

CHROM. 9908

## CHROMATOGRAPHY OF METAL CHELATES

### VII\*. HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF METAL 1,2-DIKETOBISTHIOBENZHYDRAZONES, METAL DIALKYLDITHIOCARBAMATES AND METAL 1,2-DIKETOBISTHIOSEMICARBAZONES

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#### SUMMARY

High-performance liquid chromatographic separations of Pb(II), Zn(II), Cd(II), Hg(II), Cu(II), Ni(II) and Co(III) chelates at nanogram levels by adsorption chromatography on silica gel are reported. 1,2-Diketobisthiobenzhydrazones, dialkyldithiocarbamates and 1,2-diketobisthiosemicarbazones were used as chelate-forming ligands.

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#### INTRODUCTION

In trace metal analysis, the use of chelate-forming ligands for the chromatographic separation and determination of the elements is of great importance. The separation and determination of chelated metal ions by gas chromatography (GC) has often been described<sup>1-9</sup>, but this method is limited because of the low volatility and the low thermal stability of most of the metal chelates.

In liquid chromatography, these problems do not arise and this method often therefore appears to be more suitable for the separation and determination of extracted metal chelates<sup>10,11</sup>. Ion-exchange chromatography offers an other possibility for the separation of metal ions<sup>12-14</sup>. Separations and determinations of metal ions by forced-flow ion-exchange chromatography have been described<sup>15,16</sup> but, compared with this method, the use of chelating ligands for the determination of metal ions after solvent extraction permits enrichment of the components in the organic solvent before the separation step.

This paper demonstrates the possibilities of separating metal chelates by high-performance liquid chromatography (HPLC). The following ligands were used: 1,2-diketobisthiosemicarbazones (I)<sup>17</sup>, dialkyldithiocarbamates (II)<sup>18-21</sup> and 1,2-diketobisthiobenzhydrazones (III)<sup>22,23</sup> (Fig. 1).

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\* Part VI: P. Heizmann and K. Ballschmiter, *Z. Anal. Chem.*, 266 (1973) 206.



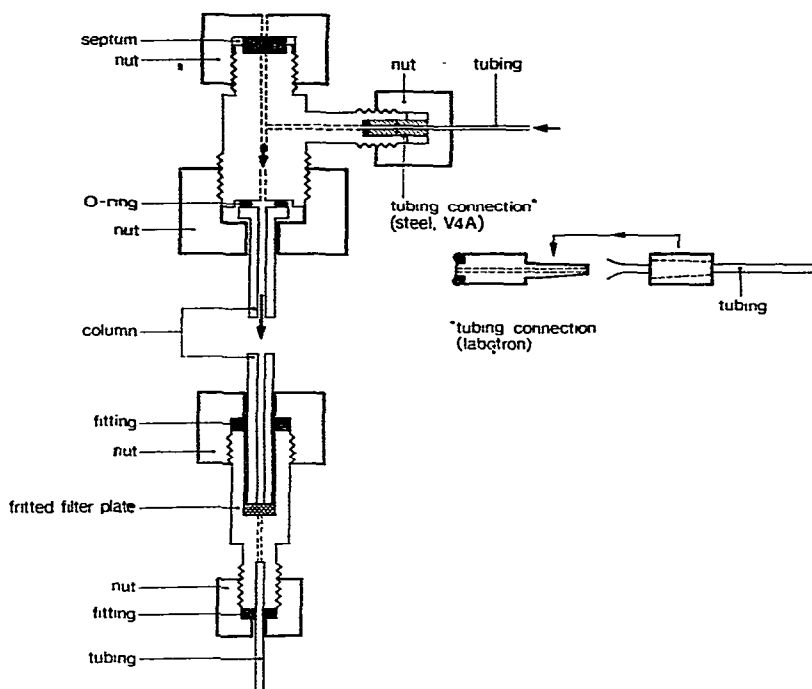


Fig. 2. Scheme of injection port and column termination (PTFE).

chelates, quantitative determinations of trace elements in aqueous solutions at the picogram level should be possible.

**Reproducibility.** Using Hg(II) and Ni(II) chelates as examples, the reproducibility was determined. Portions of 10  $\mu$ l of chelate solution were injected (septum injection) and the resulting peak areas integrated. The standard deviation was found to be 1.6% for the Hg(II) chelate (20 injections) and 2.2% for the Ni(II) chelate (15 injections). The reproducibility can be improved by using a septumless injector (standard deviation *ca.* 1%).

To avoid any contact between the chelates and metal parts, which might lead to decomposition, all experiments were run in glass columns, although commercial steel columns (5- $\mu$ m silica gel) nowadays have a much better separation efficiency.

The same peak areas were obtained when the same amounts of chelate solution [Hg(II), Cu(II), Ni(II)] were injected once into a column filled with silica gel and once into a column that contained no adsorbent (ten injections of each chelate on each type of column).

These results indicated that chromatography on LiChrosorb SI 60 does not result in decomposition or irreversible adsorption of the chelates.

#### *HPLC of metal diacetylbisthiobenzhydrazones*<sup>10</sup>

Hg(II), Ni(II), Cu(II), Zn(II) and Pb(II) diacetylbisthiobenzhydrazones can be separated, using silica as the adsorbent.

**Isocratic elution.** The influence of column length, particle diameter and flow-

TABLE I

HPLC SEPARATIONS OF Hg(II) AND Cu(II) DIACETYLBISTHIOBENZHYDRAZONES  
LiChrosorb SI 60 (40  $\mu\text{m}$ ); glass column, 35 cm; solvent, benzene; temperature, 23°.

Solvent flow-rate (ml/h)	Resolution, $R_s$ [Hg(II)/Cu(II)]	Plate height, Hg(II) chelate (mm)	Analysis time, $t_A$ (min)
3	1.8	0.9	50
6	1.6	1.3	25
15	1.1	2.2	10
30	0.9	2.6	5
60	0.7	2.8	4

rate of the eluent on the separation conditions for the Hg(II), Cu(II) and Pb(II) chelates were studied (Tables I and II). Because of its excessive retention on LiChrosorb SI 60, the determination of the Zn(II) chelate of diacetylbisthiobenzhydrazone is not possible when benzene is used as the eluent, and gradient elution is required.

Tables III and IV show the separations obtained with LiChrosorb SI 60 and Perisorb A as adsorbents and *n*-heptane-benzene solvent mixtures as the eluent. Although the eluent flow-rate is increased by adding more *n*-heptane while the flow-

TABLE II

HPLC SEPARATIONS OF Hg(II) AND Pb(II) DIACETYLBISTHIOBENZHYDRAZONES  
LiChrosorb SI 60 (20  $\mu\text{m}$ ); glass column; solvent, benzene; temperature, 23°.

Column length (cm)	Solvent flow-rate (ml/h)	Resolution, $R_s$ [Hg(II)/Pb(II)]	Analysis time, $t_A$ (min)
15	6	2.3	19
15	15	1.9	11
10	15	1.5	7
15	30	1.3	5

TABLE III

INFLUENCE OF THE SOLVENT GRADIENT ON THE RESOLUTION ( $R_s$ ) OF METAL DIACETYLBISTHIOBENZHYDRAZONES

LiChrosorb SI 60 (30  $\mu\text{m}$ ); glass column, 15 cm; solvent, benzene-*n*-heptane; temperature, 23° ( $t_A$  = analysis time).

Benzene- <i>n</i> -heptane ratio	Flow-rate (ml/h)	$R_s$				$t_A$ (min)	
		Hg/Cu	Hg/Ni	Ni/Cu	Cu/Pb	Hg/Cu	Hg/Pb
15:0	15	0.8	0.5	0.4	1.5	6	10
15:1	16	0.9	0.5	0.4	1.7	6	10
15:2	17	1.2	0.5	0.5	2.2	6	16
15:5	20	1.4	0.7	0.5	2.3	10	20
15:10	25	1.8	1.3	0.9	2.6	13	28
15:12.5	11	3.2	1.8	1.5	—	32	—

TABLE IV

INFLUENCE OF THE SOLVENT GRADIENT ON THE RESOLUTION ( $R_s$ ) OF METAL DIACETYLBIETHIOBENZHYDRAZONES

Perisorb A (30–40  $\mu\text{m}$ ); glass column, 35 cm; solvent, benzene–*n*-heptane; temperature, 23° ( $t_A$  = analysis time).

Benzene– <i>n</i> -heptane ratio	Flow-rate (ml/h)	$R_s$				$t_A$	
		Hg/Cu	Hg/Ni	Ni/Cu	Cu/Pb	Hg/Cu	Cu/Pb
15:12.5	11	1.8	1.0	0.7	2.3	8	14
15:20	16	2.2	1.5	0.8	2.2	6	12
15:25	35	2.6	2.3	1.0	—	14	—

rate of benzene remains constant, the separation ( $R_s$ ) is increased because of the reduced solvent strength. Fig. 3 shows the separation of Hg(II), Ni(II) and Cu(II) diacetylbiethiobenzhydrazones, while Figs. 4 and 5 show the separations of the Hg(II), Cu(II) and Pb(II) chelates. In comparison with Fig. 4, Fig. 5 demonstrates the much better separation efficiency of a slurry-packed 10- $\mu\text{m}$ -column; better separations within shorter analysis times are achieved. Using Perisorb A as the adsorbent, good separations can be achieved at lower solvent strengths.

**Gradient elution.** Gradient elution improves poor separations and reduces otherwise large capacity ratios ( $k'$  values). Using solvent mixtures of low solvent strength, the columns could be reconditioned to their initial state by running the

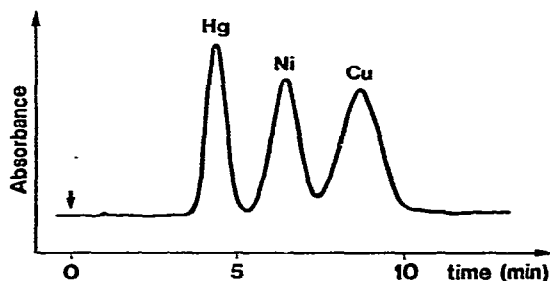


Fig. 3. HPLC separation of metal diacetylbiethiobenzhydrazones (isocratic elution). Packing, LiChrosorb SI 60 (30  $\mu\text{m}$ ); glass column, 500  $\times$  2 mm; solvent, *n*-heptane–benzene (1:1); flow-rate, 1.5 ml/min; detection, 360 nm (Perkin-Elmer LC 55); pump, Waters M 6000; temperature, 23°.

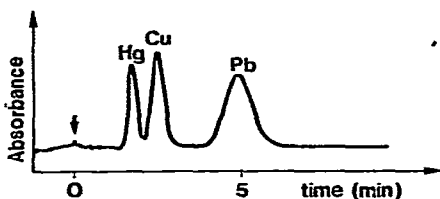


Fig. 4. HPLC separation of metal diacetylbiethiobenzhydrazones (isocratic elution). Packing, LiChrosorb SI 60 (30  $\mu\text{m}$ ); glass column, 500  $\times$  2 mm; solvent, *n*-heptane–benzene (3:7); flow-rate, 2.0 ml/min; detection, 360 nm (Perkin-Elmer LC 55); pump, Waters M 6000; temperature, 23°.

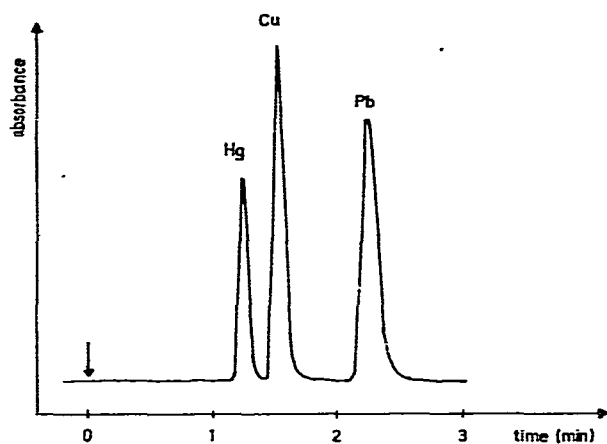


Fig. 5. HPLC separation of metal diacetylbi-thiobenzhydrazones (isocratic elution). Packing, Nucleosil 100-10; glass column,  $300 \times 2.3$  mm (pre-packed column from Macherey, Nagel & Co., Düren, G.F.R.); solvent, benzene; flow-rate, 1.5 ml/min; detection, 360 nm (Perkin-Elmer LC 55); pump, Waters M 6000; temperature, 23°.

reverse programme. By varying the gradient programme of the components of the *n*-heptane–benzene eluent system, good separations of Hg(II), Ni(II), Cu(II) and Pb(II) chelates are possible (Figs. 6 and 7).

Gradient programmes using *n*-heptane–chloroform as eluent also give good results (Fig. 8). By using this solvent mixture, a separation of the otherwise strongly adsorbed Zn(II) chelate from the other components is possible. The programme consisted of a linear gradient (10 min), the solvent being 10–60% of chloroform in *n*-heptane with a flow-rate of 4 ml/min, using a 60-cm LiChrosorb SI 60 (20  $\mu$ m) column. The  $R_{s, \text{Cu/Zn}}$  value was 2.5 and the analysis time ( $t_A$ ) was 5 min.

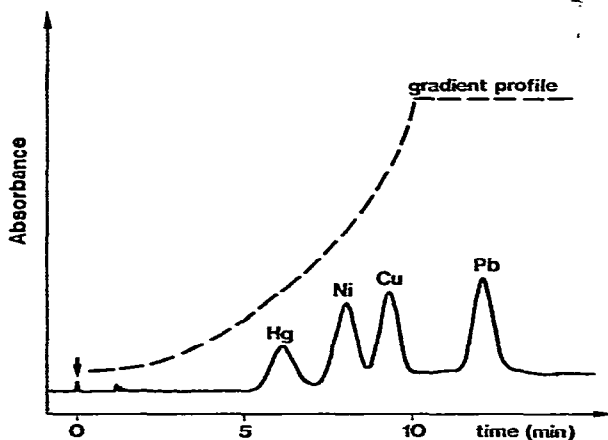


Fig. 6. HPLC separation of metal diacetylbi-thiobenzhydrazones (gradient elution). Packing, LiChrosorb SI 60 (30  $\mu$ m); glass column,  $500 \times 2$  mm; solvent, *n*-heptane–benzene; flow-rate, 1.5 ml/min; programme, curve 7 on Waters M 660 (running time 10 min); conditions, from 40% benzene in *n*-heptane to 100% benzene; detection, 360 nm (Perkin-Elmer LC 55); pumps, Waters M 6000; temperature, 23°.

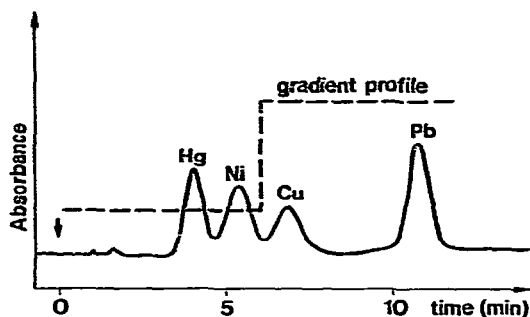


Fig. 7. HPLC separation of metal diacetylbi(2-thiobenzhydrazones) (gradient elution). Packing, LiChrosorb SI 60 ( $30\ \mu\text{m}$ ); glass column,  $500 \times 2\ \text{mm}$ ; solvent, *n*-heptane–benzene; flow-rate, 1.5 ml/min; programme, curve 11 on Waters M 660 (running time 6 min); conditions, from 50% benzene in *n*-heptane to 100% benzene; detection, 360 nm (Perkin-Elmer LC 55), pumps, Waters M 6000; temperature,  $23^\circ$ .

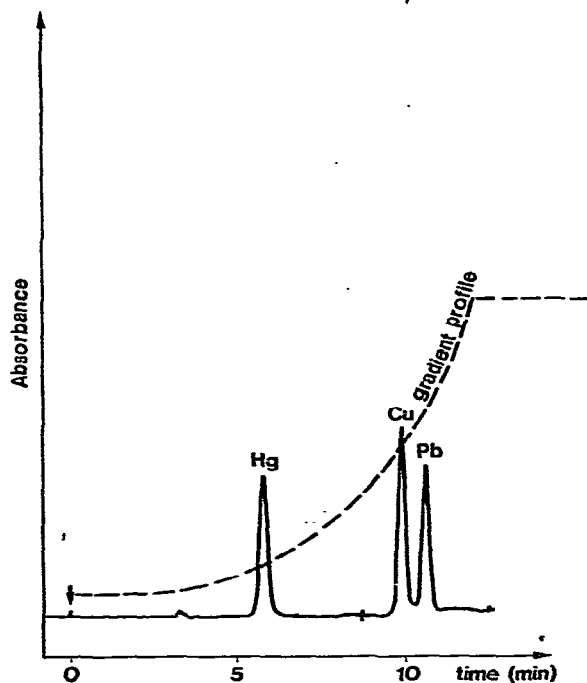


Fig. 8. HPLC separation of metal diacetylbi(2-thiobenzhydrazones) (gradient elution). Packing, LiChrosorb SI 60 ( $30\ \mu\text{m}$ ); glass column,  $500 \times 2.2\ \text{mm}$ ; solvent, *n*-hexane–chloroform; flow-rate, 3.0 ml/min; programme, curve 8 on Waters M 660 (running time 12 min); conditions, from 20% chloroform in *n*-hexane to 70% chloroform in *n*-heptane; detection, 360 nm (Perkin-Elmer LC 55); pumps, Waters M 6000; temperature,  $23^\circ$ .

#### HPLC of chelates of disubstituted dithiocarbamates (DTC)

Previously described TLC separations of metal dithiocarbamates<sup>18–21</sup> were attempted by using HPLC. Dithiocarbamates with different substituents can easily be prepared and the influence of different substituents on the separation can be studied.

*HPLC of metal diethyldithiocarbamates.* Separations of the Cu(II), Hg(II), Ni(II) and Co(III) diethyldithiocarbamates are possible within a reasonable time using silica gel as adsorbent and benzene as eluent (Table V).

TABLE V

## HPLC SEPARATIONS OF METAL DIETHYLDITHIOCARBAMATES

LiChrosorb SI 60 (30  $\mu\text{m}$ ); glass column, 15 cm; solvent, benzene; detection, 330 nm. Elution sequence: Cu > Hg > Ni > Co.

Pair	$R_s$	Solvent flow-rate (ml/h)	Analysis time, $t_A$ (min)
Cu/Hg	0.4	4	15
Cu/Ni	1.1	4	18
Ni/Co	1.6	8	15
Cu/Co	1.9	20	6
Ni/Co	1.3	20	6

The separation of the Cu(II) and Ni(II) chelates is possible on a short column (15 cm), although only at low solvent flow-rates. On the other hand, the separation of the Hg(II) and Cu(II) chelates is difficult, but can be achieved by using a 50-cm column. Separations of the Cu(II) and Co(III) chelates and the Ni(II) and Co(III) chelates pose no problems. Again, increasing the solvent flow-rate reduces analysis time. The Zn(II) chelate is eluted between the Cu(II) and the Hg(II) chelates, the Cd(II) chelate is eluted before the Cu(II) chelate and the Pb(II) chelate is eluted after the Ni(II) chelate. However, under these conditions, the three chelates show considerable tailing on silica gel.

Further HPLC results will be published later<sup>25</sup>.

*HPLC of metal benzylmethyldithiocarbamates.* As the results obtained by TLC<sup>19,21</sup> show, the chelates of the benzylmethyldithiocarbamates are also suitable for liquid chromatographic separations.

For the benzylmethyldithiocarbamates of Cu(II), Zn(II), Ni(II), Cd(II) and Co(III) there was good agreement between the TLC results and HPLC results using LiChrosorb SI 60 and solvent mixtures with low solvent strength (e.g., 3:1 benzene-cyclohexane). Fig. 9 compares the TLC  $hR_F$  values with the HPLC retention times ( $t_R$ ). The sequence of the signals in HPLC is almost identical with those in the separation by TLC.

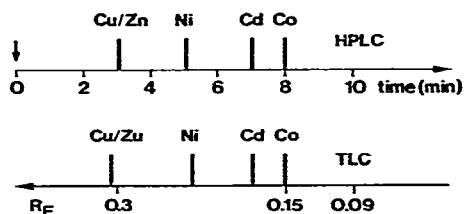


Fig. 9. Comparison between TLC and HPLC separations of metal benzylmethyldithiocarbamates. TLC: DC-Alufolie SiO<sub>2</sub>; benzene-cyclohexane (3:1). HPLC: packing, LiChrosorb SI 60 (40  $\mu\text{m}$ ); glass column, 350  $\times$  2 mm; solvent, benzene-cyclohexane (3:1).



A flow-programme separation of the Zn(II), Ni(II) and Co(III) benzylmethyl-dithiocarbamates (Fig. 10) gave good  $R_s$  values with an acceptable analysis time. After the peak maximum of the Ni(II) chelate, had been reached, the solvent flow-rate was increased from the initial 8 ml/h to 20 ml/h. The values obtained were  $R_{s, Zn/Ni} = 1.6$ ,  $R_{s, Ni/Co} = 1.6$  and  $t_A = 35$  min. Under these conditions, the Pb(II) chelate will be eluted with the Co(III) chelate.

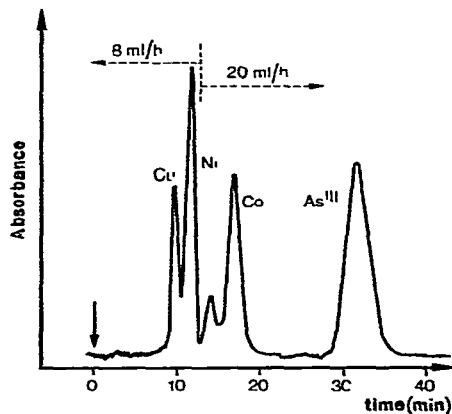
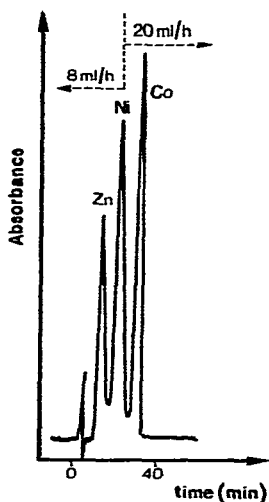


Fig. 10. HPLC separation of metal benzylmethyl-dithiocarbamates. Packing, LiChrosorb SI 60 (40  $\mu$ m); glass column, 350  $\times$  2 mm; solvent, benzene-cyclohexane (3:1); flow-rates, 8 and 20 ml/h; detection, 360 nm (Zeiss PM 2 D); pump, Labotron LDP 13 A; temperature, 23 $^{\circ}$ .

Fig. 11. HPLC separation of metal diethoxyethyl-dithiocarbamates. Packing, LiChrosorb SI 60 (40  $\mu$ m); glass column, 350  $\times$  2 mm; solvent, carbon tetrachloride-4% acetonitrile; flow-rates, 8 and 20 ml/h; detection, 360 nm (Zeiss PM 2 D); pump, Labotron LDP 13 A; temperature, 23 $^{\circ}$ .

*HPLC of metal diethoxyethyl-dithiocarbamates.* Separations of the Hg(II), Cu(II), Ni(II), Co(III) and As(III) diethoxyethyl-dithiocarbamates were studied. Using binary mixtures, we found small distortions between the TLC and HPLC separations. Separations of the Cu(II) and Ni(II) chelates were possible only at low solvent flow-rates.

Fig. 11 shows the separation of the chelates of Cu(II), Ni(II), Co(III) and As(III). The analysis was started at a solvent flow-rate of 8 ml/h, and was increased to 20 ml/h for the separation of the Co(III) and As(III) chelates. Under these conditions, the Hg(II) chelate is eluted with the Cu(II) chelate. The chelates of Sb(III) and Bi(III) show strong adsorption, resulting in excessive retention times. The chelates of Pb(II), Zn(II) and Cd(II) show large tailing effects, as demonstrated by TLC<sup>19</sup>.

Fig. 12 shows the separation of a three-component mixture at a constant solvent flow-rate of 20 ml/h.

#### *HPLC of chelates of 1,2-diketobis(4-substituted)thiosemicarbazones*

The wide analytical use of 1,2-diketobisthiosemicarbazones in trace metal analysis is prevented by the low solubility of most of the chelates in organic solvents.

However, substitution of the ligand molecule in the  $R_2$  position (Fig. 1) leads to chelates that can be extracted into organic solvents such as chloroform and ethyl acetate<sup>17,21,23</sup>.

**HPLC of metal glyoxalbis(2,2,3,3-tetramethylbutyl)thiosemicarbazones.** Using Alox T as the adsorbent and benzene as the eluent, the separation of the Hg(II), Cu(II) and Ni(II) chelates can be achieved (Fig. 13). The Pb(II) chelate decomposes via a catalytic reaction on the adsorbent. The chelates of Cd(II), Zn(II) and Co(III) are strongly adsorbed.

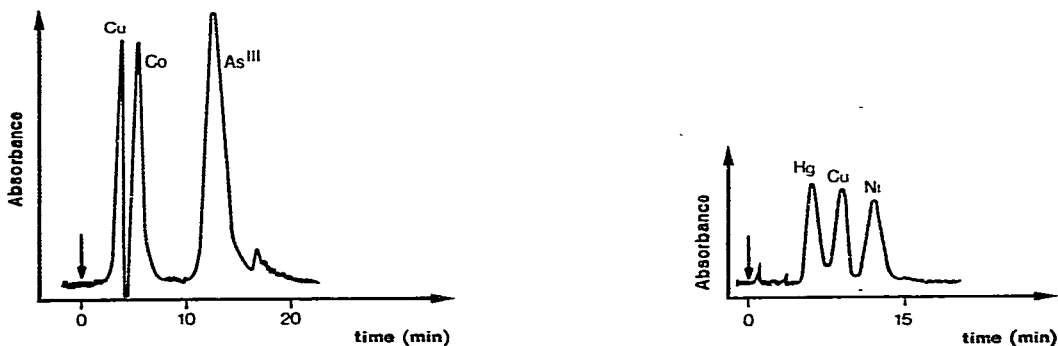


Fig. 12. As Fig. 11; flow-rate, 20 ml/h.

Fig. 13. HPLC separation of metal glyoxalbis(2,2,3,3-tetramethylbutyl)thiosemicarbazones. Packing, Alox T ( $30\ \mu\text{m}$ ); glass column,  $350 \times 2\ \text{mm}$ ; solvent, benzene; flow-rate, 8 ml/h; detection, 360 nm (Zeiss PM 2 D); pump, Labotron LDP 13 A; temperature,  $23^\circ$ .

When Perisorb A is used as an adsorbent of lower activity, only a slight separation of the Hg(II), Cu(II) and Ni(II) chelates occurs, but the Pb(II) chelate no longer decomposes and its separation from the other three components is possible ( $R_{s\ \text{Hg/Pb}} = 1.35$ ; benzene (20 ml/h) as eluent; column length 35 cm).

**HPLC of metal diacetylbis(cyclohexyl)thiosemicarbazones.** The separation of the Hg(II) and Cu(II) chelates can be achieved on a silica gel column (LiChrosorb SI 60,  $40\ \mu\text{m}$ , length 35 cm) using benzene-3% tetrahydrofuran as eluent. A better separation of these components is possible on Alox T ( $30\ \mu\text{m}$ ) alumina as the adsorbent and benzene as the eluent (column length, 10 cm; flow-rate, 20 ml/h;  $R_s = 1.4$ ;  $t_A = 5\ \text{min}$ ). Under these conditions, the Pb(II) chelate decomposes. The chelates of Zn(II), Ni(II) and Co(III) are only slightly extracted with benzene. The Cd(II) chelate shows very strong adsorption.

## CONCLUSION

The application of HPLC in trace metal analysis offers new possibilities for the separation and determination of metal chelates at nanogram levels. By varying the substituents in the ligand molecule, the separation can be influenced. Substituent effects on the selectivity of liquid chromatographic separations of metal chelates will be discussed elsewhere.

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