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A note on the identification of sulphonamides by thin-layer chromatography

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A method is described for the identification of 25 sulphonamides by thin layer chromatography. Four systems are used, the solvents being (I) chloroform–methanol (4:1), (II) chloroform–carbon tetrachloride–methanol (7:2:1), (III) ethyl acetate–methanol (9:1), (IV) acetone–methanol (4:1), the plates being coated with silica gel G mixed with (I) sodium hydroxide, (II) potassium hydrogen sulphate, (III) water, (IV) sodium hydroxide. The location reagents are (*a*) copper sulphate, (b) *p*-dimethylamino-benzaldehyde, (c) *N*(1-napthyl) ethylenediamine di-HCl, and (d) fluorescein.

Chromatographic methods for the identification of sulphonamides have been given by Klein & Kho (1962), Kho & Klein (1963), Grafe (1964), Fogg & Wood (1965), Lin, Wang & Yang, (1965); Kamp (1966) and Güven & Pekin (1966). Clarke (1968) noted that sulphonamides could be detected on citrate buffered paper chromatograms (Curry & Powell, 1954) but that the Rf values lay too close together to allow satisfactory identification—a common situation when one has a large group of compounds differing but little in chemical structure. The location reagents generally used [*p*-dimethylaminobenzaldehyde or diazotization followed by coupling with alkaline β-naphthol or *N*-(1-naphthyl)ethylenediamine], although extremely sensitive, are not specific, as they give similar colours with other compounds containing a primary arylamino group, nor will they serve to distinguish one sulphonamide from another. To do so requires the use of some reagent which will give different results with different members of the group. An ammoniacal solution of copper has been used by Güven & Pekin (1966) for this purpose.

The reaction between copper sulphate solution and an alkaline solution of a sulphoamide (Sample, 1945) is well known, and has frequently been used to differentiate between these compounds. It was thought that this might serve as a location reaction which would give different coloured spots on the chromatogram with different drugs. Details of such a method are given here.

EXPERIMENTAL

Thin-layer chromatography

Glass plates, 20 × 20 cm, with a 250 μm coat of silica gel G slurried with the appropriate solution, are dryed for 1 h at 110°. Solvent (100 ml) is placed in tanks, 21 × 21 × 10 cm with ends lined with filter paper and allowed to stand for half an hour. The liquid should be changed after each run and the tank re-equilibrated.

Systems

1. Solvent: chloroform–methanol (4:1). Alkaline plates (30 g silica gel G and 60 ml 0·1M sodium hydroxide solution).

2. Solvent: chloroform–carbon tetrachloride–methanol (7:2:1). Acid plates (30 g silica gel G and 60 ml 0·1M potassium hydrogen sulphate solution).

3. Solvent: ethyl acetate-methanol (9:1). Neutral plates (30 g silica gel G and 60 ml water).

4. Solvent: acetone–methanol (4:1). Alkaline plates, as in System 1.

Location reagents

1. Copper sulphate spray. 5% $CuSO_4$ $5H_2O$ w/v, in water.

2. *p*-Dimethylaminobenzaldehyde spray. 1 g of *p*-dimethylaminobenzaldehyde dissolved in 100 ml of ethanol, and 10 ml of concentrated hydrochloric acid added.

3. *N*(1-naphthyl)ethylenediamine dihydrochloride. 0·1% w/v in water.

4. Fluorescein spray. 1·0% w/v in a mixture of acetone and water (3:1).

Procedure

1·0 μl of a 1·0% solution of the sulphonamide in 0·1M sodium hydroxide solution is spotted on the plate and run for 30 min and the plate then dried and sprayed with the copper sulphate solution. (The acid plate in System 2 must then be sprayed with 0·1N sodium hydroxide). The colours of the spots are noted, and the plate oversprayed with the *p*-dimethylaminobenzaldehyde solution, which gives an intense yellow colour with all sulphonamides, except those with substituent groups on N(4), whether they have reacted with the copper sulphate or not. Alternatively, the compounds may be diazotized by exposing the plate to NO_2 fumes and then spraying with *N*(1-naphthyl) ethylenediamine solution to give intense purple spots.

The results are shown in Table 1.

DISCUSSION

The N(4)-substituted compounds succinylsulphathiazole and phthalylsulphathiazole hardly move from the base line in systems 1, 2 and 3. They give brown-purple spots with the copper sulphate spray, but do not react with *p*-dimethylaminobenzaldehyde nor can they be diazotized. They may be identified by their Rf values in system 4. A spot corresponding to the unsubstituted drug, present as an impurity, or formed by decomposition on the plate, is often seen. Phthalysulphacetamide, which does not react with copper sulphate, can be located as an absorbing spot if the plate is finally oversprayed with fluorescein. Sulphasalazine is an orange compound and gives a yellow-orange spot unaffected by the spray reagents used.

Some compounds may run as streaks rather than spots; this is indicated by the letter "s" in Table 1.

Some of the colours with the copper sulphate spray are faint but the alkaline solutions of the drugs, spotted on filter paper and sprayed with copper sulphate, give colours that may be readily recognized.

Table 1. *Rf values of sulphonamides in four solvent systems*

Sulphonamide	Colour	Rf values System 1	System 2	System 3	System 4
Phthalylsulphacetamide	—	0·00	0·00	0·00	0·11
Phthalylsulphathiazole	Brown purple	0·01	0·00	0·00	0·13
Succinylsulphathiazole	Brown purple	0·02	0·00	0·00	0·4
Sulphacetamide	—	0·13	0·25	0·58	0·45s
Sulphadiazine	Brown	0·33s	0·30s	0·55s	0·55s
Sulphadimethoxine	Yellow	0·66	0·45	0·70	0·72
Sulphadimidine	Orange brown	0·67	0·39	0·63	0·69
Sulphaethidole	Green	0·17	0·36	0·50s	0·43s
Sulphafurazole	Greenish brown	0·18	0·34	0·65	0·66
Sulphaguanidine	—	0·26	0·4	0·37	0·62
Sulphamerazine	Brown	0·56	0·34s	0·59	0·61
Sulphamethizole	Green	0·10	0·33	0·43s	0·45
Sulphamethoxazole	Yellow green	0·47	0·41	0·70	0·71
Sulphamethoxydiazine	Purple brown	0·63	0·39s	0·62	0·68
Sulphamethoxypyridazine	Brown	0·68	0·39	0·63	0·71
Sulphamoprine	Orange	0·71	0·49	0·73	0·77
Sulphanilamide	—	0·41	0·14	0·66	0·75
Sulphaphenazole	Light brown	0·57	0·43	0·69	0·76
Sulphapyridine	Light brown	0·58	0·22	0·61	0·69
Sulphaquinoxaline	Yellow green	0·41	0·41s	0·65	0·71
Sulphasalazine	(Orange)	0·7	0·2	0·10s	0·32s
Sulphasomidine	Yellow green	0·40	0·17	0·41	0·42s
Sulphasomizole	Grey brown	0·22	0·31	0·65	0·69
Sulphathiazole	Brown purple	0·44	0·20s	0·50	0·62
Sulphormethoxine	Faint yellow	0·69	0·50	0·69	0·73

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