

CHROM. 4007

Separation of thiamine derivatives by polyamide layer chromatography

Polyamide layer chromatography has been successfully used for the separation of many types of compounds, *e.g.*, chloramphenicol derivatives¹, sulphonamides², indole derivatives³ and DNP-amino acids⁴⁻⁶ etc. Recent advances in the preparation of polyester film supported polyamide layers have resulted in the easier handling and better separation of more compounds. Separation is based on the reversible formation of hydrogen bonds between the amide bonds of the polymer and the compound being separated⁷.

Recently, thin-layer chromatography has been the preferred method^{8,9} of separation but the adsorbents used are largely Silica Gel G. The separation of thiamine derivatives on polyamide medium has not been reported.

The development of a method for the routine determination of thiamine derivatives for the purity control and official analysis of pharmaceutical preparations is important. In view of the several advantages of polyamide layers, we attempted the separation of thiamine derivatives on polyamide.

Experimental

Materials. All samples were supplied by P. C. CHIANG of Taiwan Provincial Hygienic Laboratory. The solvents were Wako Pure Chemicals Ltd., Osaka, Japan, reagent grade. The polyamide resin was Amilan CM 1011 (poly- ϵ -caprolactam) of Toyo Rayon Co., Tokyo, Japan. The polyamide layers were prepared according to WANG AND WANG⁶.

Chromatography. The ascending method described by WANG AND WANG was followed⁶. The solvent was allowed to ascend 10 cm from the start.

Visualization. The spots are detected by iodine vapor or iodoplatinate reagent¹⁰.

TABLE I
CHROMATOGRAPHIC DATA OF THIAMINE DERIVATIVES

No.	Substance	Solvents*			
		I	II	III	IV
		Time required (min)			
		25	20	30	30
1	Thiamine hydrochloride	0.03	0.02	0.05	0.05
2	Thiamine monophosphate	0	0	0	0
3	Thiamine disulfide	0.36	0.18	0.29	0.23
4	Thiamine propyl disulfide (Alinamin)	0.77	0.74	0.72	0.62
5	Thiamine tetrahydrofurfuryl disulfide (Alinamin F)	0.67	0.67	0.68	0.57
6	Dibenzoyl thiamine	0.75	0.83	0.87	0.82
7	Thiamine β -hydroxyethyl disulfide (Neometabolin)	0.49	0.38	0.49	0.29
8	Dicethiamine hydrochloride	—	0.94	—	—
9	Thiamine 8-methyl-6-acetyl dihydrothioctate disulfide (Neuvitan)	0.72	0.77	0.80	0.72

* Solvent systems: I = Acetone; II = acetone-diethyl ether-glacial acetic acid (20:20:1); III = methyl ethyl ketone; IV = chloroform-ethyl acetate-glacial acetic acid (20:20:1).

Result and discussion

R_F values obtained with four different solvent systems are given in Table I and typical chromatograms are shown in Figs. 1 and 2. It can be seen that a sharp resolution of thiamine derivatives is obtained by polyamide layer chromatography. We tried some solvent systems which were suitable for paper and thin-layer chromatography, but we found these solvents were unsuitable for polyamide layer chromatography.

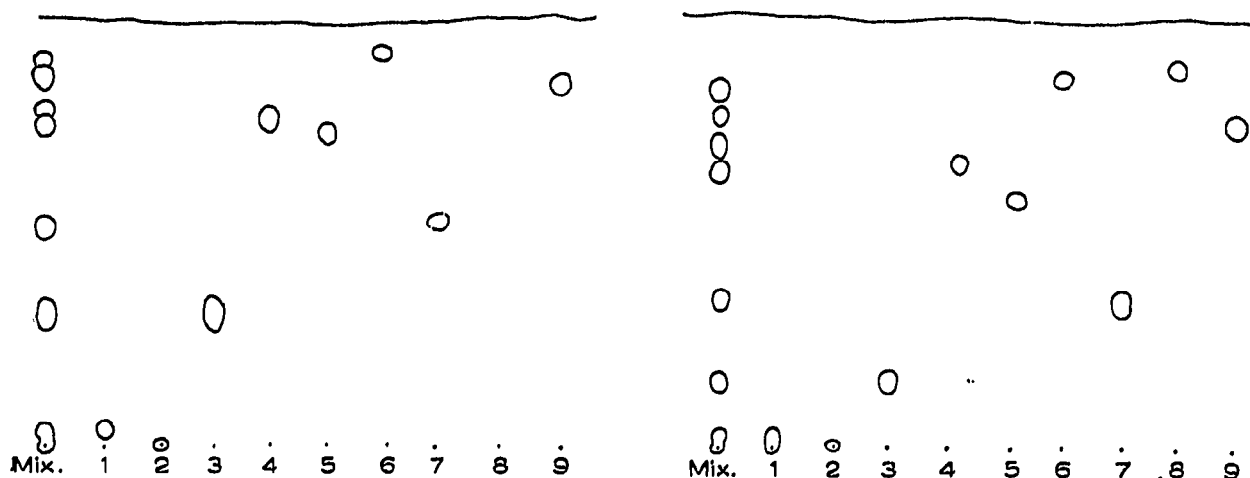


Fig. 1. Sketch of a chromatogram after detection by iodine vapor. Solvent: methyl ethyl ketone. The spots are numbered as in Table I. No. 8 could not be detected and is probably on the solvent front.

Fig. 2. Sketch of a chromatogram after detection by iodine vapor. Solvent: acetone-diethyl ether-glacial acetic acid (20:20:1). The spots are numbered as in Table I.

Iodine vapor can be used for detection but iodoplatinate reagent is much preferred because of its higher sensitivity. Ten μg of thiamine derivatives were easily detected by the iodoplatinate reagent. The violet spots changed to pale yellow after standing.

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Received February 11th, 1969