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THE THIN-LAYER CHROMATOGRAPHIC PROPERTIES OF TRACE METALS

I. SEPARATION OF SOME METALS WITH SPECIAL REFERENCE TO LEAD

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SUMMARY

The thin-layer chromatographic behaviour of a group of metals has been investigated with various solvent systems on cellulose and silica gel plates and sheets (Eastman Chromagram sheets) by the ascending technique. The results were compared to paper chromatographic separation of the same metals. The cations investigated were Co, Ni, Cu, Bi, Pb, Mn, Cd, V(V), and U(VI). Solvent systems included mixtures of acetone-isopropanol-acetic acid-HCl-water in various combinations and proportions. The separation of lead, which has a strong tendency to tail, was carried out successfully with a solvent mixture of methanol-25% HNO₃-water (8:1:1).

New spray reagents, 4-(2-thiazolylazo)resorcinol, 1-(2-thiazolylazo)-2-naphthol, 4-(2-pyridylazo)resorcinol and 1-(2-pyridylazo)-2-naphthol, were successfully introduced for the detection of the metals as distinctly coloured complexes. Visual detection limits ranged from 0.1 to 0.01 μg per spot.

INTRODUCTION

Thin-layer chromatography (TLC) offers a rapid and efficient way of separating a large number of metals in trace amounts. However, the amount of TLC work done with inorganic systems is still small. The major portion was carried out on fixed layers of silica gel with gypsum or starch used as a binder, and by the ascending technique¹⁻³. SEILER⁴ has separated mixtures of Fe, Zn, Co, Mn, Cr, Ni, and Al with acetone-conc. HCl-acetylacetone (100:1:0.5) and Hg, Bi, Cd, Pb, and Cu with *n*-butanol-1.5 N HCl-acetylacetone (100:20:0.5) on silica gel. Others have used starch^{5,6} or Kieselguhr⁷ for the separation of similar systems. SEILER⁸ studied the influence of the concentration of hydrochloric acid in acetone as a mobile phase on *R_F* values of Cu, Co, and Ni, and the influence of different solvents (dioxane, tetrahydrofuran, *n*-butanol, isopropanol, *n*-propanol, acetone, ethanol, and methanol) with a content of 10% 1 N HCl or 10% 1 N HClO₄ on the chromatographic behaviour of Cu, Co, and Ni on silica gel. The best resolution of these metals was achieved in isopropanol with 10% 1 N HCl. Recently, MERKUS⁹⁻¹¹ separated traces of Ni, Co,

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Mn, and Cu on cellulose layers with solvent mixtures of acetone-HCl-water in various ratios. For many of the metals, particularly for lead, the methods published are still unsatisfactory and at best of a qualitative nature.

In this work an attempt has been made to achieve better and faster separation of metal ions suitable for *in situ* qualitative evaluation by reflectance spectroscopy or other spectroscopic techniques. A study of the chromatographic properties of lead has been particularly emphasized because of inherent difficulties. New spray reagents for the detection of submicrogram amounts of the ions, and suitable for quantitative work, have also been investigated.

EXPERIMENTAL

Reagents and materials

Stock solutions of Bi^{3+} , Cd^{2+} , Mn^{2+} , Pb^{2+} , U^{6+} , Ni^{2+} , Co^{2+} , Zn^{2+} , Fe^{3+} (1 g/l) were prepared by dissolving the nitrates in doubly distilled water. A few drops of nitric acid were added to the solution of Bi^{3+} and Pb^{2+} ions to avoid hydrolysis and adsorption on the container walls. The stock solution of V^{5+} was prepared by dissolving sodium vanadate (meta) in water. Metallic copper wire, 99.999 % pure, was dissolved in a small amount of conc. nitric acid and diluted to a 1 g/l stock solution.

Cellulose powder MN 300 HR (Macherey, Nagel & Co., Düren, G.F.R.) was used for coating of the plates with a Desaga TLC applicator. A layer thickness of 0.25 mm was employed. The plates were airdried overnight at 25°.

Eastman precoated Chromagram sheets (Eastman Organic Chemicals, Eastman-Kodak Co., Rochester, N.Y.) were also used in this investigation. The cellulose sheets are without binder, and the silica gel sheets contain a solvent-resistant polyvinyl alcohol binder.

Chromatography

The test metals were spotted in 0.5- to 1.0- μg amounts, resulting in a spot approximately 0.5 cm in diameter. Exceptions were Pb (5 to 10 μg per spot) and Cd (4.5 μg per spot). The solutions were applied by means of 1- μl disposable micropipettes "Microcaps" (Drummond Scientific Co., Broomall, Pa.). Rectangular chambers saturated for 24 h were used. The chromatograms were developed by the ascending technique. The solvent systems used for the development of the chromatograms in this study were: acetone-6 N HCl (9:1), isopropanol-glacial acetic acid-6 N HCl-water (8:1:1:1), methanol-32 % HCl-water (8:1:1), and methanol-25 % HNO_3 -water (8:1:1). The reagent-grade organic solvents were redistilled for chromatographic use. To alter R_F values, 2,4-pentanedione and 2,5-hexanedione (Eastman Organic Chemicals, Eastman-Kodak Co., Rochester, N.Y.) were occasionally added to the solvent systems in 1 % amounts.

The completed chromatograms were either airdried at 20-27° overnight, or dried with a hair-dryer using warm air, in vertical position, then exposed to ammonia vapour for 20 min and dried again with a hair-dryer at room temperature.

Detection

For the detection of the spots the following spray reagents were prepared: 4-(2-thiazolylazo)resorcinol (TAR) (Aldrich Chemical Co. Inc., Milwaukee, Wisc.),

1-(2-pyridylazo)-2-naphthol (PAN) (J. T. Baker Chemical Co., Phillipsburg, N.J.), 4-(2-pyridylazo)resorcinol (PAR) and 1-(2-thiazolylazo)-2-naphthol (TAN) (Eastman Organic Chemicals, Eastman-Kodak Co., Rochester, N.Y.). The solutions were made 0.1 % reagent in 95 % ethanol.

An aerosol atomizer was used for spraying the chromatograms, the exact spraying procedure depending upon the reagent used.

After treatment with TAR or PAR the sprayed chromatograms were placed in a tank saturated with ammonia vapour in order to obtain a deep coloration of the spots. They were kept to dry in this condition for stabilization of the complexes.

After spraying with TAN or PAN the chromatograms were dried with the hair-dryer at room temperature and then for 1 to 2 min placed in a tank saturated with ammonia vapour.

Purity of materials

In preliminary work on the estimation of trace metals, considerable difficulties were encountered due to the presence of large amounts of zinc and iron impurities in the chromatographic adsorbents.

Impurities in cellulose powder and on cellulose and silica gel sheets were re-

TABLE I

R_F VALUES OF SOME CATIONS SEPARATED ON VARIOUS ADSORBENTS WITH SOLVENT SYSTEMS

Solvent systems: (A) Isopropanol-glacial acetic acid-6 N HCl-water (8:1:1:1) and (B) same as A + 1 ml 2,5-hexanedione. (a) Chromagram sheets prewashed with acetone-6 N HCl (9:1). (b) Chromagram sheets prewashed with solvent system B. C = comet; D = diffuse spot; T = tailing; ST = strong tailing.

Metal	$R_F \times 100^a$				
	Cellulose plate MN 300 HR	Cellulose sheet		Silica gel sheet	
		B	A	B	A
Bi(III)	77	77	80	72	71
Cd(II)	84	89	85	88	96
Co(II)	9	9	9	23	22
Cu(II)	22	20	20	46	42
Fe(III)	82	85	82	90	98
Mn(II)	8	11	11	37	35
Ni(II)	8	7	7	19	19
Pb(II)	2 T	T	27 T	ST	ST
U(VI)		26 C	26 (I)	30	33
V(V)	17	(35-21)	33 (II)	ST	ST
Zn(II)	86	20 C	19 D		
		(30-13)	(24-12)		
Wet front		91	87	91	94
Distance (cm)	15	86	88	89	92
Temperature (°C)	21	14	14	14	11
Time	3 h	25	22	24	25
	35 min	2 h	3 h	8 h	5 h
		35 min	10 min	35 min	30 min

^a Average of six to ten results.

moved by prewashing of the layers with the same solvent system used later for separation. Iron and zinc as the major heavy metal impurities were running with the solvent front in most solvent systems investigated, and can therefore be eliminated. The level of metal contamination in the hydrochloric acid was negligibly small, since only up to 10% of 6 *N* HCl by volume has been used in the solvent systems. During the purification step it was observed that the solvent system acetone-6 *N* HCl (9:1) dissolved away small quantities of the cellulose; therefore another chamber must be used for prewashing in order to avoid an increase of metallic impurities in the solvent mixture due to the washing procedure.

Great care must be taken in storing the coated plates and sheets, since the reagents used for the detection of the metals are very sensitive to iron particles present in the dust of the atmosphere.

Contamination was further reduced by using only redistilled water from an all-glass Pyrex distilling apparatus. Before use, all glassware was cleaned with hot nitric acid, and rinsed with redistilled water, followed by rinsing with ethanol.

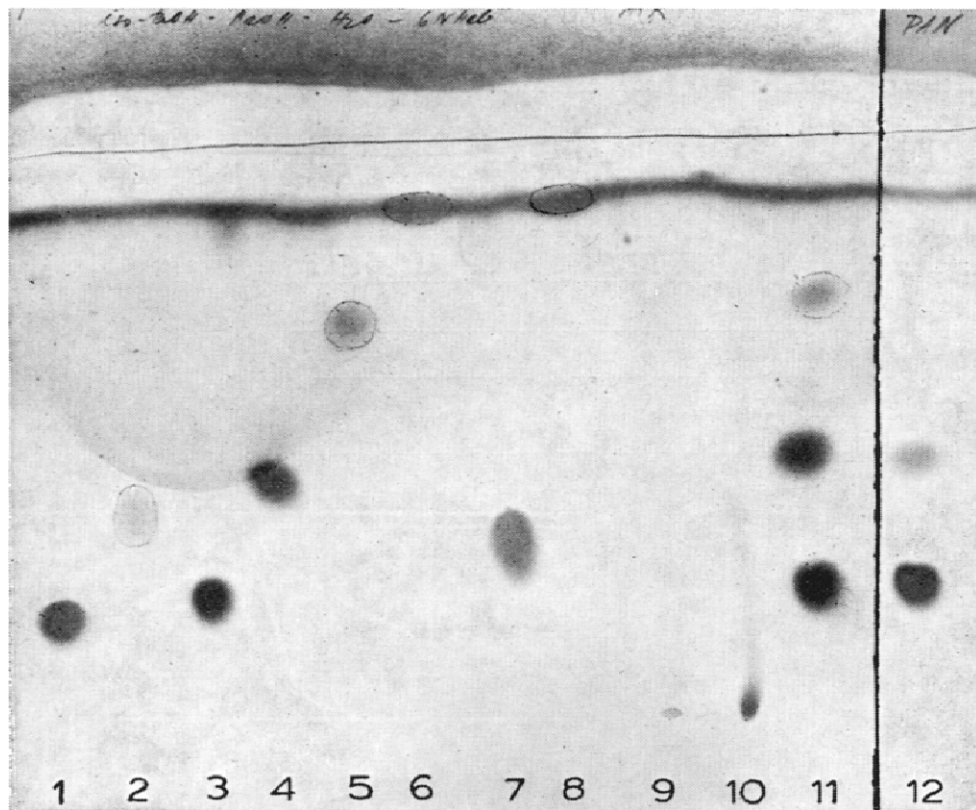


Fig. 1. The separation of metals by the ascending technique in the solvent system isopropanol-glacial acetic acid-6 *N* HCl-water (8:1:1:1) on Eastman silica gel sheets No. 6061. Concentration: 1 μg of metal per spot, except for Pb (5 μg), Cd (4.5 μg) and U(VI) (2 μg). Detection: 1-11 with PAR; 12 with PAN. Spots from left to right: 1 = Ni, deep red; 2 = Mn, light red brown; 3 = Co(II), deep red brown; 4 = Cu, deep red brown; 5 = Bi, red; 6 = Cd, red; 7 = U(VI), bright red; 8 = Zn, red; 9 = Pb, wine red; 10 = V(V), violet; (11 and 12 are mixtures of Co, Cu, and Bi; colour with PAN: Co = green, Cu = red violet, Bi, no detection). Background for PAR and PAN is yellow. Fe(III) is in the wet front strip. The dark band at the top of the chromatogram is due to impurities from the prewashing step.

RESULTS AND DISCUSSION

Separation of Bi, Cd, Co, Cu, Fe, Mn, Ni, Pb, U(VI), V(V), and Zn on various adsorbents

The solvent mixture isopropanol-glacial acetic acid-water (8:1:1) was used for the separation of Pb, Cd, Cu, Bi, and Hg by circular paper chromatography¹². With the same solvent used in conjunction with cellulose plates and sheets, Ni, Co, and Mn remained at the starting point and Bi gave a tailing spot near the origin.

The addition of hydrochloric acid to the system caused increasing migration of Ni, Co, Cu, Cd, and Bi, and the tailing of Cu and Bi spots was suppressed.

The chromatographic behaviour of the cations on various adsorbents, with the modified solvent system isopropanol-glacial acetic acid-6 N HCl-water (8:1:1:1) is depicted in Table I. No significant difference in R_F values is observed on cellulose plates and sheets. Lead is tailing in all three systems. A very close double spot formation was observed for U(VI) on cellulose sheets with the addition of 2,5-hexanedione to the solvent system. This can be explained by multiple zone formation of the solute in the solvent system rather than a change in oxidation states. The migration observed for the same cations on silica gel is generally higher. Little or no change of R_F values has been observed with the addition of 2,5-hexanedione as a complexing agent. The speed of migration of the cations is increasing in the order Ni < Co < Mn < Cu < U(VI) < Bi < Fe < Cd-Zn on cellulose layers, while on silica gel the following increase was observed: Ni < Co < U(VI) < Mn < Cu < Bi < Cd-Fe-Zn.

TABLE II

R_F VALUES OF SOME CATIONS OBTAINED ON CELLULOSE AND SILICA GEL LAYERS IN THE SOLVENT SYSTEM ACETONE-6 N HCl (9:1)

(a) Without purification of plates; (b) prewashed with acetone; (c) prewashed with the system acetone-6 N HCl (9:1). C = comet; D = diffuse spot; T = tailing.

Metal	$R_F \times 100^a$					
	Cellulose plates MN 300			Cellulose sheet	Silica gel sheet	Whatman paper No. 1
	a	b	c	c	c	
Bi(III)				83 T	93	78
Cd(II)	72			71 C		69
Co(II)	58	59	60	50	87	42
Cu(II)	67	69	73	61	90	57
Fe(III)	100	100	100	100	100	78
Mn(II)	34	35	37	29	77	26
Ni(II)	2	2	2	2	5	0
Pb(II)	T	T	T	62		58
U(VI)				61		59
V(V)				9 (I)		
				65 (II)		60
Zn(II)				85 D		78
Wet front	67	69	73	61		
Distance (cm)		16		16	16	17
Temperature (°C)		23		22	23	22
Time (min)		40		40	120	60

^a Average of eight to twelve results.

A sharp resolution was obtained on silica gel for Ni (or Co)-U(VI) (or Mn)-Cu-Bi, with R_F 0.19 (or 0.23)-0.30 (or 0.37)-0.46-0.72, respectively. The solvent mixture is also suitable for the separation of Bi from all the other metal ions on silica gel, but the development is time-consuming.

The separation of the cations on silica gel sheets is shown in Fig. 1.

The results obtained for the separation of the cations on cellulose and silica gel layers with the solvent system acetone-6 *N* HCl (9:1) are summarized in Table II, and also compared to R_F values obtained on paper.

On cellulose plates and sheets the same R_F values were obtained for Cd and Fe. The lead again showed strong tailing. The R_F values were generally higher on sheets and plates than on paper. Vanadium(V) yielded double spots with R_F 0.09 for the main spot and R_F 0.65 for the second spot, which was much smaller. On Whatman No. 1 paper only one species with R_F 0.60 was obtained. The time required for TLC separation was less than for paper chromatography, using the same solvent system.

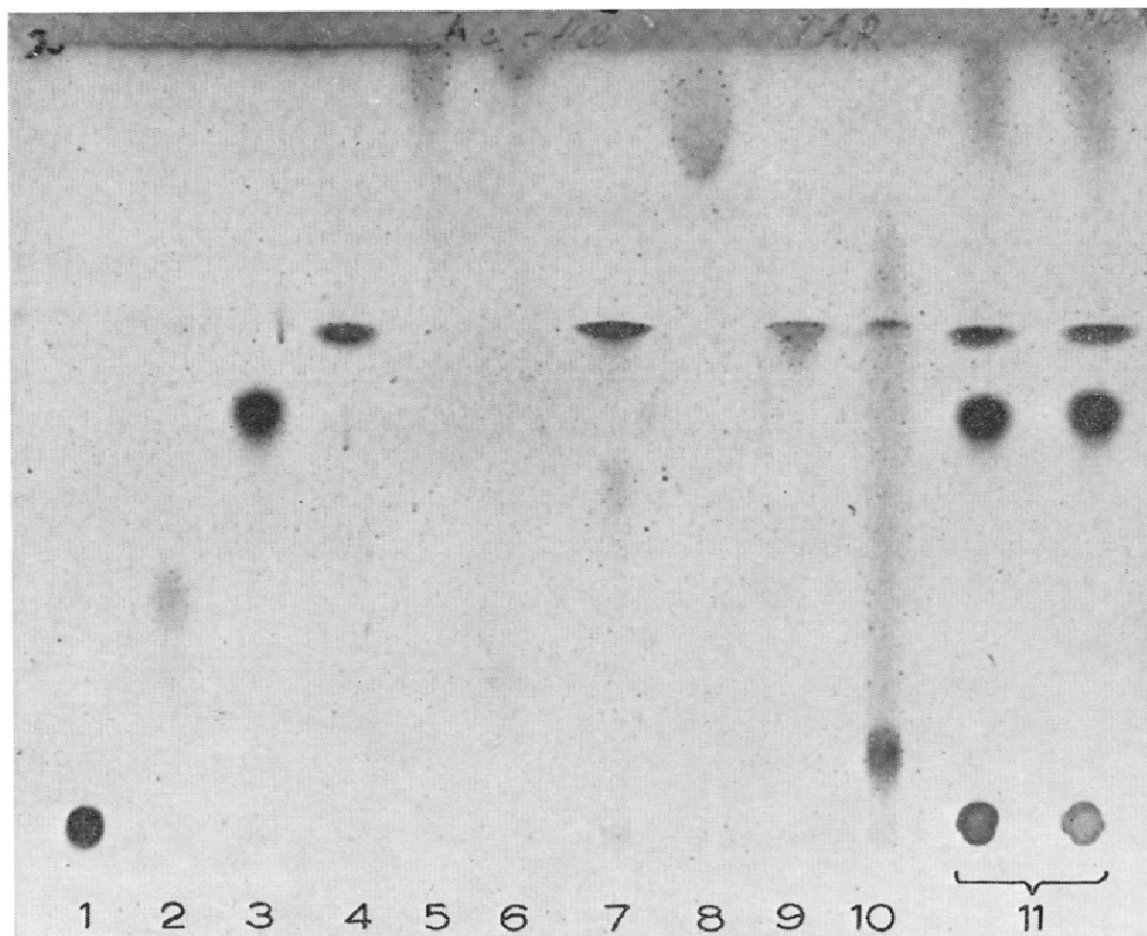


Fig. 2. The separation of metals by the ascending technique in the solvent system acetone-6 *N* HCl (9:1) on Eastman cellulose sheets No. 6064. Concentration: 1 μg of metal per spot, except for Pb (5 μg) and Cd (4.5 μg). Detection with TAR. Spots from left to right: 1 = Ni, violet; 2 = Mn, gray violet; 3 = Co, violet; 4 = Cu, violet; 5 = Bi, violet; 6 = Zn, deep red; 7 = U(VI), violet; 8 = Cd, deep red; 9 = Pb, violet; 10 = V(V), blue; 11 = mixture of Ni, Co, Cu, and Bi.

On silica gel nickel remained close to the starting line (R_F Ni = 0.05) and could be separated easily from the other cations which were running close to the organic solvent front.

The separation of the cations on cellulose sheets is shown in Fig. 2. Ni, Mn, Co, and Cu, with R_F values of 0.02, 0.29, 0.59, and 0.70, respectively, were separated within 40 min (for a distance of 16 cm). Cu, Pb, and U(VI) appeared in the wet front.

Separation of lead

The separation of lead from other metal ions was studied in more detail, due to its tendency to tail. In various studied solvent systems (see Part II, ref. 13) we found strong tailing spots of lead on cellulose as well as on silica gel. Only in the system acetone-6 N HCl (9:1) (Table II) no tailing of Pb was observed, but Cu showed the same R_F value.

The solvent mixture methanol-32% HCl-water (8:1:1) was first investigated. Contrary to satisfactory results obtained with this system on Whatman No. 1 paper¹⁴ (R_F Pb = 0.35), no useful separation was observed on cellulose plates and sheets. The addition of 2,5-hexanedione and 2,4-pentanedione, to reduce the tailing, was

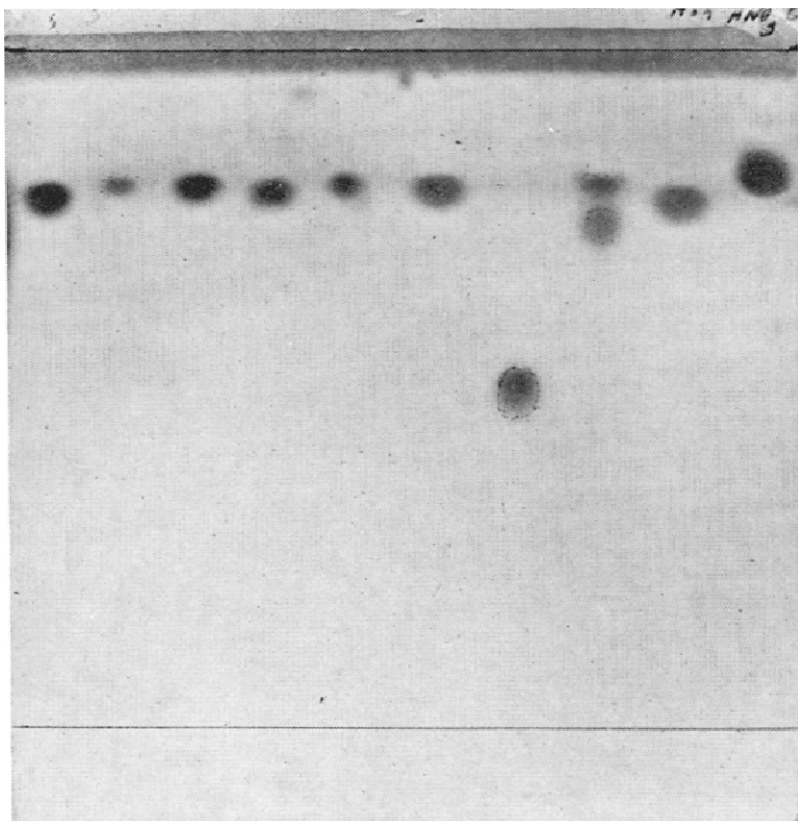


Fig. 3. The separation of lead by the ascending technique in the solvent system methanol-25% HNO_3 - H_2O (8:1:1) on Eastman cellulose sheet No. 6064. The 16-cm run took 70 min; detection was by TAR. Amounts of metal in μg per spot; corresponding R_F values are indicated in brackets. From left to right in the wet front ($R_F = 0.77$): Ni (1; 0.77), Mn (0.1; 0.79), Co(II) (0.5; 0.79), Cu (1; 0.78), Bi (0.1; 0.79), Zn (1; 0.79), Pb (5; 0.50), V(V) (1; 0.73 and 0.79), Cd (4.4; 0.77), U(VI) (5; 0.81); Fe(III) impurities are in the solvent front.

found to have little effect, contrary to previous literature citations⁴. On silica gel layers the R_F value of Pb was too high, and other cations interfered.

The solvent system methanol-25% HNO_3 -water (8:1:1) finally gave satisfactory results. No tailing was observed on cellulose layers under these conditions, and a complete separation was achieved within about 40 min (for a distance of 10 cm) from all the other cations. The R_F value of Pb was found to be 0.50. The successful separation of lead from other cations is shown in Fig. 3. Vanadium(V) gave two clear double spots, with R_F values of 0.73 and 0.79, respectively.

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REFERENCES

- 1 D. I. RYABCHIKOV AND M. P. VOLYNETS, *Zh. Anal. Khim.*, 21 (1966) 1348; *J. Anal. Chem. USSR (Engl. Transl.)*, 21 (1966) 1197.
- 2 M. LEDERER, *Chromatog. Rev.*, 9 (1967) 115.
- 3 G. NICKLESS, *Advan. Chromatog.*, 7 (1968) 142.
- 4 H. SEILER, *Helv. Chim. Acta*, 43 (1960) 1939; H. SEILER *et al.*, in E. STAHL, *Dünnschicht-Chromatographie. Ein Laboratoriumshandbuch*, 2nd Ed., Springer-Verlag, Berlin, Göttingen, Heidelberg, 1967, pp. 800-801.
- 5 V. D. CANIĆ, S. M. PETROVIĆ AND A. K. BEM, *Z. Anal. Chem.*, 213 (1965) 251.
- 6 V. D. CANIĆ AND S. M. PETROVIĆ, *Z. Anal. Chem.*, 211 (1965) 321.
- 7 P. KÜNZI, J. BÄUMLER AND J. OBERSTEG, *Deut. Z. Ges. Gerichtl. Med.*, 52 (1962) 605.
- 8 H. SEILER, *Helv. Chim. Acta*, 45 (1962) 381.
- 9 F. W. H. M. MERKUS, *Pharm. Weekblad*, 103 (1968) 1037.
- 10 F. W. H. M. MERKUS, *Pharm. Weekblad*, 104 (1969) 55.
- 11 F. W. H. M. MERKUS, *J. Chromatog.*, 41 (1969) 497.
- 12 T. BARNABAS AND J. BARNABAS, *Naturwiss.*, 42 (1955) 15.
- 13 V. MIKEŤUKOVÁ AND R. W. FREI, *J. Chromatog.*, 47 (1970) 435.
- 14 V. MIKEŤUKOVÁ AND K. KÁCL, *Intern. Congr. Pure Appl. Chem.*, XXIst, Prague, Sept. 4-10, 1967; *Toxicol. Chem. Abstr.*, T-67.

J. Chromatog., 47 (1970) 427-434