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THIN-LAYER CHROMATOGRAPHY AND ULTRAVIOLET SPECTROPHOTOMETRY OF MIXTURES OF SULFONAMIDES

PRACTICAL APPLICATIONS

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SUMMARY

A method is proposed for the analysis of mixed sulfonamides by thin-layer chromatography and ultraviolet spectrophotometry. Solutions of sample mixtures are spotted on fluorescent Silica Gel H plates and developed in chloroform–methanol (88:12). The developed spots are delineated under short-wave ultraviolet light, scraped from the plate and extracted with 0.1 N NaOH. The centrifuged extracts are read with a recording spectrophotometer. A new method of calculation is introduced which permits to calculate the recovery of each chromatogram and to derive from it the recoveries of the individual compounds.

INTRODUCTION

Thin-layer chromatography, in addition to being very widely used for identification purposes, has been occasionally employed for the quantitative estimation of components of mixtures. A review of quantitative TLC methods was made by SPENCER AND BEGGS[1] and, in the field of mixed sulfonamides, by CIERI[2] in a recent publication. The procedure usually followed is to scrape and extract the developed spots and subsequently analyze the extracts colorimetrically or spectrophotometrically. Reference standards, in quantities nearly equal to those of the spotted compounds or covering the expected range, are similarly chromatographed, extracted and, if necessary, reacted.

Because of the empirical approach followed, absolute recoveries are not generally calculated and consequently the recovery of the spotted compounds is not known. There have still been indications[1] that these are never completely recovered from TLC plates. A rather extensive study of the absolute recoveries of five sulfonamides from developed TLC plates has been reported[3], using spectrophotometry as the determinative step. The recoveries of the sulfonamides, extracted in acidic alcohol or 0.1 N NaOH, varied from 86.4 to 99.7% but were most commonly in the 89–93%

range. The recoveries of different sulfonamides in each chromatogram were generally very close with the exception of sulfacetamide, which often had lower recoveries than the others. The percent recoveries were not substantially affected by changes in the quantity of sulfonamide per spot in the considered range of 100–200 μg per spot, but remained at a rather constant level. The losses were attributed to scattering of particles of the adsorbent containing sulfonamides during, or after spotting and to some adsorptive retention of each compound by the chromatographic medium.

The results obtained in such study[2] and in a previous investigation[3] strongly suggest the possibility that a constant quantity of a compound is retained by a unit area (or perhaps unit volume) of adsorbent. Under given development conditions the concentration of a compound per unit area of a developed spot is also reasonably constant, regardless of the quantities spotted, since the sizes of such spots vary proportionately with the quantities of the compounds. The developed spots of different compounds may, however, have different concentrations of compound per unit area, as was found in a previous work[3], and thus produce different retention losses.

An accurate estimation of the losses related to adsorption cannot be made since, due to the possibility that small quantities of the compounds may detach from the plate, the amount spotted in each instance is not known with great precision. The relative extent of such losses, for compounds chromatographed under specified conditions, can be, however, reasonably well established by comparing their recoveries in each of several chromatograms. The study of the five sulfonamides previously investigated[2] was then continued for the purpose of obtaining more conclusive information on their relative recoveries. The developed spots, each containing a sulfonamide in the 80–120 μg range, were extracted with 0.1 N NaOH.

After the completion of the additional recovery study a method was developed for the analysis of mixed sulfonamides by TLC and UV spectrophotometry. The proposed method contains an outstanding innovation in that it permits to calculate the recovery of each sample chromatogram and to derive from it the recoveries of the different sulfonamides comprising the chromatogram. The recovery of a sample chromatogram is obtained from the ratio of the sum of the absorbances, all referred to a given volume, of the extracts of the sulfonamide spots to the absorbance of a dilution of the unchromatographed sample solution containing, in the reference volume, the amount of total sulfonamides theoretically delivered by the micropipet used. The calculated recovery of each chromatogram is then corrected, when necessary, in such a way that the recoveries of the individual sulfonamides are related by the same ratios that were found to characterize the recoveries of the chromatographed standard sulfonamides. The introduction of such a method of calculation, it is hoped, will eliminate or at least reduce considerably the error associated with the variability of the volume delivered by the micropipet and of the losses occurring during spotting. Seven synthetic mixtures of known composition and four commercial preparations have then been analyzed by the proposed method.

EXPERIMENTAL

Apparatus, reagents and TLC plates
    See previous article[2].

UV spectrophotometry

Read UV absorbance of all solutions with a recording spectrophotometer from 350 to 220 mμ in matched 0.5- or 1-cm cells. Use the same set of cells throughout the experiment.

Reference solutions

Following the procedure outlined in the previous article[2], prepare a reference solution containing about 10 μg/ml 0.1 N NaOH for each of the following compounds: sulfacetamide (SC), sulfathiazole (SZ), sulfadiazine (SD), sulfamerazine (SM) and sulfamethazine (SH). Read UV absorbances, calculate the absorbance, at the maximum near 255 mμ, of a solution containing 10.00 μg/ml and designate as $_M A_x$, where the letter x identifies a particular sulfonamide.

Recovery of the chromatographed standard sulfonamides

Weigh accurately about 100 mg each of the five sulfonamides listed above, in a 100-ml volumetric flask. Add 5 ml of alcohol and 2 ml of strong ammonia water, swirl well to dissolve compounds, fill to mark with alcohol and mix. Similarly prepare two solutions containing respectively about 80 and 120 mg of each of the five sulfonamides in 100 ml.

Analyze each solution as follows. Pipet a 2-ml aliquot into a 100-ml volumetric flask, fill the flask to mark with alcohol and mix. Pipet 10 ml of the diluted solution to a small beaker, evaporate to dryness on a steam bath, transfer residue to a 100-ml volumetric flask with small portions of 0.1 N NaOH, fill to mark with 0.1 N NaOH and mix. Read UV absorbances and designate as $_g A_t$ the reading at the maximum near 255 mμ, the letter g identifying the wavelength of maximum of this solution.

Spot five 100-μl aliquots of the undiluted solution, each over an area about 4 cm wide, and develop in chloroform–methanol (88:12) until the solvent front reaches the top of the plate. Delineate the developed spots, transfer each to a 10-ml volumetric flask and read UV absorbances with 0.1 N NaOH in the reference cells, as described in the previous article[2]. Scrape also two or more blank spots, extract with 10 ml 0.1 N NaOH and read UV absorbances of the centrifuged extracts. Record the absorbances of each sulfonamide spot at the maximum near 255 mμ and also at wavelength g (see above), subtract an average absorbance blank, and designate the blank corrected readings as $_M A_{sx}$ and $_g A_{sx}$, the letter x identifying a particular sulfonamide. Calculate the percent recovery ($\%R_x$) of each sulfonamide by the formula:

$$\%R_x = (1000)(_M A_{sx})/(_M A_x)(W_x)(V_p),$$

where W_x indicates the weighed amount of the sulfonamide in mg and V_p the average volume in ml delivered by the micropipet. Average the percent recoveries of the five sulfonamides, in each chromatogram, and designate as $\%R_{ms}$. Calculate, also for each chromatogram, the average percent recovery ($\%R_{mc}$) by the formula:

$$\%R_{mc} = 10 \sum_{x=1}^{n} (_g A_{sx})/n(_g A_t) (V_p),$$

where n equals the number of sulfonamide spots, five in this instance. The other terms have the same meaning as previously explained in this section.

Analysis of synthetic mixtures

Prepare several three- or four-component synthetic mixtures, each simulating a commercial preparation, as described below.

Designate as L_x the labeled amount of a sulfonamide in the commercial preparation, the letter x identifying a particular sulfonamide. Calculate then the e_x value of each component, to two decimal figures, by the formula $e_x = L_x/L_m$, where L_m indicates the labeled amount of the sulfonamide (or sulfonamides) whose quantity is the lowest in the mixture. Transfer to a volumetric flask accurately weighed amounts of the sulfonamides such that the quantity of each, in mg per ml of the resulting solution, equals its e_x value or does not differ from it by more than 20%. Designate as W_x the weighed amount of a sulfonamide in mg, as W_t the sum of the W_x values, as V_s the labeled volume of the flask in ml and as e_t the sum of the e_x values. Prepare also some synthetic mixtures that do not have a commercial equivalent; in this case call L_x the intended quantity of a sulfonamide in a mixture.

Analyze each mixture as follows. Add to flask 5 ml of alcohol and 2 ml of strong ammonia water, fill to mark with alcohol and mix. Pipet 5 ml of the solution to a glass-stoppered flask and add to it an accurately measured volume of alcohol such that the volume of the resulting solution in ml equals ten times the e_t value. After mixing, pipet 2 ml of the solution to a small beaker and evaporate to dryness on a steam bath. Transfer the residue to a 100-ml volumetric flask with small portions of 0.1 N NaOH, fill flask to mark with 0.1 N NaOH and mix. Read absorbances and designate the reading at maximum near 255 mμ as $_gA_t$, the letter g indicating the wavelength of maximum of this solution. Read also the absorbances, at wavelength g, of the reference sulfonamides, calculate the absorbance of each at a concentration of 10.00 μg/ml and designate as $_gA_x$. Calculate the total sulfonamide content (S_t) in mg by the formula:

$$S_t = (_gA_{st}) \ (V_s) \ (e_t)/\sum_{x=1}^{n} [(e_x/e_t) \ (_gA_x)]$$

where n indicates the number of sulfonamides in the mixture. To obtain the percent total sulfonamide content multiply S_t by $100/W_t$.

Spot three 100-μl aliquots of the undiluted solution, develop plate and delineate spots under UV light. Transfer each spot to a volumetric flask estimated to produce, when filled to mark, an absorbance in the 0.4–0.9 range and designate as V_x the labeled volume of a flask containing a given sulfonamide spot. Add to flask 0.1 N NaOH to half of its volume, swirl well for 1 min, fill to mark with 0.1 N NaOH, mix and centrifuge. Extract also, and similarly centrifuge, two or more blank spots in each of the volumes used for the extraction of the sulfonamide spots. Read absorbances, with 0.1 N NaOH in the reference cells, and designate as $_MA_{sx}$ and $_gA_{sx}$, respectively, the blank corrected readings of the extract of a sulfonamide spot, at its maximum and at wavelength g. For each chromatogram determine the recovery value (R_v) by the formula:

$$R_v = \sum_{x=1}^{n} (_gA_{sx}) \ (V_x)/(10) \ (e_t) \ (_gA_t).$$

where n indicates the number of spots in the chromatogram. Calculate then the content in mg of each sulfonamide (S_x) by the formula:

$$S_x = (_M A_{sx})(V_s)/(_M A_x)(R_v + k)^*$$

The term k equals o if the mixture does not contain sulfacetamide. If this compound is present in the mixture, $k = (1 - n)/100\ n$ when calculating the recovery value of sulfacetamide and $k = 1/100\ n$ when calculating the recovery value of any of the others. Multiply the S_x values by $100/W_x$ to obtain the percentage of each sulfonamide relative to the amount weighed.

Analysis of commercial tablets

From the label declarations calculate the e_x and e_t values of each sample. Weigh twenty or more tablets, calculate the average weight of a tablet and grind to uniform powder. Weigh a portion of the powder into a volumetric flask and analyze as in the preceding section. Calculate the S_t and S_x values and multiply by the ratio of the average tablet weight to the amount of sample weighed, to obtain the average content per tablet of the total and individual sulfonamides. Determine the percentage of total and individual sulfonamides relative to the label declarations.

TABLE I

RECOVERY OF STANDARD SULFONAMIDES

Aliquot	Content of spot	$\%R_{SC}$	$\%R_{SZ}$	$\%R_{SD}$	$\%R_{SM}$	$\%R_{SH}$	$\%R_{ms}$	$\%R_{mc}$
1	100 μg	92.6	93.3	92.4	94.3	94.4	93.4	93.8
2		92.6	91.6	95.8	94.5	93.3	93.6	93.8
3		92.6	91.9	94.6	94.0	93.9	93.4	93.6
4		91.8	90.1	93.6	92.7	93.9	92.4	93.0
5		91.2	91.0	93.1	92.7	93.6	92.3	93.0
Av. (5)		92.2	91.6	93.9	93.6	93.8	93.0	93.4
1	120 μg	90.6	92.0	92.5	92.4	91.0	91.7	92.2
2		90.7	94.6	92.7	93.9	90.6	92.5	92.5
3		90.5	94.9	90.5	92.4	91.4	91.9	92.2
4		89.8	94.9	90.1	92.6	91.9	91.9	92.3
5		88.7	91.3	90.5	90.5	88.0	89.8	90.3
Av. (5)		90.1	93.5	91.3	92.4	90.6	91.6	91.9
1	80 μg	91.1	91.9	94.9	93.0	94.0	93.0	93.0
2		90.8	91.2	93.7	92.9	93.3	92.4	92.5
3		91.5	90.8	92.5	91.1	93.0	91.8	91.8
4		90.7	90.8	88.9	89.2	90.6	90.0	90.1
5		91.4	94.5	91.9	93.3	92.3	92.7	92.0
Av. (5)		91.1	91.8	92.4	91.9	92.6	92.0	91.9
Av. (15)		91.1	92.3	92.5	92.6	92.3	92.2	92.4

* The correction factor k is introduced to account for the lower observed recoveries of sulfacetamide. The assumption is made, based on the results of the recovery study, that the recoveries of sulfathiazole, sulfadiazine, sulfamerazine and sulfamethazine are equal in a chromatogram and that the recovery of sulfacetamide is 0.01 lower than that of any of the others. Thus calling R_{sc} the recovery of sulfacetamide and R_0 the recovery of any of the other sulfonamides the following relationships can be set up:
$R_{sc} = R_0 - 0.01$ and $R_{sc} + (n - 1)R_0 = nR_v$
After proper substitutions these formulas are obtained:
$R_{sc} = R_v + (1 - n)/100\ n$ and $R_0 = R_v + 1/100\ n$.

TABLE II

ANALYSIS OF SYNTHETIC MIXTURES OF SULFONAMIDES

Compound	Individual sulfonamides by quantitative TLC									
	Amount weighed (mg)	Aliquot No. 1			Aliquot No. 2			Aliquot No. 3		
		Found (mg)	% of amount weighed	R_v	Found (mg)	% of amount weighed	R_v	Found (mg)	% of amount weighed	R_v
Sulfadiazine	109.1	105.6	96.8		107.5	98.5		108.2	99.2	
Sulfamerazine	93.4	91.3	97.8	(0.9180)	92.6	99.1	(0.8860)	92.0	98.5	(0.9003)
Sulfamethazine	102.1	104.0	101.9		100.5	98.4		100.4	98.3	
otal	304.6	300.9	98.8		300.6	98.7		300.6	98.7	
Sulfathiazole	101.0	101.0	100.0		99.7	98.7		102.1	101.1	
Sulfadiazine	97.2	97.7	100.5	(0.9397)	97.8	100.6	(0.9524)	94.5	97.2	(0.9442)
Sulfamerazine	91.4	90.7	99.2		90.9	99.5		92.2	100.9	
otal	289.6	289.4	99.9		288.4	99.6		288.8	99.7	
Sulfacetamide	99.6	99.3	99.7		100.6	101.0		98.8	99.2	
Sulfadiazine	108.5	110.5	101.8	(0.9094)	108.0	99.5	(0.9169)	107.9	99.4	(0.9219)
Sulfamerazine	97.5	94.9	97.3		96.3	98.8		97.9	100.4	
otal	305.6	304.7	99.7		304.9	99.8		304.6	99.7	
Sulfacetamide	213.5	214.9	100.7		214.2	100.3		212.3	99.4	
Sulfadiazine	200.0	199.3	99.6	(0.9450)	199.1	99.5	(0.9508)	200.3	100.1	(0.9682)
Sulfamerazine	102.5	105.7	103.1		102.7	100.2		105.5	102.9	
Sulfamethazine	101.6	101.6	100.0		104.7	103.0		103.4	101.8	
otal	617.6	621.5	100.6		620.7	100.5		621.5	100.6	
Sulfacetamide	194.3	188.3	96.9		191.1	98.4		191.7	98.7	
Sulfadiazine	105.2	107.8	102.5	(0.9419)	105.8	100.6	(0.9095)	105.7	100.5	(0.9212)
Sulfamerazine	92.9	94.6	101.8		93.3	100.4		93.7	100.9	
Sulfamethazine	98.2	100.3	102.1		101.2	103.0		100.2	102.0	
otal	490.6	491.0	100.1		491.4	100.2		491.3	100.1	
Sulfacetamide	108.0	106.8	98.9		109.2	101.1		107.1	99.2	
Sulfadiazine	106.3	106.6	100.3	(0.9520)	106.3	100.0	(0.9186)	105.7	99.4	(0.9291)
Sulfamerazine	110.0	113.1	102.8		108.5	98.6		112.8	102.5	
Sulfamethazine	93.9	91.0	96.9		94.0	100.1		92.5	98.5	
otal	418.2	417.5	99.8		418.0	100.0		418.1	100.0	
Sulfathiazole	108.0	109.5	101.4		108.9	100.8		107.4	99.4	
Sulfadiazine	106.7	105.7	99.1	(0.9390)	106.1	99.4	(0.9709)	104.4	97.8	(0.9304)
Sulfamerazine	94.4	91.7	97.1		95.4	101.1		95.3	101.0	
Sulfamethazine	106.3	105.7	99.4		101.7	95.7		105.1	98.9	
otal	415.4	412.6	99.3		412.1	99.2		412.2	99.2	

DISCUSSION

The results of Table I confirm the conclusions of the previous investigation[2], which indicated that the recoveries of the different sulfonamides in a chromatogram are nearly equal. The situation arises probably from the fact that the concentration per unit area of spot is almost the same for all the five sulfonamides, when developed under the described conditions. Sulfacetamide has generally the lowest recovery in a chromatogram but, as previously discussed[2], this may be due to partial degradation of this compound during the marking of the spots under UV light rather than to

| | | | Total sulfonamides by direct dilution | | | | | | |
| Average (3) | | | | Determination No. 1 | | Determination No. 2 | | Average (2) | |
Found (mg)	% of amount weighed	R_v	Total amount weighed (mg)	Found (mg)	% of amount weighed	Found (mg)	% of amount weighed	Found (mg)	% of amount weigh
107.1	98.2		304.6	301.5	99.0	300.7	98.7	301.1	98.9
92.0	98.5	(0.9014)							
101.6	99.5								
300.7	98.7								
109.9	99.9		289.6	288.7	99.7	287.2	99.2	288.0	99.4
96.7	99.4	(0.9454)							
91.3	99.9								
288.9	99.7								
99.6	100.0		305.6	305.2	99.9	305.2	99.9	305.2	99.9
108.8	100.3	(0.9161)							
96.4	98.9								
304.8	99.7								
213.8	100.1		617.6	617.1	99.9	624.6	101.1	620.9	100.5
199.6	99.8	(0.9547)							
104.6	102.0								
103.2	101.6								
621.2	100.6								
190.4	98.0		490.6	493.7	100.6	488.7	99.6	491.2	100.1
106.4	101.1	(0.9242)							
93.9	101.1								
100.6	102.4								
491.3	100.1								
107.7	99.7		418.2	420.4	100.5	416.4	99.6	418.4	100.0
106.2	99.9	(0.9332)							
111.5	101.4								
92.5	98.5								
417.9	99.9								
108.6	100.6		415.4	412.6	99.3	410.6	98.8	411.6	99.1
105.4	98.8	(0.9378)							
94.1	99.7								
104.2	98.0								
412.3	99.3								

higher retention by the adsorbent. Even the recoveries of sulfacetamide do not differ substantially from those of the other four sulfonamides; if the fifteen determinations of each are averaged, the recovery of sulfacetamide is about 1% lower than that of any of the others (Table I). The difference is considered significant only because it is the outcome of a constant trend, observed in this as well as in the previous investigation[2].

Another important conclusion drawn from the results of Table I is that, in each chromatogram, the calculated recovery (designated as R_{mc}) very nearly equals the average of the actual recoveries of the individual sulfonamides (designated as R_{ms}),

TABLE III

ANALYSIS OF COMMERCIAL TABLETS OF SULFONAMIDES

Compound	Label per tablet (mg)	Aliquot No. 1			Aliquot No. 2			Aliquot No. 3		
		Found (mg)	% of label	R_v	Found (mg)	% of label	R_v	Found (mg)	% of label	R_v
Ifadiazine	162	158.0	97.5		157.7	97.3		160.7	99.2	
Ifamerazine	162	162.8	100.5	(0.9283)	165.0	101.8	(0.9084)	161.5	99.7	(0.8959)
Ifamethazine	162	165.2	102.0		163.2	100.7		163.5	100.9	
Total	486	486.0	100.0		485.9	100.0		485.7	99.9	
Ifathiazole	32.4	29.3	90.4		29.4	90.7		29.9	92.3	
Ifadiazine	32.4	30.4	93.8	(0.9286)	30.1	92.9	(0.9216)	29.9	92.3	(0.9117)
Ifamerazine	32.4	30.2	93.2		30.3	93.5		30.2	93.2	
Total	97.2	89.9	92.5		89.8	92.4		90.0	92.6	
facetamide	166.5	168.5	101.2		169.1	101.6		167.2	100.4	
fadiazine	166.5	154.9	93.0	(0.8562)	158.8	95.4	(0.8834)	156.2	93.8	(0.8460)
famerazine	166.5	164.6	98.8		160.7	96.5		165.2	99.2	
Total	499.5	488.0	97.7		488.6	97.8		488.6	97.8	
facetamide	200	214.6	107.3		211.4	105.7		215.1	107.6	
fadiazine	100	103.0	103.0	(0.9398)	104.4	104.4	(0.9642)	103.6	103.6	(0.9237)
famerazine	100	104.1	104.1		105.4	105.4		104.9	104.9	
famethazine	100	98.5	98.5		99.1	99.1		97.0	97.0	
Total	500	520.2	104.0		520.3	104.1		520.6	104.1	

the maximum observed difference between any of such two terms being 0.7%. This justifies the assumption, made for the estimation of the constituents of sample mixtures (Tables II and III), that the calculated recovery indicates the actual recovery of a chromatogram. In these calculations a new term is introduced, designated as R_v, which relates the recovery of a chromatogram to the labeled rather than to the determined volume of the micropipet used. This approach eliminates the need of knowing the volume of the micropipet and consequently allows standardization of the dilution factor. The R_v terms do not then indicate absolute recoveries; such recoveries can, however, be easily obtained, if desired, by multiplying the R_v values by the ratio of the labeled volume to the determined volume of the micropipet.

The total sulfonamide content of the sample mixtures is calculated with the assumption that the quantities of the individual components are related by the theoretical or labeled ratios. This permits estimation of the total sulfonamide content with a maximum possible error of about 1%, even when the quantities of the individual components differ by as much as 10% from those expected from the theoretical ratios, as indicated by an examination of the results of Table II.

The reliability of the method of calculating the individual components is also indicated by the results of Table II. Even when the recovery values (R_v) of chromatograms of the same solution are considerably different, the calculated quantities of each sulfonamide do not differ appreciably. The highest observed error in a single determination of a sulfonamide is 4.3%, but very seldom is such an extreme value reached; if three determinations are averaged, the maximum observed error is reduced

			Total sulfonamides by direct dilution						
Average (3)		R_v	Total declrd. mg per tablet	Determination No. 1		Determination No. 2		Average (2)	
Found (mg)	% of label			Found (mg)	% of label	Found (mg)	% of label	Found (mg)	% of label
158.8	98.0	(0.9109)	486	486.9	100.2	484.6	99.7	485.8	99.9
163.1	100.7								
164.0	101.2								
485.9	100.0								
29.5	91.0	(0.9206)	97.2	89.4	92.0	90.7	93.3	90.1	92.6
30.1	92.9								
30.2	93.2								
89.8	92.4								
168.3	101.1	(0.8619)	499.5	487.7	97.6	487.7	97.6	487.7	97.6
156.6	94.1								
163.5	98.2								
488.4	97.8								
213.7	106.9	(0.9426)	500	522.9	104.6	518.2	103.6	520.6	104.1
103.7	103.7								
104.8	104.8								
98.2	98.2								
520.4	104.1								

to 2.4%. The correction to the recovery value, made when sulfacetamide is present in a mixture, helps also to maintain the margin of error within the indicated limits. Without the correction factor the calculated quantities of sulfacetamide would be slightly lower and those of the other sulfonamides slightly higher than those reported in Table II. It may also be noted at this point that the sum of the calculated individual components generally very nearly equals the total sulfonamide content of the unchromatographed solution.

Although still negligible with 0.5-cm cells, the absorbance blanks were slightly higher than found in the previous investigation[2]. The average absorbance blank was about 0.01 with 0.5-cm cells and about 0.02 with 1-cm cells. This indicates that, while caused mostly by suspended colloidal particles, the absorbance blanks are also partially contributed by variable amounts of impurities present in the adsorbent. Prewashing of the silica gel is still not necessary, as long as the average absorbance blanks are not higher than those here indicated. To limit the possible error caused by the variability of the absorbance blank, the absorbances of the sulfonamide spots should be at least 0.3 in 0.5-cm cells and 0.6 in 1-cm cells.

Future applications of the proposed method do not necessitate, in our opinion, determining again the recoveries of the reference sulfonamides, as long as no changes are made to the specified conditions. A new recovery study of the standards would be needed only if a mixture contains one or more components other than those investigated or if the spotted quantities should be outside the considered range of 80–200 μg.

It is hoped that other types of mixtures can be similarly characterized by the recovery ratios of their constituents and that they can be consequently analyzed by the simple procedure here introduced.

REFERENCES

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