Polyamide layer chromatography of chloramphenicol and its derivatives

In a previous note, we reported the chromatography of sulfonamides on a polyamide layer[1]. Another application of polyamide layer chromatography is described below.

ENDRES AND HORMANN[2] described polyamide column chromatography of nitro-compounds and WANG AND HUANG[3] succeeded in separating DNP-amino acids by polyamide layer chromatography. Chloramphenicol is an antibiotic with a nitro group on the benzene nucleus and would be sorbed by polyamide. Although several authors[4–6] have described the paper chromatography of chloramphenicol, the only literature on TLC of chloramphenicol was by LIBOSVAR *et al.*[7], who used a thin layer of aluminum oxide for the control of the classical chloramphenicol synthesis.

We have used a layer of polyamide according to WANG[8] to separate chloramphenicol and its derivatives by two solvent systems, *viz.*:

(A) *n*-butanol–chloroform–acetic acid (10:90:0.5),

(B) *n*-butanol–water–acetic acid (82:18:0.5).

In preliminary experiments, we used benzene, *n*-butanol, chloroform, ethyl acetate and combinations of these solvents. In these systems, *n*-butanol–chloroform (10:90) and *n*-butanol–water (82:18) are suitable, but chloramphenicol succinate has a tendency to "tailing". We found that addition of only 0.5% glacial acetic acid or formic acid (90%) to the solvent mixture was effective in depressing the "tailing".

The R_F values and chromatograms obtained with these two solvent systems are shown in Table I and Figs. 1a and b.

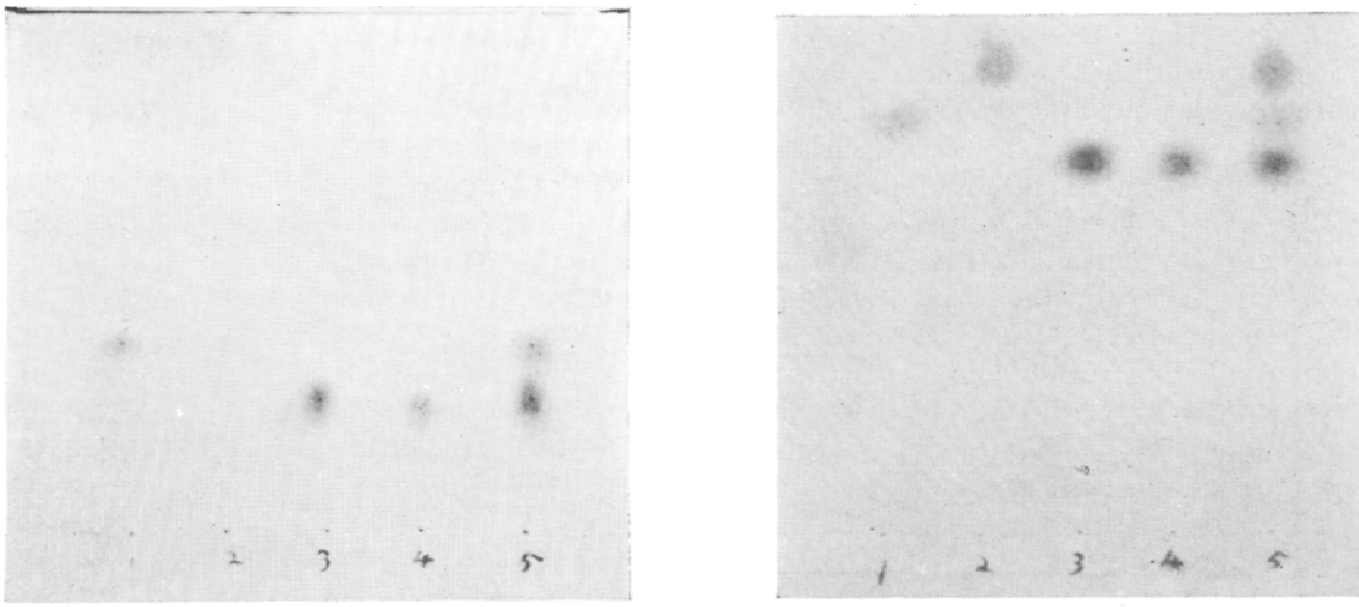
For visualization, the chromatograms were sprayed with 0.25% stannous chloride in 1 *N* HCl, then, after drying, with 2% *p*-dimethylaminobenzaldehyde in 1.2 *N* HCl. All compounds appear as bright yellow spots after a few hours. We found the minimum detectable quantities were as follows: chloramphenicol 10 γ, chloramphenicol palmitate 40 γ, and chloramphenicol succinate 30 γ.

TABLE I

R_F VALUES OF CHLORAMPHENICOL AND ITS DERIVATIVES ON POLYAMIDE LAYERS

	Solvent A n-Butanol–chloroform–acetic acid (10:90:0.5)	*Solvent B n-Butanol–water–acetic acid (82:18:0.5)*
Chloramphenicol	0.35	0.80
Chloramphenicol palmitate	0.95	0.90
Chloramphenicol succinate	0.25	0.72

In Fig. 1b we can recognize the presence of chloramphenicol in chloramphenicol palmitate and we also found two spots due to impurities or decomposition products in chloramphenicol sodium succinate (lyophilized), one of which is apparently chloramphenicol.



(a) (b)

Fig. 1. Chromatograms of chloramphenicol and its derivatives. Polyamide layer according to WANG[8]. (a) Solvent system: *n*-butanol–chloroform–acetic acid (10:90:0.5); 1 h at 29°. (b) Solvent system: *n*-butanol–water–acetic acid (82:18:0.5); 5 h at 29°. 1 = Chloramphenicol, cryst.; 2 = chloramphenicol palmitate, cryst.; 3 = chloramphenicol acid succinate, cryst.; 4 = chloramphenicol sodium succinate, lyophylized; 5 = mixture of 1, 2, and 3.

We also applied this method to pharmaceutical preparations (chloramphenicol capsules, chloramphenicol tablets, chloramphenicol palmitate granules, chloramphenicol sodium succinate for injection etc.) from local pharmacies and found the solvent systems and method suitable.

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1 Y. T. LIN, K. T. WANG AND T. I. YANG, *J. Chromatog.*, 20 (1965) 610.
2 H. ENDRES AND H. HORMANN, *Angew. Chem. Intern. Ed. Engl.*, 2 (1963) 254.
3 K. T. WANG AND J. M. K. HUANG, *Nature*, (1965) in press.
4 R. J. BLOCK, E. L. DURRUM, G. ZWEIG, *Paper Chromatography and Paper Electrophoresis*, Academic Press, New York, 1958, p. 396.
5 G. N. SMITH AND C. S. WORREL, *Arch. Biochem.*, 28 (1950) 1.
6 W. T. SOKOLSKI, H. KOFFLER AND P. A. TETRAULT, *Arch. Biochem.*, 43 (1953) 236.
7 J. LIBOSVAR, J. NEDBAL AND V. HACH, *C.A.*, 57 (1962) 16452d.
8 K. T. WANG, *J. Chinese Chem. Soc. (Taiwan)*, 8 (1961) 241.

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