SEPARATION AND IDENTIFICATION OF SULFONAMIDES BY THIN-LAYER CHROMATOGRAPHY

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Pure single sulfonamides can be easily identified by a number of reactions. When, however, several sulfonamides are present in a mixture it is generally necessary to perform a separation prior to the identification process. This is particularly true for those sulfonamides which do not have an analytically characteristic group that can be identified in a routine manner.

Paper chromatography[1–9] and electrophoresis[10] have proved to be very useful techniques for this purpose. Although paper chromatography has so far given very good results for many difficult separations, some combinations of sulfonamides have not yet been adequately resolved by this method. Furthermore, paper chromatography (strip and circular) is somewhat time consuming (6 to 24 h), which is a drawback, especially when used in routine work.

In recent years thin-layer chromatography (T.L.C.) has proved to be exceptionally useful for qualitative analysis and purity testing of many types of compounds, providing new facilities for their simple separation. The advantages of T.L.C. over paper chromatography have been emphasized in a number of papers[11–17].

With all this in mind the purpose of this paper was to elaborate a method for the rapid separation and identification of the most commonly used sulfonamides by this new technique. Some sulfonamides were separated by WOLLISH *et al.*[18] by T.L.C.

EXPERIMENTAL

Apparatus and reagents

All the experiments were carried out with the No. 600, Desaga, Heidelberg, thin-layer chromatography apparatus.

Adsorbent: Kieselgel G for thin-layer chromatography, Merck No. 7731.

Solvents: Chloroform p.a., methanol p.a., ether pro narcosi, redistilled.

Reagents: All reagents used were of p.a. purity grade.

Reference substances: Sulfacetamide-sodium, Sulfadiazine, Sulfaguanidine, Sulfamerazine, Sulfamethazine, Sulfamethoxypyridazine, Sulfanilamide, Sulfathiazole-sodium, Sulfathiazole, Sulfisoxasole, Acetylsulfisoxasole, Plisulfan. Purity grade: B.P. 1958 and U.S.P. XVI resp.

Procedure

Prepare Kieselgel G layers on glass plates (20 × 20 cm) by the technique used by STAHL[19] in such a way that the time from the addition of water to the adsorbent

to its spreading on the glass plates is 5 min. Activate the plate by drying in the oven for 30 min at 105°. Mark the starting line (15 mm from the lower edge of the plate) and the front (10 cm from the start) with a thin glass rod and apply 1 μl (= 0.4 μg of each sulfonamide) of the ethanol solution of the sulfonamide mixture to be tested, and of the respective solution of reference substances, by means of a micropipette, along the starting line. Insert the plate in the chamber (21 × 9 × 21 cm) containing 100 ml of the solvent and saturated with a filter paper sheet, dipped in the solvent and cover the chamber with the lid. When the solvent reaches the front, which, with the chloroform–methanol mixture, takes about 30 min, and with ether about 45 min, take out the plate and allow it to dry for a few minutes. Spray the plate with a freshly prepared 1 % solution of sodium nitrite in 0.1 *N* hydrochloric acid, dry in the oven at about 100° for 5 min and spray with 0.2 % β-naphthol solution in 0.1 *N* sodium hydroxide. Evaluate the chromatogram by comparing the spots of the sulfonamides in the sample mixture with those of the reference substances.

The degree of activity of the adsorbent was checked by means of the Desaga color test, using benzene as solvent (Sudan Red: R_F 0.18, Butter Yellow: R_F 0.45, Indophenol: R_F 0.07).

DISCUSSION AND RESULTS

From the various studies carried out so far on the paper chromatographic separation of sulfonamides it was believed that good separation of these substances would also be achieved on the inorganic adsorbent Kieselgel G on the basis of partition, using alkaline solvent systems. Results, however, of separations obtained in preliminary experiments with solvent systems such as *n*-butanol–ammonia–water, cyclohexane–chloroform–diethanolamine, chloroform–methanol–ammonia, *n*-butanol–ammonia–water were unsatisfactory. The same was found to be true for acid solvent systems, *e.g.* *n*-butanol–acetic acid–water.

Assuming that less or more hydrophobic compounds could be separated on inorganic plates on the basis of adsorption with hydrophobic solvents, experiments were carried out using a series of solvents such as chloroform, ether, cyclohexane, benzene, acetone, as well as their mixtures in various proportions.

As can be seen in Fig. 1 optimal possible separation effects for most of the sulfonamides were obtained when ether alone was used as solvent. Satisfactory separation conditions were obtained also with the solvent mixture chloroform–methanol (100:10) (Fig. 2).

The reproducibility of R_F values in T.L.C. has up to now been criticised considerably, but it is generally agreed that the R_F reproducibility can be considered satisfactory within the limits $\pm$ 0.05.

In our experiments special attention was paid to establishing optimal conditions which render the R_F values reproducible within these limits, and it was confirmed that when the given conditions of work are strictly adhered to, the reproducibility of R_F values is very good. In spite of this fact we consider that when carrying out an identification test standard sulfonamides or sulfonamide mixtures should be run on each plate with the sample to be tested.

In Table I the limits of R_F values of 12 sulfonamides tested are given.

As can be seen from Figs. 1 and 2, the technique proposed gives sharp round spots without tailing and with a satisfactory sensitivity. With the diazo reagent as

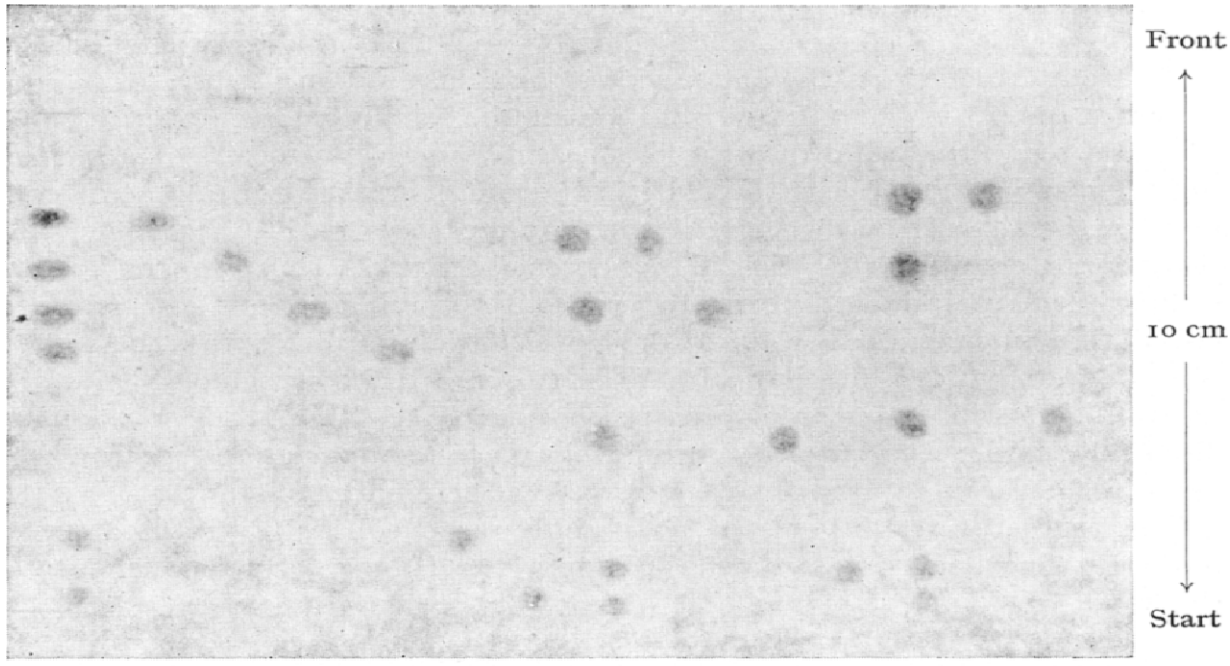

Fig. 1. Solvent: ether. M_1 = mixture of sulfonamides 12, 5, 4, 2, 8 and 3. M_2 = mixture of sulfonamides 11, 7, 6, 9 and 3. M_3 = mixture of sulfonamides 10, 5, 1, 9 and 3. For explanation of numbers, see Table I.

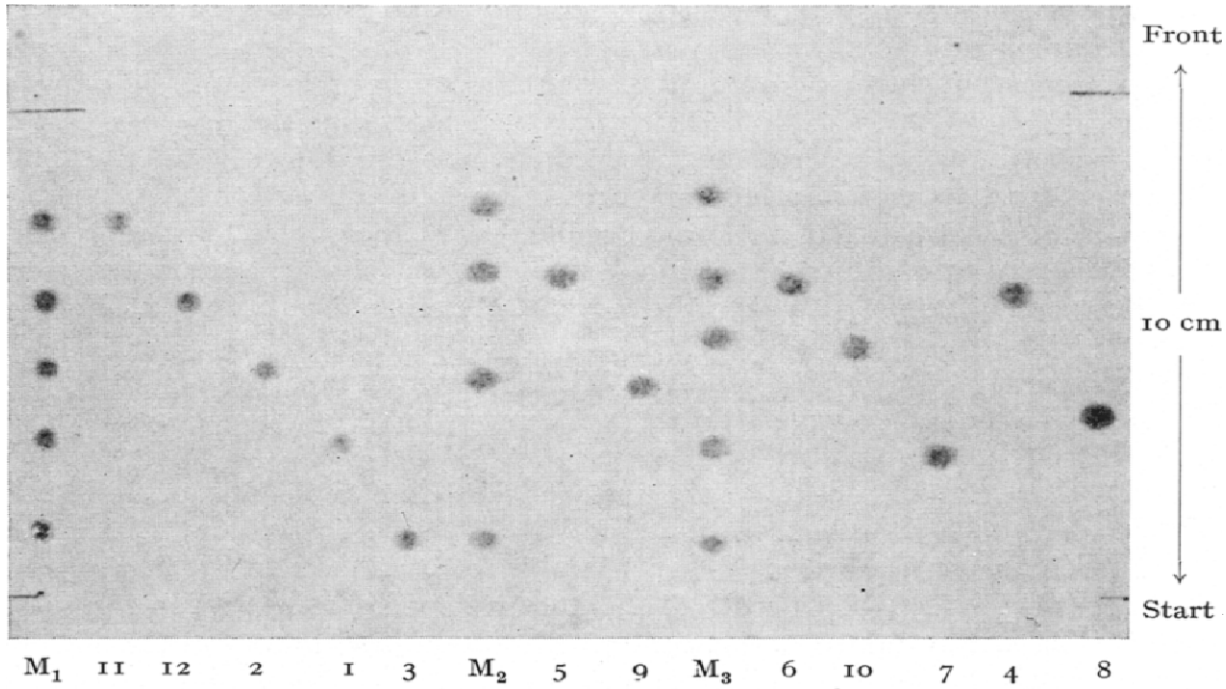

Fig. 2. Solvent: chloroform–methanol (100:10). M_1 = mixture of sulfonamides 11, 12, 2, 1 and 3. M_2 = mixture of sulfonamides 11, 5, 9 and 3. M_3 = mixture of sulfonamides 11, 6, 10, 7 and 3. For explanation of numbers, see Table I.

well as *p*-dimethylaminobenzaldehyde, 0.25 μg of the sulfonamide could still be easily detected (paper chromatography: 5 μg). The technique was applied to the identification of commercial preparations, *e.g.* Sulfacombin (Sulfadiazine, Sulfadimidine and Sulfathiazole) and Trisulfon (Sulfadiazine, Sulfathiazole and Sulfamerazine).

Work is in progress on the quantitative determination of sulfonamides and their mixtures and will be described in a subsequent communication from this laboratory.

TABLE I

LIMITS OF R_F VALUES OF VARIOUS SULFONAMIDES

Solvents: 1 = ether; 2 = chloroform–methanol (100:10).

No. (Figs. 1 and 2)	*Sulfonamide*	*R_F values in solvent*	
		1	*2*
1	Sulfacetamide sodium	0.34–0.38	0.34–0.39
2	Sulfadiazine	0.51–0.55	0.48–0.52
3	Sulfaguanidine	0.03–0.05	0.13–0.18
4	Sulfamerazine	0.58–0.62	0.56–0.62
5	Sulfamethazine	0.70–0.74	0.61–0.66
6	Sulfamethoxypyridazine	0.36–0.40	0.66–0.70
7	Sulfanilamide	0.59–0.63	0.34–0.38
8	Sulfathiazole	0.12–0.16	0.36–0.40
9	Sulfathiazole sodium	0.09–0.12	0.45–0.48
10	Sulfisoxasole	0.79–0.83	0.49–0.53
11	Acetylsulfisoxazole	0.74–0.77	0.79–0.83
12	Plisulfan	0.78–0.81	0.63–0.67

SUMMARY

By means of thin-layer chromatography on Kieselgel G layers it is possible to separate a number of sulfonamides in 30–45 min, using ether or a mixture of chloroform–methanol (100:10) as solvent. When using the diazo reagent or *p*-dimethylaminobenzaldehyde, as little as 0.25 μg of the sulfonamide can be easily detected.

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