QUANTITATIVE ANALYSIS OF SULFONAMIDE MIXTURES BY THIN-LAYER CHROMATOGRAPHY

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In a recent paper[1] thin-layer chromatography (TLC) was applied to the separation of 12 commonly used sulfonamides. The purpose of this paper is to provide by means of TLC a rapid separation and quantitative determination of some commercial sulfonamide mixtures.

Procedures for the assay of sulfonamide mixtures in the U.S.P. and the N.F. are based on the separation of sulfonamides by paper chromatography and the colorimetric determination of the extracted sulfonamides by the BRATTON–MARSHALL reaction[2]. A total of at least 14 paper strips are needed for the analysis of one triple sulfa preparation and many operations are involved. It should not be forgotten that not only does the large tank used require a long equilibration period but this is followed by 18 h development time.

Several authors have recently proposed procedures for the colorimetric determination of sulfonamide mixtures after a somewhat simplified paper chromatographic separation[3–6]. MARZYS[7] developed a spectrophotometric method for sulfonamide mixtures, while OLIVARI[8] determines sulfonamide mixtures directly on the chromatogram. However, all these procedures are rather tedious and time consuming for routine work and the application of TLC to this kind of analysis seemed very tempting.

The proposed procedure uses simple solvent systems for the separation of the most commonly used sulfonamide mixtures. After extraction from the adsorbent each sulfonamide is determined colorimetrically by means of the BRATTON–MARSHALL reaction[2].

The procedure can be applied to the determination of mixtures containing sulfathiazole, sulfamerazine, sulfamethazine; sulfacetamide, sulfamerazine and sulfadiazine (U.S.P., N.F.); and sulfadiazine, sulfamerazine and sulfamethazine (U.S.P.).

EXPERIMENTAL

Apparatus

Thin-layer chromatography apparatus No. 600, Desaga, Heidelberg.

Agla micrometer syringe, Burroughs Welcome & Co., London.

Solvents and solvent systems

Chloroform–methanol (90 ml + 10 ml).

Chloroform–methanol–ammonia solution (25 %) (90 ml + 15 ml + 2.4 ml).

Ether.

Reagents

Kieselgel G for thin-layer chromatography, Merck No. 136021.

Acid sodium nitrite reagent; a freshly prepared 0.1 % solution in 0.1 *N* HCl.

N-1-Naphthylethylenediamine dihydrochloride reagent: 0.1 % solution in distilled water.

Ammonium sulfamate reagent: 0.5 % solution in distilled water.

Sodium nitrite reagent: a freshly prepared 0.1 % solution in distilled water.

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

Reference substances

Sulfacetamide, sulfadiazine, sulfamerazine, sulfamethazine and sulfathiazole. Purity grade: U.S.P. and B.P., respectively.

Procedure

Tablets. Twenty tablets were weighed and finely powdered. A quantity of the powder equivalent to one sulfonamide tablet was taken and transferred to a 50 ml volumetric flask. 40 ml of a mixture of 50 ml 70 % ethanol and 2 ml 25 % ammonia solution were added to the flask which was shaken for 15 min, then made up to the mark with the solvent mixture and centrifuged (15 min at 2,500 r.p.m.). Kieselgel G coated plates (20 × 20 cm) were prepared by the technique proposed by STAHL[9] and activated by drying in an oven for 60 min at 105°. Three times 3 μl spots were applied along the starting line as 1 cm horizontal lines of the extracts of the sulfonamide mixture to be assayed and 3 μl spots of the corresponding standard solutions were similarly applied with a microsyringe and the chromatogram was run with the corresponding solvent system. When the solvent reached the front (*ca.* 45 min) the plate was dried in an oven for 10 min at 105° in the cases where the solvent mixture containing ammonia had been used. The spots were located by spraying only faintly with acid sodium nitrite reagent followed by the N-1-naphthylethylenediamine reagent and the spots of each separated sulfonamide were scraped off into a 25 ml glass stoppered flask, to which was added 5 ml 0.1 *N* HCl. Each flask was shaken well for about 20 min, centrifuged and 3 ml aliquots of each supernatant sample and standard solution were pipetted into a 25 ml flask. 1 ml sodium nitrite reagent was added to each flask followed after 3 min by 1 ml ammonium sulfamate reagent. After a further 2 min 1 ml N-1-naphthylethylenediamine reagent was added. After standing for 15 min the absorbancies of the sample and standard solution were determined at 545 mμ, relative to a reagent blank.

Weight of single sulfonamide (g) per tablet:

$$\frac{E_s \times \text{mg standard in 3 ml} \times 27.77}{E_{st}} \times \frac{\text{Mean weight of tablets}}{\text{Weight of samples}}.$$

Suppositories. Ten suppositories were weighed and pressed into a homogeneous mass. A quantity of the mass corresponding to *ca.* 250 mg total sulfonamides was weighed out and extracted by shaking in a separatory funnel with 100 ml ether, 10 ml water and 2 ml ammonia solution (25 %). After allowing the layers to separate the aqueous layer was drained into a 25 ml volumetric flask, and the ether layer washed in the separatory funnel several times with a few ml of water, the washings

being added to the solution already in the flask which was then filled up to the mark with water. Portions of 3 μl of the sulfonamide mixture and the corresponding standard solutions were applied to the Kieselgel G plate which was then treated as described above for tablets.

Weight of single sulfonamide (g) per suppository:

$$\frac{E_s \times \text{mg standard in 3 ml} \times 27.77}{E_{st}} \times \frac{\text{Mean weight of suppository}}{\text{Weight of sample}}.$$

Suspension. A quantity of the suspension corresponding to about 500 mg of total sulfonamides, was weighed accurately into a 50 ml volumetric flask to which was added 40 ml of the ethanol–ammonia solution mixture (50:2), and after mixing well the volume was adjusted to the mark with the same solvent mixture. After centrifuging, 3 μl portions of the supernatant of the sulfonamide mixture to be analysed and the corresponding standard solutions were applied to the plate. The method is then the same as for tablets.

Weight of single sulfonamide (g) per g of suspension sample:

$$\frac{E_s \times \text{mg standard in 3 ml} \times 27.77}{E_{st}}.$$

DISCUSSION AND RESULTS

Although ether proved to be a very good solvent for qualitative purposes[1], for quantitative separations we preferred other solvents. From the results obtained it appeared that the volatility of ether diminished the accuracy of the method and at the same time affected the reproducibility of results. The solvent systems chloroform–methanol and chloroform–methanol–ammonia, which had given promising results in the qualitative separation of some sulfonamide mixtures[1], were tried for this reason. A perfect quantitative separation of sulfathiazole, sulfamerazine and sulfadiazine was obtained with the chloroform–methanol (90:10) mixture (Fig. 1), whereas for the other two sulfonamide mixtures, *viz.* sulfacetamide, sulfamerazine and sulfamethazine, and sulfadiazine, sulfamerazine and sulfamethazine, the solvent system chloroform–methanol–ammonia was found to be better. This solvent system was most stable and gave a high resolution of the sulfonamides when 90 ml chloroform were well mixed with 15 ml methanol and 2.4 ml ammonia solution (25 %) (Figs. 2 and 3). Separation of the mixture sulfathiazole, sulfadiazine, sulfamethazine and sulfamerazine was best with ether alone (Fig. 4).

The determination of the separated and extracted sulfonamides was first carried out on the basis of U.V. absorption. Although such technique has been employed previously[10] our results were rather discouraging as we encountered several difficulties such as high absorbancy of the blank and solubility of the Kieselgel G in the hydrophilic solvent used for the extraction of the sulfonamides, which gave rise to colloidal solutions. For these reasons we considered that a colorimetric procedure would be much more favorable for this technique and the BRATTON–MARSHALL reaction was adopted.

Experiments were carried out with standard mixtures of sulfonamides each

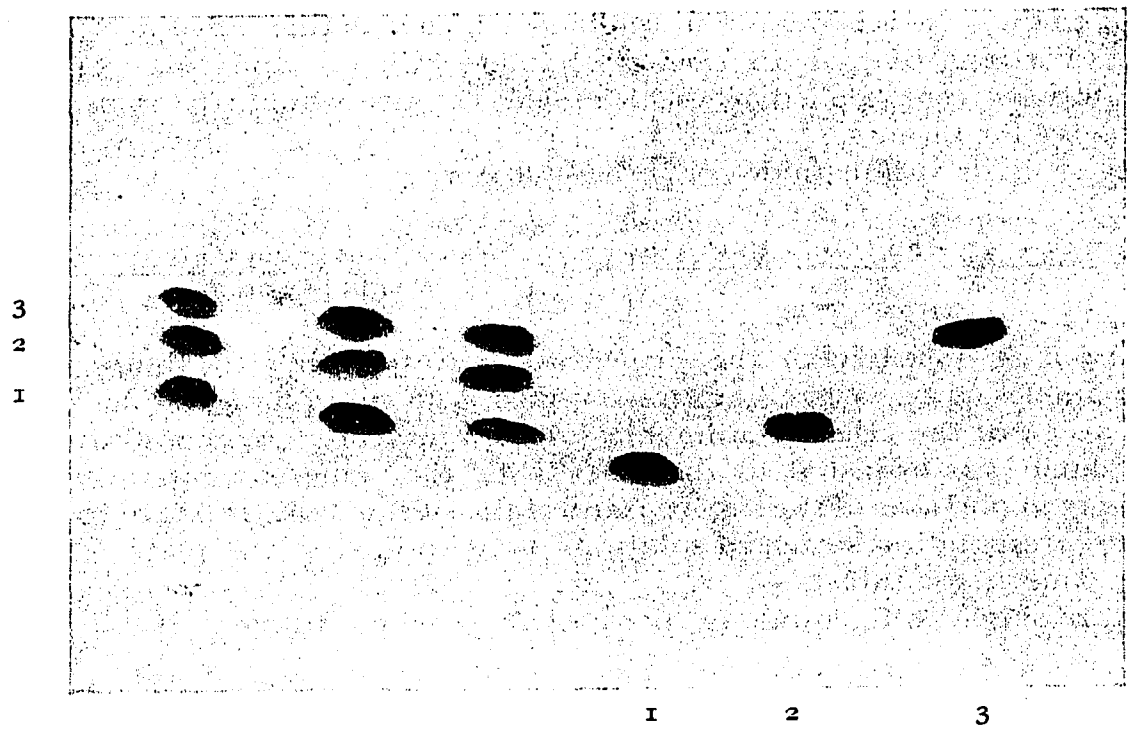

Fig. 1. Chromatographic separation of sulfathiazole (1), sulfadiazine (2) and sulfamerazine (3

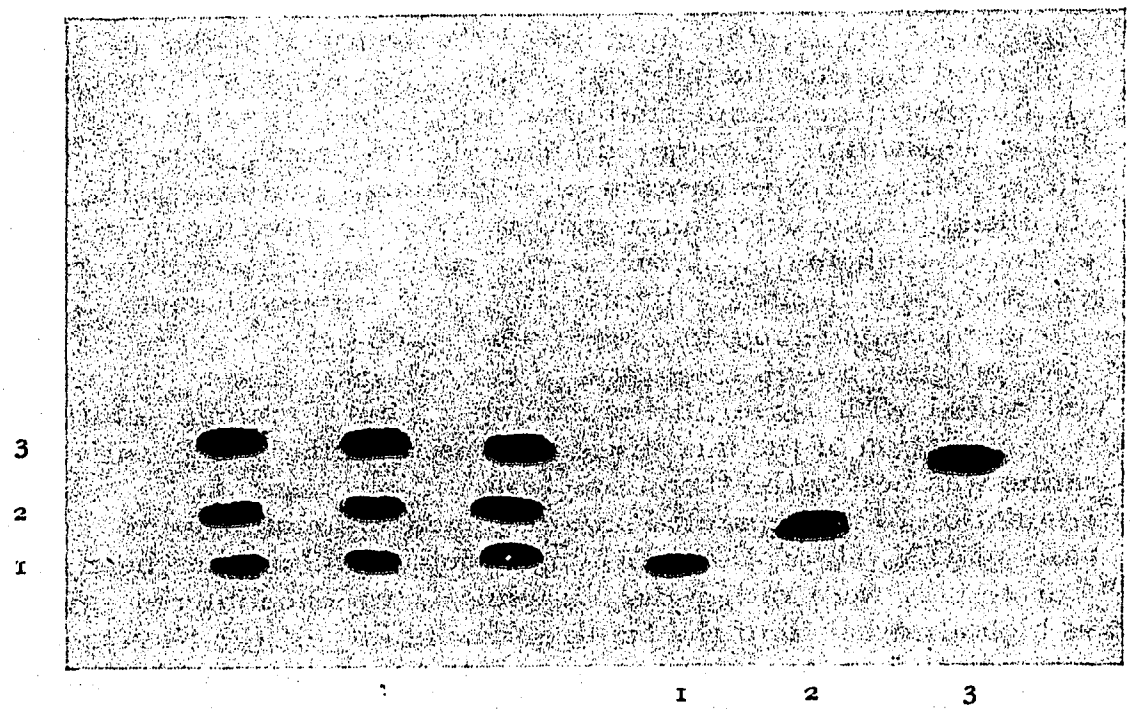

Fig. 2. Chromatographic separation of sulfacetamide (1), sulfadiazine (2) and sulfamerazine (3)

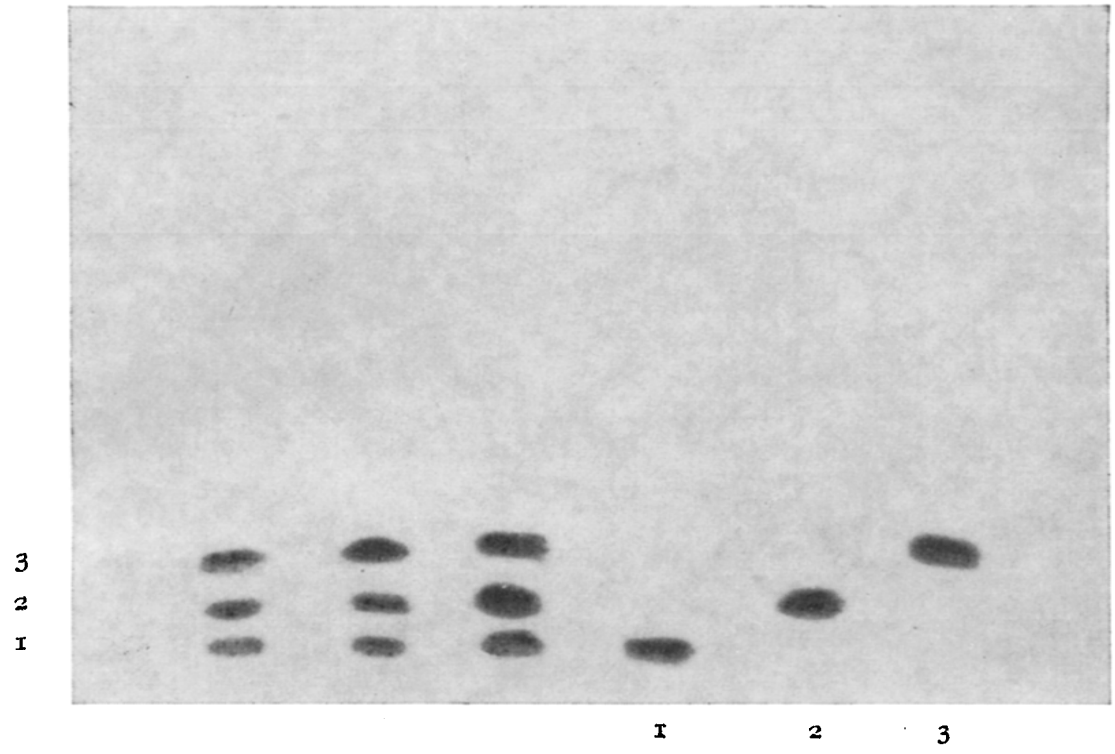

Fig. 3. Chromatographic separation of sulfadiazine (1), sulfamerazine (2) and sulfamethazine (3).

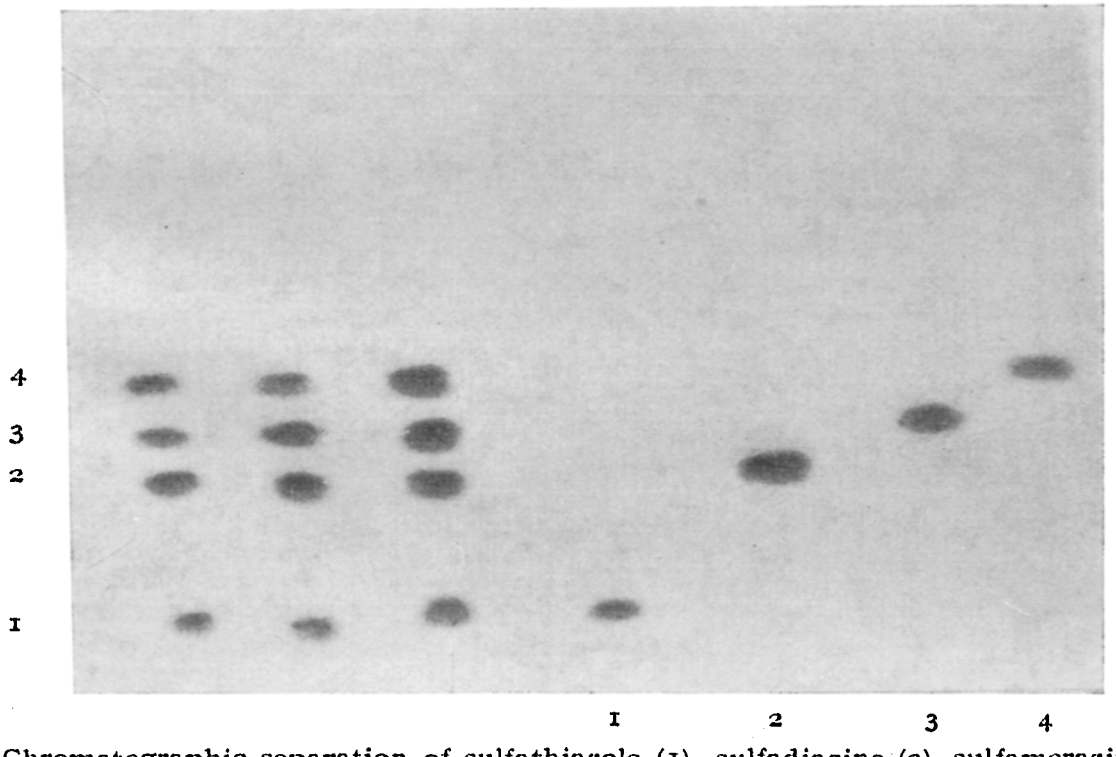

Fig. 4. Chromatographic separation of sulfathiazole (1), sulfadiazine (2), sulfamerazine (3) and sulfamethazine (4).

TABLE I

ANALYSIS OF STANDARD MIXTURES I, II AND III

Analysis No.	μg found			μg found			μg found		
	Sulfadiazine 6.60 μg added	Sulfamerazine 6.60 μg added	Sulfathiazole 4.78 μg added	Sulfadiazine 10.02 μg added	Sulfamerazine 10.02 μg added	Sulfamethazine 10.02 μg added	Sulfacetamide 9.9 μg added	Sulfamerazine 9.9 μg added	Sulfadiazine 9.9 μg added
1	6.27	4.54	6.31	9.46	10.46	10.17	9.52	9.84	9.33
2	6.20	4.53	6.69	10.28	9.49	9.92	9.78	9.16	10.30
3	6.03	4.94	6.74	10.12	10.09	9.41	10.39	10.19	9.84
4	6.33	4.58	6.55	10.12	10.15	10.06	9.27	9.77	9.28
5	6.69	4.78	6.48	9.99	9.55	10.24	10.14	10.39	10.24
6	6.85	4.91	6.62	9.98	9.99	10.05	10.45	9.83	10.42
7	6.08	4.32	6.30				9.19	9.55	9.33
8	6.32	4.40	7.04				9.67	9.13	10.20
9	6.50	4.85					9.55	9.95	10.00
ean value	6.36	4.65	6.60	9.99	9.95	9.97	9.78	9.97	9.88
andard viation =0.05)	±0.22%	±0.22%	±0.21%	±0.28%	±0.40%	±0.32%	±0.47%	±0.32%	±0.34%
mits of error	±3.4%	±3.8%	±3.3%	±2.8%	±4.1%	±3.2%	±3.8%	±3.2%	±3.4%

TABLE II

ANALYSIS OF COMMERCIAL MULTIPLE PHARMACEUTICAL PREPARATIONS

Preparation and concentration		Found mg	% recovery
Tablets	*mg*		
Sulfadiazine	167	174.0	104.2
Sulfamerazine	167	169.3	101.4
Sulfamethazine	167	168.1	100.7
Sulfadiazine	185	187.4	101.3
Sulfamerazine	130	133.4	102.6
Sulfathiazole	185	187.4	101.3
Sulfathiazole	125	125.0	100.0
Sulfadiazine	125	126.8	101.44
Sulfamethazine	125	127.2	101.8
Sulfamerazine	125	126.8	101.5
Suspension	*g/100 ml*		
Sulfadiazine	3.33	3.32	99.70
Sulfamerazine	3.33	3.66	110.0
Sulfamethazine	3.33	3.21	96.39
Suppositories	*mg*		
Sulfadiazine	167	159.3	95.4
Sulfamethazine	167	158.9	95.2
Sulfamerazine	167	162.8	97.5

containing equal parts of the constituent compounds which are common in commercial pharmaceutical preparations. Standard mixtures were:

I. Sulfadiazine 186 mg; sulfamerazine 130 mg and sulfathiazole 185 mg.
II. Sulfadiazine, sulfamerazine and sulfamethazine each 167 mg.
III. Sulfacetamide, sulfamerazine and sulfadiazine each 167 mg.

These mixtures were analysed by the proposed method by two analysts and the limits of error for each sulfonamide was determined. Results are given in Table I.

Analyses were also made of available commercial multiple sulfonamide pharmaceutical preparations. The results obtained indicate both good checks and satisfactory adherence to the labeled declarations for each component compound (Table II).

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SUMMARY

A simple method for the thin-layer chromatographic separation and determination of some commonly used sulfonamide mixtures is proposed.

After separation on plates coated with Kieselgel G each sulfonamide is extracted and determined colorimetrically by means of the BRATTON–MARSHALL reaction. The total time of the analysis is less than 5 h. Statistical analysis of the results obtained with standard mixtures gave very good results.

REFERENCES

1 T. BIĆAN-FIŠTER AND V. KAJGANOVIĆ, *J. Chromatog.*, 11 (1963) 492.
2 A. C. BRATTON AND E. K. MARSHALL, Jr., *J. Biol. Chem.*, 128 (1939) 537.
3 N. MAIENTHAL, J. CAROL AND F. M. KUNZE, *J. Assoc. Offic. Agr. Chem.*, 44 (1961) 313.
4 M. TH. VAN DER VENNE AND J. B. T'SIOBBEL, *Chromatographie Symposium II, 1962*, Soc. Belge Sci. Pharm., Brussels, 1963, p. 191.
5 F. M. KUNZE AND L. ESPINOZA, *J. Assoc. Offic. Agr. Chem.*, 46 (1963) 899.
6 G. THOMAS, J. RANSY, P. ROLAND AND A. VAN DEN BULCKE, *J. Pharm. Belg.*, [N.S.] 5 (1950) 263.
7 A. MARZYS, *Analyst*, 86 (1961) 460.
8 G. OLIVARI, *Boll. Chim. Farm.*, 97 (1958) 552.
9 E. STAHL, *Dünnschichtchromatographie*, Springer, Berlin, 1962.
10 H. GÄNSHIRT, *Arch. Pharm.*, 296 (1963) 129.