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Note

p-Dimethylaminocinnamaldehyde as a spray reagent for sulphonamides in combined Avicel–Kieselguhr thin-layer chromatography

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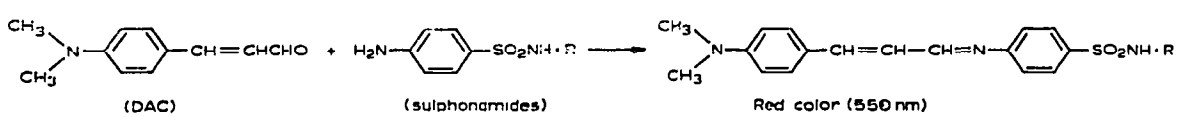
p-Dimethylaminobenzaldehyde (DAB) has been used as a colour reagent for aromatic primary amines in many cases. It was found by the author that the use of *p*-dimethylaminocinnamaldehyde (DAC) in alcohol with the addition of a mineral acid gave a more sensitive and deeper colouration. The colouration given by DAB was yellow, while the colouration given by DAC was red* with aromatic primary amines. Its application in this connection has therefore been investigated and it has been considered as a location agent for identifying sulphonamides. Preliminary experiments showed that DAC gave much better results than DAB as a colour reagent. The standard method of colour development was as follows. The reagent solutions for sulphonamides were (A) DAB (50 mg) + concentrated sulphuric acid (1 ml) + 95% ethanol to 100 ml; and (B) DAC (50 mg) + concentrated sulphuric acid (1 ml) + 95% ethanol to 100 ml. In the quantitative test, 1 ml of sulphonamide solution (200 μg/ml) plus 1 ml of reagent solution were diluted to 20 ml with 95% ethanol containing 1% sulphuric acid and the colour was developed. In the qualitative test, to a few drops (5–10 μg) of sulphonamide solution were added 1–2 drops of reagent solution and the colour developed. (The results are given in Table I and Fig. 1.)

TABLE I

COLOURS AND ABSORPTION MAXIMA OF SULPHONAMIDES DEVELOPED BY DAB AND DAC

Sulphonamide	*DAB*			*DAC*		
	Colour	$\lambda_{max.}$ (*nm*)	*Absorbance*	*Colour*	$\lambda_{max.}$ (*nm*)	*Absorbance*
Sulphanilamide	Yellow	450	0.065	Red	550	0.95
Sulphisoxazole	Yellow	450	0.045	Red	550	0.66
Sulphadimethoxine	Yellow	450	0.035	Red	550	0.56
Sulphamethomidine	Yellow	450	0.035	Red	550	0.52

* Mechanism of colour reaction:



(DAC) (sulphonamides) Red color (550 nm)

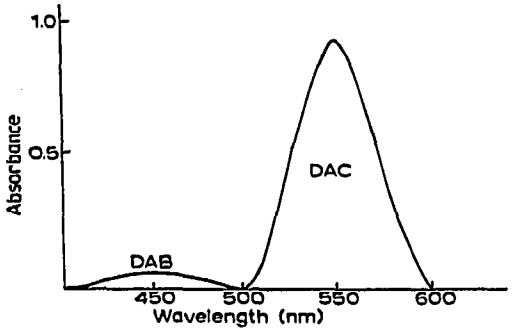


Fig. 1. Absorption curves for sulphonamides (as sulphanilamide) developed by DAB and DAC.

In a previous paper[1] we described the separation of amino acids by Avicel thin-layer chromatography. This durable Avicel layer gave good results and is suitable for application in TLC.

Several investigators have described the TLC of sulphonamides on silica gel[2–7] and polyamide[8], and reported that TLC gave better results than paper chromatography.

We have now applied combined Avicel–Kieselguhr TLC and used DAC as the detection agent for various sulphonamides, and have found it possible to separate sulphonamides by using the following three mobile phases: toluene–chloroform–glacial acetic acid–water (20:20:10:0.5); *o*-dichlorobenzene–acetone–0.5 *N* ammonia solution (20:30:1); and isooctane–methyl ethyl ketone (1:1).

TABLE II

R_F VALUES OF SULPHONAMIDES ON AVICEL–KIESELGUHR THIN-LAYER CHROMATOGRAPHY (300 μm) AT 23°

Solvent systems: 1 = toluene–chloroform–glacial acetic acid–water (20:20:10:0.5); 2 = *o*-dichlorobenzene–acetone–0.5 *N* ammonia solution (20:30:1); 3 = isooctane–methyl ethyl ketone (1:1).

*No.**	*Sulphonamide*	$R_F \times 100$		
		Solvent 1	*Solvent 2*	*Solvent 3*
1	Sulphanilamide	5	49	47
2	Sulphamethazine	47	61	68
3	Sulphathiazole	13	26	34
4	Sulphadiazine	30	28	52
5	Sulphamerazine	41	45	61
6	Sulphisoxazole	30	10	78
7	Sulphisomidine	10	18	31
8	Sulphamethizole	22	5	58
9	Sulphaguanidine	2	24	7
10	Sulphamethoxypyridazine	40	47	62
11	Sulphadimethoxine	58	50	85
12	Sulphaphenazole	44	42	85
13	Sulphisomezole	41	35	83
14	Sulphamethomidine	53	43	80

* Numbers of sulphonamides correspond to spots in Fig. 5.

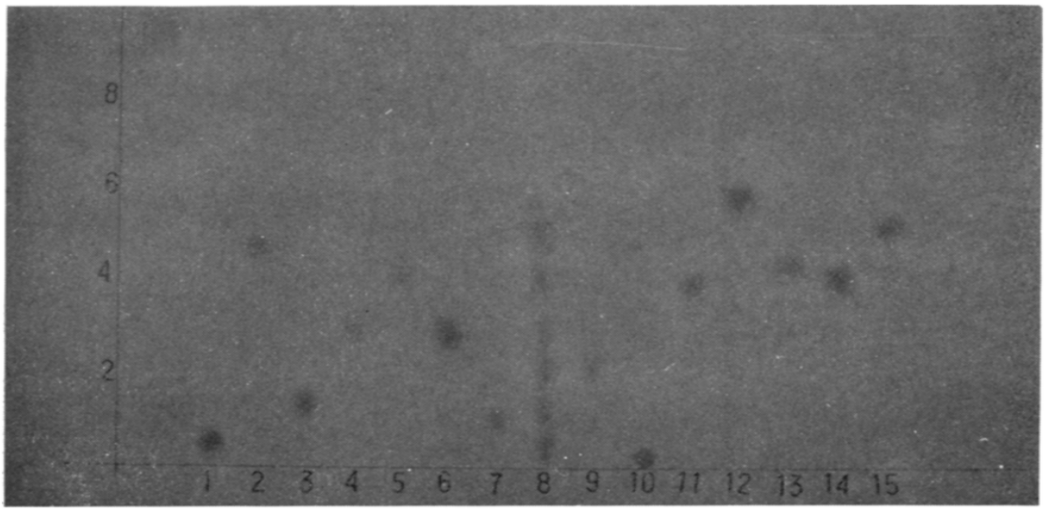


Fig. 2. Thin-layer chromatogram of sulphonamides. Solvent system: toluene–chloroform–glacial acetic acid–water (20:20:10:0.5). Length of run: *ca.* 10 cm; 30 min at 23°. Colour reagent: reagent solution B. Samples: 1 = sulphanilamide; 2 sulphamethazine; 3 = sulphathiazole; 4 = sulphadiazine; 5 = sulphamerazine; 6 = sulphisoxazole; 7 = sulphisomidine; 8 = sulphonamide mixture; 9 = sulphamethizole; 10 = sulphaguanidine; 11 = sulphamethoxypyridazine; 12 = sulphadimethoxine; 13 = sulphaphenazole; 14 = sulphisomezole; 15 = sulphamethomidine.

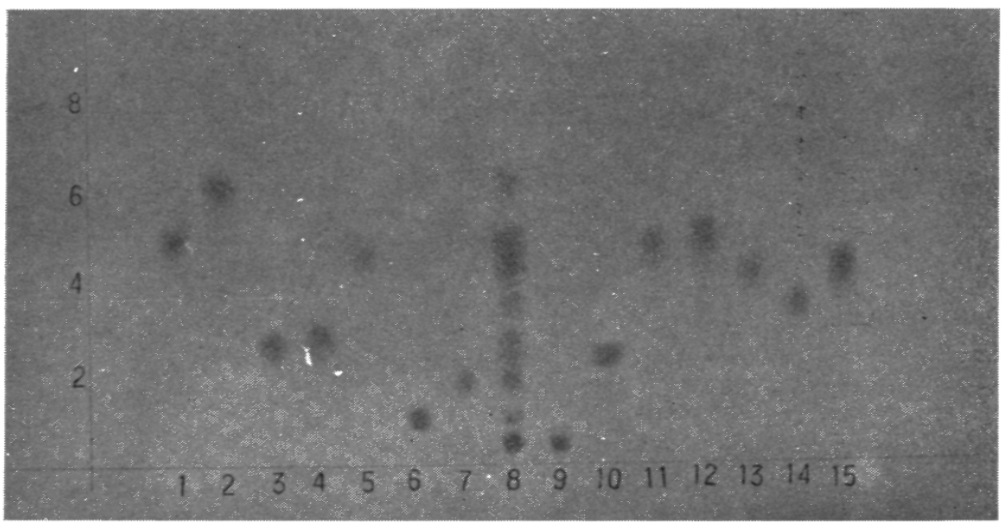


Fig. 3. Thin-layer chromatogram of sulphonamides. Solvent system: *o*-dichlorobenzene–acetone–0.5 *N* ammonia solution (20:30:1). Length of run: *ca.* 10 cm; 20 min at 23°. Colour reagent: reagent solution B. Spots as in Fig. 2.

In the mixed layer, Avicel also serves as a strong binder and makes the layer very durable and easy to handle. Also, the layer did not crack or peel and could be conserved easily.

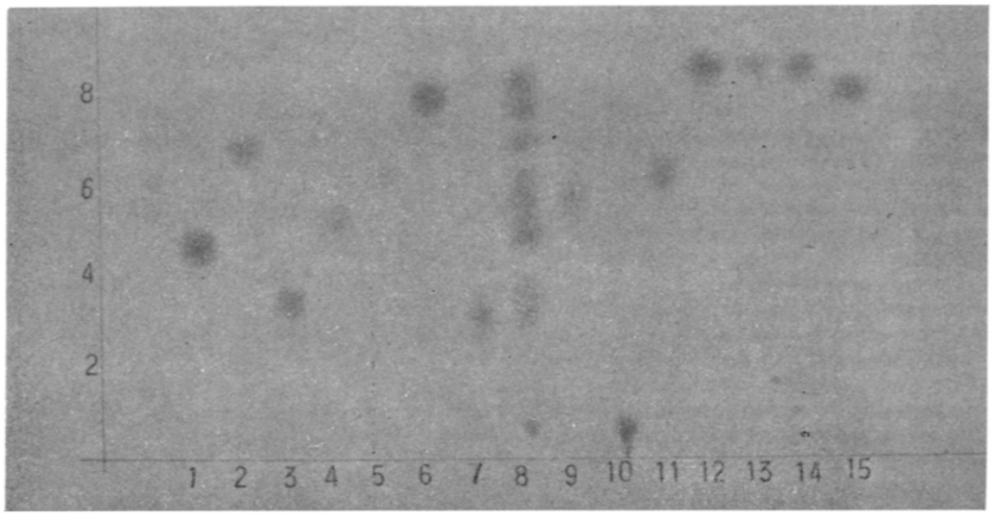


Fig. 4. Thin-layer chromatogram of sulphonamides. Solvent system: isooctane–methyl ethyl ketone (1:1). Length of run: *ca.* 10 cm; 40 min at 23°. Colour reagent: reagent solution B. Spots as in Fig. 2.

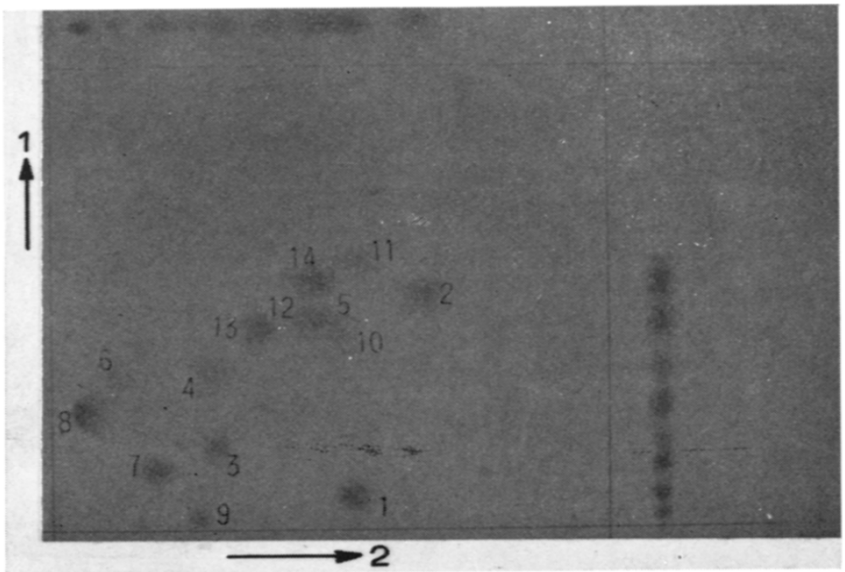


Fig. 5. Two-dimensional Avicel–Kieselguhr thin-layer chromatogram of 14 mixed sulphonamides. Solvent systems: (1) toluene–chloroform–glacial acetic acid–water (20:20:10:0.5); (2) *o*-dichlorobenzene–acetone–0.5 *N* ammonia solution (20:30:1). Length of run: *ca.* 10 cm each side. Colour reagent: reagent solution B. Spots: 1 = sulphanilamide; 2 = sulphamethazine; 3 = sulphathiazole; 4 = sulphadiazine; 5 = sulphamerazine; 6 = sulphisoxazole; 7 = sulphisomidine; 8 = sulphamethizole; 9 = sulphaguanidine; 10 = sulphamethoxypyridazine; 11 = sulphadimethoxine; 12 = sulphaphenazole; 13 = sulphisomezole; 14 = sulphamethomidine.

The combined Avicel–Kieselguhr layers were prepared according to Stahl's method, using an Avicel PH 101 (Asahi Chemical Industry Co., Japan) to Kieselguhr G (E. Merck, Darmstadt, G.F.R.) ratio of 1:1 to make a 20% homogeneous aqueous emulsifying solution. The general method (2.5 g Avicel–Kieselguhr on each 20 × 20 cm glass plate) for combined Avicel–Kieselguhr layer chromatography was followed, using 1–2 μg of sample in each spot.

The R_F values and chromatograms obtained with the three solvent systems are shown in Table II and Figs 2, 3, 4 and 5.

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REFERENCES

1 S.-C. Lee, *Chemistry (Chin. Chem. Soc.)*, No. 4 (1972) 121.
2 E. G. Wollish, M. Schmall and M. Hawrylyshyn, *Anal. Chem.*, 33 (1961) 1138.
3 S. Klein and B. T. Kho, *J. Pharm. Sci.*, 51 (1962) 9.
4 B. T. Kho and S. Klein, *J. Pharm. Sci.*, 52 (1963) 404.
5 T. Fuwa, T. Kido and H. Tanaka, *Arch. Pract. Pharm. (Tokyo)*, 23 (1963) 101.
6 T. Bićan-Fišter and V. Kajganović, *J. Chromatogr.*, 11 (1963) 492.
7 T. Bićan-Fišter and V. Kajganović, *J. Chromatogr.*, 16 (1964) 503.
8 Y. T. Lin, K. T. Wang and T. I. Yang, *J. Chromatogr.*, 20 (1965) 610.