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THIN-LAYER CHROMATOGRAPHY AND ULTRAVIOLET SPECTROPHOTOMETRY OF SULFONAMIDE MIXTURES

A STUDY OF THE ABSOLUTE RECOVERIES

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SUMMARY

Recovery patterns were studied for individual sulfonamides separated from a mixture by thin-layer chromatography and then determined by ultraviolet spectrophotometry. To make the investigation more complete experimental conditions such as quantity of sulfonamide per spot, extracting solvent and pathlength were varied. The recoveries are never complete, but fairly constant. Factors affecting recoveries and accuracy of results are discussed.

INTRODUCTION

Single-component sulfonamide preparations can be analyzed by spectrophotometry, colorimetry or by titration with nitrous acid[1]. The total sulfonamide content of preparations containing two or more sulfonamides can also be estimated with a fair degree of accuracy by any of the same three methods. In some special cases the individual sulfonamides can be determined by spectrophotometry[2,3] or by spectrophotometric–colorimetric procedures[4,5] without separation from mixtures. Gas chromatography has also been applied to the quantitative analysis of some sulfonamide mixtures[6,7]. Several methods of analysis of mixed sulfonamides, published in recent years, involve the estimation of the compounds separated by paper chromatography[8–13] or thin-layer chromatography[14–16]. The 15th edition of the *U.S. Pharmacopoeia*[17] and the 10th edition of the *National Formulary*[18] adopted a PC procedure for the analysis respectively of trisulfapyrimidines and sulfadiazine–sulfamerazine mixtures. These methods[17,18] were retained in the two successive editions of the *U.S. Pharmacopoeia* and the *National Formulary*. In all the PC and TLC methods mentioned[8–18] the determinative step for the estimation of the individual sulfonamides is either colorimetry or spectrophotometry. Spot size comparison allows only a rough estimation of each compound[8,15]. The colorimetric procedures used are: reaction of the sulfonamides with vanillin–hydrochloride[8], with *p*-dimethylaminobenzaldehyde[8,15] or, following diazotization, with N-(1-naphthyl)-ethylenediamine[8,10–12,14,17,18], naphthylethylenediamine chlorohydrate[13] and N,N-diethyl-

N'-(1-naphthyl)-ethylenediamine oxalate[15]. Direct spectrophotometric determinations of the separated sulfonamides have been carried out by HEINANEN *et al.*[8], OLIVARI[9], WAGNER AND WANDEL[15] and SARSUNOVA *et al.*[16]. Generally the spectrophotometric or colorimetric determinations are performed on the extracts or eluates of the developed spots, previously cut from the paper or scraped off from the thin-layer plate after being located under UV light or by spraying the chromatograms with suitable solutions. OLIVARI[9] gradually exposes the developed chromatostrips to the radiation beam of a spectrophotometer and records the total absorbance of each spot.

It is common practice, in methods based on the colorimetric or spectrophotometric examination of the extracts of PC or TLC spots, to chromatograph the compounds to be used for reference purposes under the same conditions as the sample, generally in the same paper or plate, to compensate for possible losses and other factors affecting absorbance readings. Chromatography of the standards was not found necessary by MAIENTHAL *et al.*[11] and by KUNZE AND ESPINOZA[12] in their PC procedures but must always be performed in quantitative TLC methods mainly because, as indicated by SPENCER AND BEGGS[10], compounds are apparently never completely recovered from thin-layer plates. While it seems certain that recoveries are not complete it is not too well known how reproducible they are and how they are affected by changes in experimental conditions.

This investigation originated primarily from the desirability of obtaining information on the absolute recoveries of sulfonamides chromatographed on thin-layer plates and on the reproducibility of such recoveries. The other aim of the study was to improve the accuracy of direct spectrophotometric determinations of the content of TLC spots. Spectrophotometric methods are quicker and generally more accurate than colorimetric procedures. When applied in conjunction with TLC, however, spectrophotometry has often given unsatisfactory results. WAGNER AND WANDEL[15] indicate that spectrophotometric determinations were less accurate than the ones based on colorimetry. SARSUNOVA *et al.*[16], who used only spectrophotometry, do not furnish sufficient data for an evaluation of its accuracy in this connection. BICAN-FISTER AND KAJGANOVIC[14] attempted to analyze the separated sulfonamides by spectrophotometry but were discouraged by the poor results obtained and decided in favor of a colorimetric procedure. The main reason for the reported poor accuracy of spectrophotometric determinations of compounds extracted or eluted from TLC spots is apparently the high and variable UV absorbance contributed by the adsorbent. Aqueous or alcoholic extracts of blank TLC spots, even after centrifugation or filtration through high retentive paper, have been found to have high and very erratic absorbances; see again the review part of the article of SPENCER AND BEGGS[10]. The high absorbances have been attributed by many authors to soluble absorbing substances, which could be removed by washing the adsorbent with methanol or other suitable solvent. BICAN-FISTER AND KAJGANOVIC[14] believe, however, that they are caused by colloidal-size particles suspended in the solutions. A similar conclusion was reached by SPENCER AND BEGGS[10], who succeeded in minimizing the absorbance blanks by filtering the extracts of the TLC spots through prewashed Millipore filters. In the course of the work preliminary to this investigation it was found that they can also be eliminated by reading the centrifuged aqueous or alcoholic extracts in a narrow path (0.5 cm or less). Centrifuged chloroform extracts of blank TLC spots do not have UV absorbance, even if read in 2-cm cells; for this reason this solvent

was preferred to alcohol by CIERI[20] to extract coumarins and furocoumarins from developed TLC plates.

The use of the 0.5 cm cells and the desirability of keeping high the absorbances indicated the necessity for each developed sulfonamide spot to contain 100 μg and possibly more of a sulfonamide. Separations of such quantities, rather high in TLC, were achieved by spotting the aliquots to be chromatographed over a wide area (3 cm or more). In order to make the study more extensive some experimental conditions, such as composition of the mixtures, extracting solvent and volume of extraction, were varied. Centrifugation of the extracts was preferred to Millipore filtration since it was considerably quicker. The Millipore filters have to be washed free of UV absorbing materials, an operation which could be time consuming when a large number of determinations must be carried out. The sulfonamides, whose recoveries in TLC methods are studied, are the following: sulfacetamide, sulfathiazole, sulfadiazine, sulfamerazine and sulfamethazine.

EXPERIMENTAL

Apparatus

The standard equipment for TLC of Desaga and Brinkmann was used. The Chromato-Vue apparatus was supplied by Black Light-Eastern Corp. (Long Island, N.Y.) and the spot-collecting tube (Fig. 1) by Pesce Co. (Kennett Square, Pa.).

Reagents and solutions

Silica Gel H was obtained from Brinkmann Instruments Inc. (Westbury, Long Island, N.Y.) and white phosphorus for TLC from Research Specialties Co. (Richmond, Calif.). The acidic alcohol consisted of 0.4% 1 N H_2SO_4 in 95% alcohol. The developing solvent used was: chloroform–methanol (88:12).

Preparation of TLC plates

30 g of Silica Gel H and 100 mg of white phosphor were weighed in a flask. The mixture was slurried with 70 ml of 0.1 N NaOH and applied to the plates to a thickness of 0.25 mm. The plates were dried in air and kept in a dust-free cabinet.

PROCEDURE

Reference solutions

For each of the sulfonamides investigated, prepare reference solutions and read UV absorbances as follows. Weigh accurately about 100 mg in a 100 ml volumetric flask. Add 5 ml of alcohol and 2 ml of strong ammonia water. Swirl to dissolve compound, fill to mark with alcohol and mix.

Dilute with alcohol to obtain a solution containing about 0.4 mg/ml. Pipet four 5 ml aliquots to separate 100 ml volumetric flasks and evaporate to dryness on a steam bath with the help of a current of air. To two of the four flasks, each containing about 2 mg of sulfonamide, add 20 ml of acidic alcohol, swirl well or warm briefly on steam bath to dissolve residue, fill to mark with acidic alcohol and mix. Record the absorbances in 0.5 cm cells from 350 to 220 mμ. Average the absorbances at the maximum and use the resulting value to calculate the absorbance maximum, in 0.5 cm

cells, of an acidic alcohol solution containing 20.00 μg/ml. For sulfathiazole only, calculate, in addition to the absorbance maximum, the absorbance at 270 mμ of a solution containing 20.00 μg/ml. To the other two flasks add 20 ml 0.1 *N* NaOH, swirl well, fill to mark with 0.1 *N* NaOH, mix and record absorbances from 350 to 220 mμ in 0.5 cm cells. As previously described, calculate the absorbances, in 0.5 cm cells at the maximum near 255 mμ, of a 0.1 *N* NaOH solution containing 20.00 μg/ml.

By further dilutions with alcohol prepare a solution containing about 0.1 mg/ml. Continue exactly as in preceding paragraph but read absorbances in 2 cm cells. Calculate the absorbance values, in 2 cm cells at the indicated points, of acidic alcohol and 0.1 *N* NaOH solutions containing 5.00 μg/ml. These values should agree closely with those previously determined.

Volume delivered by micropipet

Dilute a sulfonamide solution with alcohol to obtain a concentration of 0.5 mg/ml (solution R). Pipet two 1 ml aliquots to separate 100 ml volumetric flasks, evaporate to dryness, dissolve residue and fill to mark with 0.1 *N* NaOH. Record the UV absorbances from 350 to 220 mμ in 2 cm cells and average the absorbances at the maximum (A_R). With the micropipet to be used for TLC spotting, transfer six 100 μl aliquots of solution *R* to separate 10 ml volumetric flasks, evaporate to dryness, dissolve residue and fill to mark with 0.1 *N* NaOH. Record UV absorbances from 350 to 220 mμ in 2 cm cells and read absorbances at the maximum (A_p).

Calculate the volume in μl delivered by the micropipet by the formula:

$$V_p = 100\, A_p/A_R$$

The volume found (six determinations) was 95.95 $\pm$ 0.64 μl.

Standard mixture No. 1

Weigh accurately about 125 mg each of sulfacetamide (SC), sulfadiazine (SD), sulfamerazine (SM) and sulfamethazine (SH) in a 100 ml volumetric flask. Add 5 ml of alcohol and 2 ml of strong ammonia water, swirl well, fill to mark with alcohol and mix (Solution M1).

Prepare serial dilutions in alcohol to obtain a solution containing about 25 μg total sulfonamide (or 6.25 μg of each sulfonamide) per ml. Pipet three 5 ml aliquots (about 125 μg total sulfonamides) to separate 25 ml volumetric flasks and evaporate to dryness. Dissolve the residues and fill to mark with acidic alcohol, mix and read absorbances in 2 cm cells from 350 to 220 mμ. Record absorbances at maximum ($_tA_a$) and calculate the percentage total sulfonamides in solution with the formula:

$$\%\ \text{Total sulfonamides} = 50{,}000\ {}_tA_a/({}_mA_{a,5,2})\,(W_t)$$

where W_t is the total weight in mg of the sulfonamides taken for analysis and ($_mA_{a,5,2}$) indicates the average of the absorbances, at the maximum in 2 cm cells, of the acidic alcohol reference solutions of the four sulfonamides, each at a concentration of 5.00 μg/ml. Pipet three more 5 ml aliquots to 25 ml volumetric flasks, evaporate to dryness and continue as above but use 0.1 *N* NaOH instead of acidic alcohol and record absorbances at the maximum near 255 mμ ($_tA_b$). Similarly calculate the percentage of total sulfonamides by the formula:

$$\%\ \text{Total sulfonamides} = 50{,}000\ {}_tA_b/({}_mA_{b,5,2})\,(W_t).$$

Spot six 100 μl aliquots of solution M1 on two prepared silica gel plates, three per plate. Spot each aliquot by repeated applications over an area 3 cm wide, leaving 2 cm margins between spotting areas and at the ends. Use a current of air to dry drops between applications. Develop until the solvent front has reached the top of the plates. View developed plates under short wave UV light and circle spots with a dissecting needle, including small margins, whenever possible. The order of succession of the developed sulfonamides is shown in Fig. 2. Scrape each marked spot and transfer

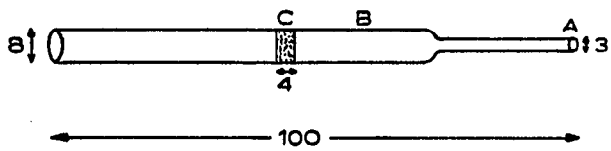

Fig. 1. Collecting tube for spots. Numbers indicate sizes in mm.

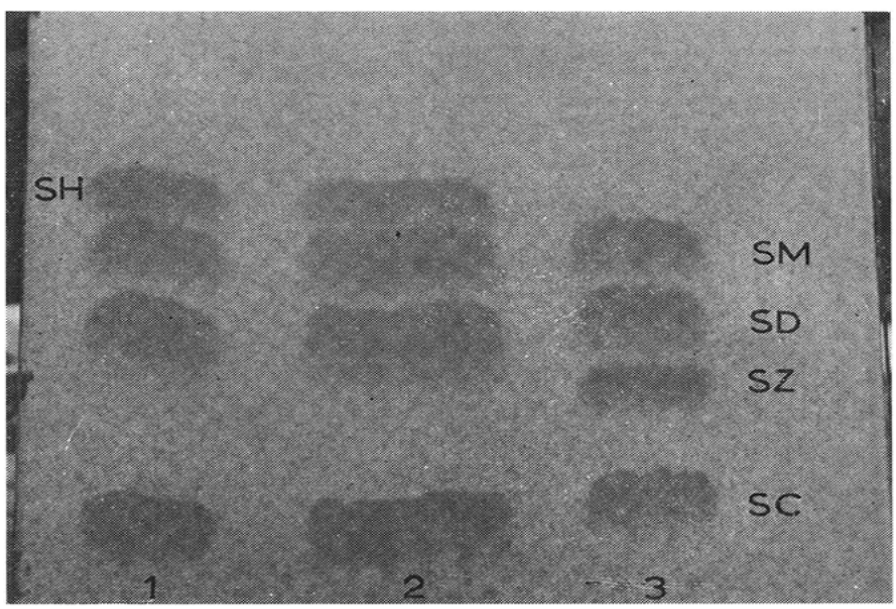

Fig. 2. Photograph of a developed chromatogram. For explanation of abbreviations, see text. Quantity of sulfonamide per spot: aliquots 1 and 3, 100 μg; aliquot 2, 200 μg.

to 25 ml volumetric flasks as follows. Attach collecting tube (Fig. 1) to a vacuum source and completely draw the content of a spot into the bulb (Fig. 1B), using the tip to loosen the adsorbent layer. Without disconnecting suction, move the collecting tube inside a flask. Remove suction and force material from tube to flask with repeated tappings. With a very gentle current of air blow into the flask any material still adhering to the tube. Identify the flasks containing the scraped spots of each aliquot. To twelve of the flasks, containing the scraped spots of three developed aliquots, add 15 ml of acidic alcohol. Stopper, shake well for 2 min, fill to mark with acidic alcohol and mix. Centrifuge 15 ml portions of the extracts in conical tubes and carefully decant about 12 ml of the clear solutions into small beakers. Read the absorbances from 350 to 220 mμ in 2 cm cells with acidic alcohol in the reference cells and record absorbances at the maximum ($_xA_a$). The letter x identifies a particular sulfonamide.

Extract with 25 ml acidic alcohol and similarly centrifuge and decant into beakers six blank spots, approximately equal in size to an average developed spot. The blank spots can be scraped off at the sides of a developed plate or from an undeveloped plate. Record absorbance in 2 cm cells from 350 to 220 mμ with acidic alcohol in the reference cells. Average the six blank absorbances at 270 mμ ($_BA_a$). Calculate the percentage recovery of the individual sulfonamides with the formula:

$$\%\, R_x = 1250({}_xA_a - {}_BA_a) / ({}_xA_{a,5,2})\,(W_x)\,(V_p)$$

where W_x is the weight in mg of the sulfonamide in the mixture, ($_xA_{a,5,2}$) the absorbance in 2 cm cells at the maximum of the sulfonamide reference solution containing 5.00 μg/ml, and V_p the average volume in ml delivered by the micropipet. To the remaining twelve flasks, add 15 ml of 0.1 N NaOH and continue as above always substituting 0.1 N NaOH for acidic alcohol. Record absorbances at maximum near 255 mμ ($_xA_b$) and calculate percentage recovery of the individual sulfonamides by the formula:

$$\%\, R_x = 1250({}_xA_b - {}_BA_b) / ({}_xA_{b,5,2})\,(W_x)\,(V_p)$$

The term ($_xA_{b,5,2}$) indicates the absorbance in 2 cm cells at the maximum near 255 mμ of the sulfonamide reference solution containing 5.00 μg/ml, and $_BA_b$ is the average of six blank absorbances at 255 mμ. The meaning of the other terms has already been explained. For each aliquot average the recoveries of the four sulfonamides and identify as R_{ms}. Calculate, also for each aliquot, the average recovery from the formulas:

$$\%\, R_{mc} = 10\left[\sum_{x=1}^{n} ({}_xA_a) - n({}_BA_a)\right] / n({}_tA_a)\,(V_p) \text{ for the alcohol solutions}$$

$$\%\, R_{mc} = 10\left[\sum_{x=1}^{n} ({}_xA_b) - n({}_BA_b)\right] / n({}_tA_b)\,(V_p) \text{ for the 0.1 } N \text{ NaOH solutions}$$

where n represents the number of the sulfonamides in the mixture, four in this instance, and V_p the average volume of the micropipette in ml.

Standard mixtures Nos. 2 and 3

Weight accurately about 200 mg each of the four sulfonamides used for preparing

TABLE I

BLANK ABSORBANCE READINGS

Blanks with 0.5 cm cells were negligible.

No.	*Acidic alcohol*		*No.*	*0.1 N NaOH*	
	Absorbance (270 mμ, 2 cm)	*Deviations from average*		*Absorbance (255 mμ, 2 cm)*	*Deviations from average*
1	0.046	+0.011	1	0.029	−0.007
2	0.031	−0.004	2	0.043	+0.007
3	0.033	−0.002	3	0.020	−0.016
4	0.030	−0.005	4	0.040	+0.004
5	0.034	−0.001	5	0.047	+0.011
6	0.038	+0.003	6	0.037	+0.001
	Average 0.035 (n = 6)			Average 0.036 (n = 6)	

TABLE II

COMPOSITION OF SOLUTIONS

	Quantity weighed (mg)		
	M1	*M2*	*M4*
SC	125.7	200.2	100.9
SD	124.9	201.5	101.0
SM	124.8	199.9	99.6
SH	129.4	200.4	—
SZ	—	—	100.1
Total	504.8	802.0	401.6

TABLE III

% TOTAL SULFONAMIDES IN SOLUTION BY UV SPECTROPHOTOMETRY

	No.	*Acidic alcohol*	*No.*	*0.1 N NaOH*
Solution M1	1	99.1	1	99.5
	2	100.4	2	99.1
	3	98.6	3	99.9
	Average	99.4	Average	99.5
Solution M2	1	100.5	1	100.4
	2	100.1	2	100.5
	3	100.1	3	99.5
	Average	100.2	Average	100.1
Solution M4	1	100.6	1	100.4
	2	99.9	2	100.1
	3	100.6	3	99.8
	Average	100.4	Average	100.1

solution M1 in a 100 ml volumetric flask. Dissolve in 5 ml of alcohol and 2 ml of strong ammonia water and fill to mark with alcohol (solution M2). Dilute 5.0 ml of solution M2 to 10.0 ml with alcohol (solution M3). Analyze both solutions with the same procedure used for solution M1 but transfer the scraped spots to 10 ml rather than 25 ml volumetric flasks. Centrifuge all the solution, decant about 8 ml and read the absorbances in 0.5 cm cells. The aliquots of solution M2 must be spotted over an area about 5 cm wide; consequently, only two aliquots can be spotted on one plate. Calculate percentage total sulfonamides in solution only for solution M2. For both solutions, calculate the individual recoveries of the developed sulfonamides and the R_{ms} and R_{mc} values with formulas similar to those given under solution M1.

Standard mixture No. 4

Weigh accurately about 100 mg each of sulfacetamide (SC), sulfathiazole (SZ), sulfadiazine (SD) and sulfamerazine (SM) in a 100 ml volumetric flask. Dissolve with

5 ml of alcohol and 2 ml of strong ammonia water, dilute to mark with alcohol and mix (solution M4). Analyze the solution by the same procedure as used for solutions M2 and M3.

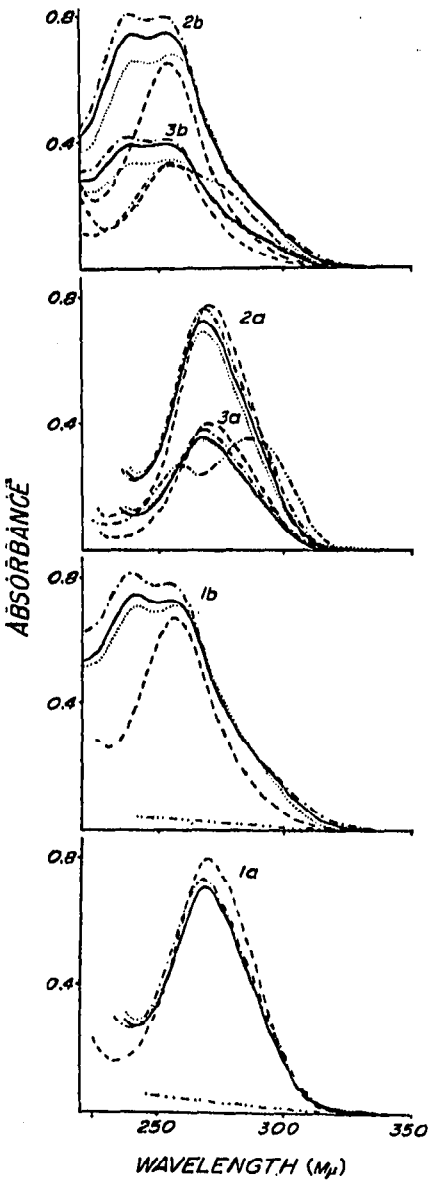

Fig. 3. UV absorbance curves of centrifuged extracts of sulfonamides separated by TLC. The letter *a* indicates acidic alcohol solution, the letter *b* 0.1 *N* NaOH solutions. Concentrations, volumes of extraction and cell paths; (1) 125 μg, 25 ml, 2 cm; (2) 200 μg, 10 ml, 0.5 cm; (3) 100 μg, 10 ml, 0.5 cm. – – – –, SC; –·–·–·–, SD; ———, SM; · · · · · ·, SH; – – · · – – · · – –, SZ; — · · — · · —, B.

DISCUSSION

As the results of Table IV indicate, the recoveries of sulfonamides extracted from the scraped spots of developed TLC plates are never complete. Losses are generally in the 7–11% range but occasionally they are higher or lower. The recoveries of

TABLE IV

% RECOVERIES OF CHROMATOGRAPHED SULFONAMIDES

Solution M1 read in 2 cm cells, solutions M2, M3 and M4 read in 0.5 cm cells.

Solution	*Aliquot*	*Extracting Solvent*	% R_{SC}	% R_{SD}	% R_{SM}	% R_{SH}	% R_{SZ}	*S.D.*	% R_{ms}	% R_{mc}
M1	1	Acidic alcohol	87.8	88.9	87.2	86.4	—	1.1	87.6	87.9
	2		88.2	94.3	93.7	93.8	—	2.9	92.5	92.5
	3		87.4	92.9	89.2	89.4	—	2.3	89.7	90.1
	Av.		87.80	92.0	90.0	89.9	—	1.7	89.9	90.2
	S.D.		0.40	2.8	3.3	3.7				2.3
	4	0.1 *N* NaOH	87.3	89.7	93.1	92.8	—	2.8	90.7	92.3
	5		91.9	96.0	94.2	92.1	—	1.9	93.5	95.2
	6		92.7	95.5	94.4	92.5	—	1.4	93.8	95.2
	Av.		90.6	93.7	93.90	92.47		1.5	92.7	94.2
	S.D.		2.9	3.5	0.70	0.35				1.7
M2	1	Acidic alcohol	90.9	96.2	95.8	93.6		2.4	94.1	93.9
	2		89.2	95.5	94.1	89.1		4.0	91.5	92.0
	3		87.1	94.1	92.8	91.6		3.0	91.4	90.9
	Av.		89.1	95.3	94.2	91.4		2.8	92.5	92.3
	S.D.		1.9	1.0	1.5	2.3				1.5
	4	0.1 *N* NaOH	89.2	96.1	92.7	88.6		3.5	91.7	91.6
	5		88.5	95.0	93.5	90.6		2.9	91.9	92.0
	6		89.9	96.4	96.0	93.3		3.0	93.9	93.8
	Av.		89.20	95.83	94.1	90.8		3.0	92.5	92.5
	S.D.		0.70	0.74	1.7	2.4				
M3	1	Acidic alcohol	91.7	92.4	92.8	90.7	—	0.92	91.90	91.9
	2		91.9	96.6	92.0	92.8	—	2.2	93.3	93.1
	3		93.6	95.8	94.6	94.1	—	0.94	94.52	94.2
	Av.		92.4	94.9	93.1	92.5	—	1.2	93.2	93.1
	S.D.		1.0	2.2	1.3	1.7	—			1.2
	4	0.1 *N* NaOH	93.9	95.3	93.7	94.7	—	0.74	94.40	94.1
	5		92.2	98.2	99.7	93.2	—	3.7	95.8	95.7
	6		92.5	96.7	97.8	93.9	—	2.4	95.2	95.1
	Av.		92.87	96.7	97.1	93.95	—	2.1	95.2	94.97
	S.D.		0.91	1.5	3.1	0.75	—			0.81
M4	1	Acidic alcohol	89.1	92.2	93.2	—	91.4	1.7	91.5	92.2
	2		91.4	92.5	94.7	—	90.9	1.7	92.4	93.2
	3		88.7	91.7	93.7	—	90.4	2.1	91.1	91.6
	Av.		89.7	92.13	93.87	—	90.90	1.8	91.7	92.33
	S.D.		1.5	0.40	0.76	—	0.50			0.81
	4	0.1 *N* NaOH	91.5	97.7	96.5	—	95.4	2.7	95.3	94.8
	5		92.5	96.5	97.0	—	95.7	2.0	95.4	95.0
	6		92.5	96.0	97.0	—	95.7	1.9	95.3	95.1
	Av.		92.17	96.73	96.83	—	95.60	2.2	95.3	94.97
	S.D.		0.58	0.87	0.29	—	0.17			0.15

spotted undeveloped sulfonamides were not studied in this investigation but preliminary work indicated that losses also occur and that they are slightly lower than those of the developed compounds. Attempts to increase recoveries, also made during the preparative work, were unsuccessful. The introduction of additional steps, such as washing the glass surface with a wad of cotton after removal of a spot, rinsing collection tube (Fig. 1) after transfer of a spot or performing multiple extractions of the scraped spots did not yield recoveries higher than those obtained with the simpler technique finally adopted and described in the section EXPERIMENTAL.

The reasons for the losses are not too well understood. They are probably caused, for the greater part, by a superficial loosening of silica gel particles at the spotting areas. The loosened particles scatter in air or fall when the plate is raised, carrying along the sulfonamides attached to them. Scattering of some fine silica gel particles, with small additional loss of sulfonamides, may also occur during extraction of the spots. It was often observed that, when the solvent was added, a fine mist came out of the flasks containing the silica gel spots. The possibility also exists that a small amount of sulfonamide is adsorptively retained by the silica gel and cannot be extracted but there is no definite experimental evidence to prove it. Whatever the causes for the incomplete recoveries, they seem to affect nearly equally all the sulfonamides studied. If the recoveries of the different sulfonamides for each aliquot are compared with each other, it appears that generally differences are small and not adequate to indicate a trend for higher or lower recoveries for a particular sulfonamide. Sulfacetamide is the only one that constantly shows slightly lower recoveries than the other sulfonamides in a given aliquot. The lower recoveries of sulfacetamide may be due to a partial decomposition during exposure to UV light, as suggested by previous investigators[11,12]. The recoveries of a sulfonamide in differently developed aliquots are also generally in good agreement thus indicating that the quantity of sulfonamides spotted by the micropipet is fairly constant. Unusually great losses of the amount spotted may, however, occur in the event of extensive loosening of the adsorbent layer during spotting. The occurrence of such high losses can be detected in a given aliquot by a low R_{mc} value (calculated average recovery). The R_{mc} values which could be computed even if the composition of the mixture is unknown and which are generally very close to the R_{ms} values (actual average recovery) in this experiment varied from 87.9 to 95.7%. Deviations from these values, especially in the low side, should alert the analyst to the possibility of high errors.

Recoveries are about the same with both extracting solvents, acidic alcohol or 0.1 *N* NaOH. Both solvents are then equally suitable for the extraction of sulfonamides from TLC spots. Percentage recoveries are also independent of the quantity of sulfonamide per spot, at least in the considered range of 100–200 μg. It is consequently not necessary, in sample analysis, that the standard and sample spots contain very nearly equal quantities of sulfonamides, as long as they remain in the indicated range. Since this study was limited to the 100–200 μg range, it is not known, however, whether this constancy in percentage recovery holds outside these limits.

The absorbance blanks are apparently caused by colloidal particles remaining suspended in the centrifuged extracts of the spots and not by soluble absorbing substances present in the adsorbent. Prewashing of the Silica Gel H is consequently not necessary and does not lower absorbance blanks. These blanks are independent of the volumes of extraction and can be decreased only by decreasing the cell paths.

They are completely eliminated if 0.5 cm cells are used. If it should become necessary to use higher pathlengths, the absorbances of the sulfonamide extracts should be kept very high (0.8 or more) in order to minimize the error that may be caused by the variability of the absorbance blank.

To limit the extent of this investigation, the recoveries of sulfonamides developed on other adsorbents were not studied. Silica Gel H was preferred to Silica Gel G or other adsorbents containing a calcium sulfate binder because the spots could be more readily scraped and transferred. The white phosphorus was incorporated in the silica gel to facilitate detection of the sulfonamides under UV light. The phosphorus does not contribute any UV absorbance. The silica gel was slurried in 0.1 *N* NaOH rather than water to increase the differences in R_F value between some of the sulfonamides, following a suggestion by NEW[21]. If overlapping of some of the spots still occurs in an experiment, the aliquot will have to be spotted over an area slightly wider than indicated in the method.

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