Reprinted from

Journal of Chromatography
Elsevier Publishing Company, Amsterdam · Printed in The Netherlands

CHROM. 4342

Polyamide–silica gel thin-layer chromatography of water-soluble vitamins

Thin-layer chromatography has proved to be a valuable analytical technique in the separation of vitamins. However, relatively few publications appeared concerning the separation of water-soluble vitamins by this technique; the use of silica gel (*e.g.* refs. 1–4), aluminum oxide[4], starch[5] and polyamide[6] has been reported.

Recently, polyamide–silica gel mixed layers have been successfully used for the separation of red food dyes[7]. Therefore the mixed-layer method was further applied to separate eleven water-soluble vitamins. For comparison thin-layer chromatography using only polyamide and silica gel was performed under the same conditions. Separation on polyamide–silica gel mixed layers was found to be preferable.

Experimental

Preparation of polyamide–silica gel (2:1) mixed layer. Twenty grams of polyamide chip (Nylon 6, type 1022B of UBE Industrial Ltd., Osaka, Japan) were dissolved in 80 ml of 90% formic acid; then 20 ml of distilled water were added. After warming (below 40°) and stirring, a homogeneous solution was obtained; after cooling it to room temperature, 10 g of Silica Gel G (E. Merck) were added. Two hundred milliliters of the above-mentioned solution were poured into a dish (14.5 × 19.5 × 2.5 cm), and a glass plate (12 × 14 × 0.1 cm) was dipped into it. Both sides of the glass were covered homogeneously. The glass was hung for 2 min over the dish to let the excess solution drain back before suspending it in a water-saturated cabinet (50 × 50 × 50 cm) for half a day. It was then taken out of the cabinet and heated at 100° for 30 min.

Preparation of polyamide layer. The above-mentioned method was employed but without the addition of Silica Gel G.

Preparation of silica gel layer. Plates of Silica Gel G were prepared by using Desaga S 11 spreader, pre-set to give an applied layer 250 μ thick, and then were heated at 100° for 30 min.

Chromatographic procedure

Two percent orotic acid dimethylamine (*ca.* 40%) solution, 2% ascorbic acid solution and 0.5% of other vitamin solutions were applied to the start line 1.5 cm from the bottom of the layer. The plates were developed in the dark by ascending techniques. The chamber had been equilibrated with the respective solvent for 30 min before use.

Visualization. Rutin, riboflavin, riboflavin 5′-phosphate sodium and cyanocobalamine can be recognized under long-wave length UV light at 365 mμ. The layers were sprayed with a 0.07% Rhodamine B alcoholic solution and all the spots could be observed under UV light at 254 mμ.

Results and discussion

R_F values of polyamide–silica gel mixed layers, silica gel layers and polyamide layers with two solvent systems are given in Table I. It has been found that the results obtaining using the mixed layers show better separation and sharper spots. The spots on the silica gel layers are rather diffused and larger. In the preparation of polyamide

TABLE I

R_F VALUES OF VITAMINS ON DIFFERENT LAYERS

P–S = polyamide–silica gel layer; S = silica gel layer; P = polyamide layer.

No.	Samples	10% NaCl solution			10% sodium acetate solution		
		P–S	S	P	P–S	S	P
1	Orotic acid	0.00	0.86	0.00	0.00	0.90	0.00
2	Rutin	0.01	0.43[a]	0.30[a]	0.10	0.54[a]	0.05
3	*p*-Aminobenzoic acid	0.06	0.64	0.11	0.65	0.80	0.78
4	Riboflavin	0.18	0.22	0.29	0.20	0.29	0.24
5	Cyanocobalamine	0.27	0.10	0.04	0.40	0.30	0.06
6	Thiamine HCl	0.40	0.15	0.86	0.32	0.10	0.93
7	Riboflavin 5′-phosphate sodium	0.43	0.22	0.79	0.47	0.29	0.67
8	Nicotinamide	0.53	0.43	0.60	0.52	0.55	0.70
9	Pyridoxine HCl	0.60	0.45	0.74	0.58	0.59	0.77
10	Nicotinic acid	0.74	0.47	0.77	0.73	0.62	0.88
11	Ascorbic acid	0.98	0.88	0.90	0.90	0.85	0.98
Time required (min)[b]		110	10	40	110	15	55

[a] Tailing.

[b] Time required to ascend 10 cm from origin.

layers, slow drying of layers in the water-saturated cabinet is essential to reduce the developing time of these layers.

The content of polyamide (*ca.* 66%) in these mixed layers is greatly increased compared to that of the previous report (12%)[7] for getting suitable results. The layer did not crack or peel and can be stored easily. The method is suitable for the identification of various water-soluble vitamins.

Department of Pharmacy, Taipei Medical College, Taipei, Taiwan (Republic of China)

HUNG-CHEH CHIANG
YU LIN
YING-CHANG WU

1 H. GÄNSHIRT AND A. MALZACHER, *Naturwiss.*, 47 (1960) 279.
2 E. NÜRNBERG, *Deut. Apotheker-Ztg.*, 101 (1961) 142.
3 E. NÜRNBERG, *Deut. Apotheker-Ztg.*, 101 (1961) 268.
4 S. ISHIKAWA AND G. KATSUI, *Vitamin*, 29 (1964) 203.
5 S. E. PETROVIĆ, B. E. BELIA AND D. B. VUKAJLOVIC, *Anal. Chem.*, 40 (1968) 1007.
6 H.-P. CHUANG, H.-C. CHIANG AND K.-T. WANG, *J. Chromatog.*, 41 (1969) 487.
7 H.-C. CHIANG, *J. Chromatog.*, 40 (1969) 189.

Received August 11th, 1969

J. Chromatog., 45 (1969) 161–162