds. A particularly promising approach to multi-element determinations by HPLC involves the pre-column formation of chelates in a multi-element sample followed by separation of the chelates by reversed-phase liquid chromatography (RPLC). RPLC studies of metal chelates have commonly employed chelating agents, such as dithiocarbamic acids[1–5],dithizone[6], 8-hydroxyquinoline[7], β-diketones[8], 4-(2-pyridylazo)resorcinol (PAR)[9–11] and porphyrins[12,13].

2-(5-Bromo-2-pyridylazo)-5-(N-propyl-N-sulphopropylamino)phenol (5-Br-PAPS) (Fig. 1)[14] is a new class of spectrophotometric reagent. It has the same che-
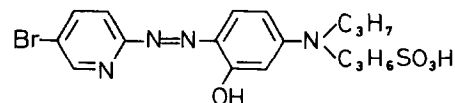

Fig. 1. Structure of 5-Br-PAPS.

lating system as PAR but is more sensitive for the determination of most metals. 5-Br-PAPS has several characteristics suitable for the purpose of RPLC studies: it is a non-selective chelating agent that forms water-soluble chelates with most transition metals; also, the formation constants of most 5-Br-PAPS chelates are as high as those of PAR, suggesting that they would be stable under RPLC conditions.

In this work we evaluated the use of 5-Br-PAPS as a pre-column chelating agent for RPLC multi-element determinations. The results demonstrate that the RPLC determination of Cu(II), Co(II), Ni(II), Cr(III) and V(V) as 5-Br-PAPS chelates at the parts per billion level is feasible.

EXPERIMENTAL

Instrumentation and reagents

The liquid chromatograph used consisted of a Nihon Seimitsu Kagaku (Tokyo, Japan) NSP-800-3U pump, a hexane damper, a Rheodyne injector and a Japan Spectroscopic (Tokyo, Japan) Uvidec-100-II W visible absorbance detector, together with a Shimazu (Kyoto, Japan) U-125MU recorder. A Merck LiChroCART column (125 mm × 4 mm I.D.) filled with LiChrosorb RP-18 (7 μm) was used. The detector was operated at 575 nm, the mobile phase flow-rate was 0.6 ml/min and the recorder chart speed was 1.25 cm/min.

Analytical-reagent grade methanol was distilled in all-glass apparatus and distilled, deionized water was purified by non-boiling distillation using a Millipore Milli-Q system. All solvents were filtered through 0.45-μm Millipore filters before use. Analytical-reagent grade 5-Br-PAPS was obtained from Dojindo (Kumamoto, Japan) and all metal salts and other reagents used were of analytical-reagent grade. The mobile phase was methanol–water (65:35) containing 10^{-4} M 5-Br-PAPS, 0.1 M lithium chloride and 0.01 M acetate adjusted to pH 4.0 by the addition of acetate buffer prior to the addition of methanol.

Procedure

A sample solution containing Cu(II), Co(II), Ni(II), Cr(III) and V(V) was placed in a test-tube [if it contains irreversible chromium(III) complexes, the solution should be boiled for 30 min after acidification to 0.2 M hydrochloric acid], 0.5 ml of $5 \cdot 10^{-3}$ M 5-Br-PAPS solution was added and the solution was adjusted to pH 4.0 by the addition of 1 M ammonium acetate solution. The solution was diluted to about 7 ml and heated at 95°C for 20 min. After cooling, the solution was transferred into a 10-ml volumetric flask and diluted to the mark. An aliquot (usually 20 μl) of this solution was then injected into the chromatograph.

RESULTS AND DISCUSSION

A typical chromatogram for the separation of the 5-Br-PAPS chelates of Cu(II), Co(II), Ni(II), Cr(III) and V(V) is shown in Fig. 2. It is clear that these metals are very easily separated.

Visible detection of the 5-Br-PAPS chelates

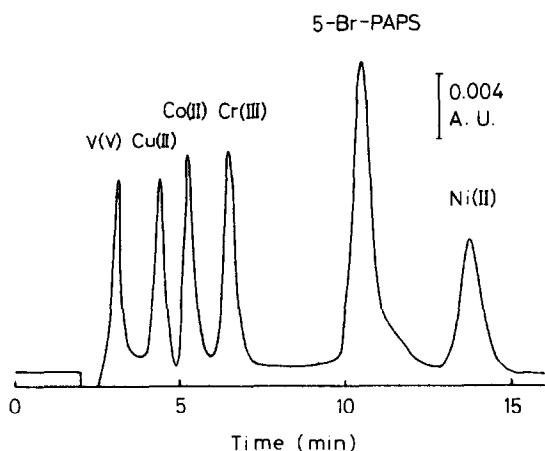The absorption spectra of the 5-Br-PAPS chelates of Cu(II), Co(II), Ni(II),

Fig. 2. HPLC separation of metal 5-Br-PAPS chelates. Column, LiChrosorb RP-18 (7 μm) (125 mm × 4 mm I.D.); mobile phase, methanol–water (65:35, v/v) containing 10^{-4} M 5-Br-PAPS, 0.1 M LiCl and 0.01 M acetate buffer (pH 4.0); flow-rate, 0.6 ml/min; detector wavelength, 575 nm; detector sensitivity, 0.04 a.u.f.s.; injection volume, 20 μl. V(V), 4 ng; Cu(II), 8 ng; Co(II), 4 ng; Cr(III), 4 ng; Ni(II), 8 ng.

Cr(III) and V(V) were obtained in methanol–water (65:35) and in aqueous media at pH 4.0. The spectroscopic properties of the chelates are summarized in Table I. It is evident that the absorption maxima of the chelates lie in the range 560–592 nm. The RPLC separation of the chelates was investigated at various detector wavelengths in the range 560–592 nm; the optimum chromatogram was obtained at 575 nm.

Composition and pH of the mobile phase
    A number of combinations of organic solvents with water, such as methanol–, ethanol–, 2-propanol– and acetone–water, were investigated as mobile phases, and methanol–water systems were found to be the most suitable for the separation of the 5-Br-PAPS chelates. A simple methanol–water mobile phase, how-

TABLE I

VISIBLE SPECTROSCOPIC DATA FOR METAL 5-Br-PAPS CHELATES

Metal	Max. wavelength (nm)	Molar absorptivity ($l\ mol^{-1}\ cm^{-1}$)
Cu(II)	562*	45 800*
	562**	64 800**
Co(II)	592*	96 600*
	592**	93 400**
Ni(II)	560*	93 000*
	560**	123 000**
Cr(III)	592*	89 400*
	592**	94 100**
V(V)	589*	54 700*
	585**	54 000**

* In aqueous media, pH 4.0.
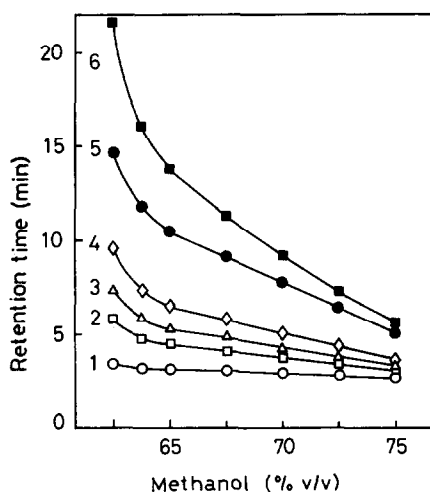** In methanol–water (65:35), pH 4.0.

Fig. 3. Variation of retention time with concentration of methanol in the mobile phase. 1, V(V); 2, Cu(II); 3, Co(II); 4, Cr(III); 5, 5-Br-PAPS; 6, Ni. All other conditions as in Fig. 2 except methanol concentration.

ever, gave poor peak shapes and inadequate resolution because part of the chelates were decomposed in the column. When acetate buffer, 5-Br-PAPS and lithium chloride were added to the methanol–water in order to suppress the decomposition of the chelates, excellent peak shapes and resolution were obtained.

The effect of the methanol content in the mobile phase on the retention of the chelates is shown in Fig. 3. The retention time of each chelate decreased on increasing the methanol content; the optimum results were obtained with 65% (v/v) methanol.

Preliminary experiments indicated that the retention time increased on addition of counter ions to the mobile phase. The RPLC separation of the chelates was assessed with a variety of counter ions, such as sodium, lithium and tetraalkylam-

TABLE II

EFECT OF COUNTER IONS ON THE RETENTION TIME OF METAL 5-Br-PAPS CHELATES

All conditions as for Fig. 2 except counter ions.

Salt (0.1 M)	Retention time (min)					
	V(V)	Cu(II)	Co(II)	Cr(III)	5-Br-PAPS	Ni(II)
LiCl	3.20	4.47	5.32	6.54	10.5	13.8
NaCl	3.18	4.57	5.26	6.48	10.63	14.09
TMABr*	3.06	4.28	4.19	5.06	8.98	10.61
TEABr**	3.05	4.02	3.97	4.73	8.47	10.45
TPABr***	3.56	4.41	4.11	5.08	10.71	15.26
TBABr§	4.85	6.02	4.35	5.30	16.75	30.86

* Tetramethylammonium bromide.
** Tetraethylammonium bromide.
*** Tetrapropylammonium bromide.
§ Tetrabutylammonium bromide.

monium ions. The results are shown in Table II. It is apparent that sodium or lithium is the most suitable counter ion for the separation of the chelates. Lithium ion was selected because the solubility of lithium chloride in methanol is much higher than that of sodium chloride. This property of lithium chloride makes it possible to use high methanol and lithium chloride contents in the mobile phase. With the use of tetraalkylammonium ion as the counter ion, the retention time of the chelates increased with increasing carbon number in the alkyl group, but inadequate resolutions were obtained.

The effect of lithium chloride concentration in the mobile phase on the retention of the chelates is shown in Fig. 4. The retention time of the chelates increased abruptly for Ni(II) and gradually for the other metals on increasing the lithium chloride concentration. Consequently, the retention times of the chelates depend on the central metal ion, increasing in the order V(V) < Cu(II) < Co(II) < Cr(III) < Ni(II) with 0.1 M lithium chloride in the mobile phase. It is thought that the retention time of the chelates increases on increasing the distribution of the chelates to the stationary phase, based on the formation of an ion pair between the chelate anions and the lithium ion. The optimum chromatogram for the chelates was obtained with 0.1 M lithium chloride in the mobile phase.

The effect of the pH of the mobile phase on the retention of the chelates was investigated and the results are shown in Fig. 5. It is evident that the retention times of the chelates of Co(II), Cr(III) and V(V) remained unchanged in the pH range 3–5, but those of the chelates of Cu(II) and Ni(II) increased in the pH range 4.5–5. The peak heights of the chelates of Cu(II), Cr(III) and V(V) decreased above pH 4.5, but those of the chelates of Co(II) and Ni(II) remained unchanged in the pH range 3.5–5. The optimum chromatogram was obtained with the addition of acetate buffer (pH 4.0) to the mobile phase.

The influence of 5-Br-PAPS concentration on the retention of the chelates was examined at pH 4.0. The retention time of the chelate of Cu(II) increased significantly, but the others only slightly, on increasing the 5-Br-PAPS concentration in the mobile phase. The separation of the chelates of Cu(II) and Co(II) was consequently
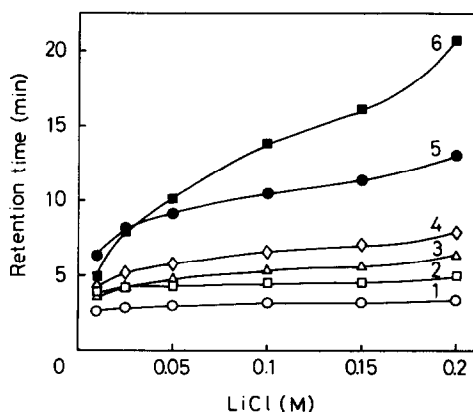

Fig. 4. Variation of retention time with lithium chloride concentration in the mobile phase. 1, V(V); 2, Cu(II); 3, Co(II); 4, Cr(III); 5, 5-Br-PAPS; 6, Ni(II). All other conditions as in Fig. 2 except lithium chloride concentration.
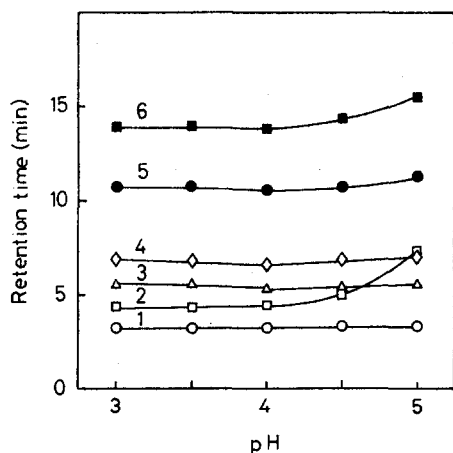
Fig. 5. Variation of retention time with pH of the mobile phase. 1, V(V); 2, Cu(II); 3, Co(II); 4, Cr(III); 5, 5-Br-PAPS; 6, Ni(II). All other conditions as in Fig. 2 except the pH of the mobile phase.

poor at 5-Br-PAPS concentrations above $1.5 \cdot 10^{-4}$ M. The decrease in peak height of the chelates of Co(II) and V(V) was considerable at 5-Br-PAPS concentrations below 10^{-5} M. The mobile phase containing 10^{-4} M 5-Br-PAPS gave excellent peak shape and resolution.

Calibration graph

The calibration graphs of peak area *versus* metal concentration were straight lines in the metal concentration ranges 1–10 ng for Co(II), Cr(III) and V(V) and 2–20 ng for Cu(II) and Ni(II) with 20-μl injections.

Detection limits

The detection limits for metal ions as 5-Br-PAPS chelates wih visible absorption detection at 575 nm are given in Table III. The detection limits were calculated as the concentrations that gave a signal twice the background noise (signal-to-noise ratio = 2). The chromatogram of unreacted 5-Br-PAPS solution was used as a blank

TABLE III

DETECTION LIMITS FOR DETERMINATION OF METALS AS 5-Br-PAPS CHELATES USING HPLC

Detector sensitivity, 0.005 a.u.f.s.; all other conditions as for Fig. 2.

Parameter	Detection limit*				
	V(V)	Cu(II)	Co(II)	Cr(III)	Ni(II)
Concentration (ppb)	1	1.5	5	2	2.5
Amount injected (ng)	0.02	0.03	0.1	0.04	0.05

* Detection limits were calculated as the concentrations that gave a signal twice the background noise (signal-to-noise ratio = 2).

chromatogram. It is thought that the detection limits for the individual metal ions are governed by coelution with blank impurities or unreacted 5-Br-PAPS. However, the separation of 5-Br-PAPS and the 5-Br-PAPS chelates is feasible when high 5-Br-PAPS/5-Br-PAPS chelate ratios are present at low metal ion levels. It is expected that the detection limits could be significantly lowered by the use of sample enrichment techniques.

CONCLUSION

RPLC seems to be a valuable method for the separation of mixtures of metal 5-Br-PAPS chelates. The simultaneous RPLC determination of Cu(II), Co(II), Ni(II), Cr(III) and V(V) as 5-Br-PAPS chelates at the parts per billion level can be achieved by this method. It is also clear that many additional metals may be determined by this method because 5-Br-PAPS forms water-soluble chelates with most transition metals.

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