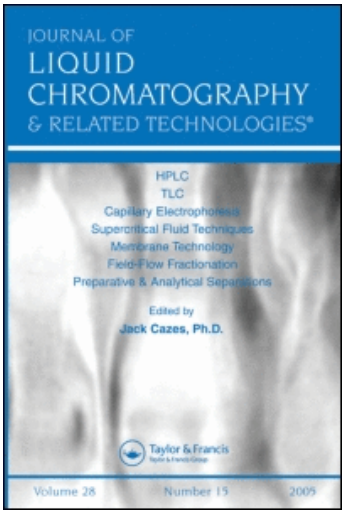

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Determination of Sulfanilamide and Sulfisoxazole in Drug Preparations by Quantitative High Performance TLC

Joseph Sherma[a]; Melinda Duncan[a]
[a] Department of Chemistry, Lafayette College, Easton, Pennsylvania

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DETERMINATION OF SULFANILAMIDE AND SULFISOXAZOLE IN DRUG PREPARATIONS BY QUANTITATIVE HIGH PERFORMANCE TLC

Joseph Sherma and Melinda Duncan
Department of Chemistry
Lafayette College
Easton, Pennsylvania 18042

ABSTRACT

A densitometric TLC method was developed for quantification of sulfanilamide and sulfisoxazole in creams, supppositories, and tablets. The sulfas were extracted into acidic ethanol, diluted to an appropriate volume, and separated by silica gel HPTLC. The fluorescence quenching of the sulfa zones in samples and standards was compared by in situ scanning. Recoveries of the drugs from authentic samples ranged from 96-105%. Recoveries from products with old expiration dates were low. Identity of the sulfa drugs in products was confirmed by application of fluorescamine and Bratton-Marshall detection reagents.

INTRODUCTION

In an earlier paper (1) we reported a quantitative TLC method based on fluorescence densitometry for determination of aminacrine hydrochloride in pharmaceutical preparations used to treat vaginal infections. These products also contain sulfanilamide or sulfisoxazole as therapeutic components. This paper describes the

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determination of these sulfa drugs by scanning of quenched zones on high performance silica gel thin layers containing fluorescent phosphor. The analysis of vaginal creams and suppositories and a sulfisoxazole tablet is demonstrated. The identity of the sulfa drug is confirmed by detection with the Bratton-Marshall reagent or fluorescamine.

EXPERIMENTAL

Standard Solutions

Sulfanilamide and sulfisoxazole standards were obtained from Sigma Chemical Company. Stock solutions (1.00 µg/µl) were prepared in ethanol-glacial acetic acid (99:1 v/v). Working standards of 100 ng/µl and 400 ng/µl concentrations were prepared by quantitative 1 to 10 and 4 to 10 dilutions of the stock solutions with the acidic ethanol.

Sample Preparation

Sample preparation procedures were essentially the same as those described for aminacrine determinations (1), except that ethanol-glacial acetic acid (99:1 v/v) was used in place of ethanol-concentrated HCl (99:1 v/v). For creams containing a label value of 15% sulfanilamide, a 1.33g sample was heated for 10 minutes with 100 ml of acidic ethanol in a 250 ml beaker, the solution was cooled and transferred to a 200 ml volumetric flask, and a 20.0 ml aliquot was quantitatively diluted to 50.0 ml using a pipet and volumetric flask. The theoretical concentration of the

final solution is 400 ng/µl. Gelatin-encapsulated suppositories with a label value of 1.050g of sulfanilamide were heated with 100 ml of acidic ethanol and the solution filtered into a 200 ml volumetric flask. After a quantitative 10 to 50 dilution, the theoretical concentration of the solution is 420 ng/µl.

Creams (1.00g) with a label value of 10% sulfisoxazole were dissolved in 40 ml of acidic ethanol, the solution was transferred to a 100 ml volumetric flask, and a 4 to 10 dilution was made to produce a final solution with a 400 ng/µl concentration. Suppositories containing 600 mg of sulfisoxazole were treated exactly as described above for sulfanilamide-containing suppositories, but a 17 to 50 dilution was made to produce a final solution with a 408 ng/µl concentration. Tablets containing 500 mg of sulfisoxazole were crushed using a mortar and pestle, and the powder was transferred with rinsing to a 100 ml beaker and heated for 10 minutes with 50 ml of acidic ethanol. The cooled solution was filtered into a 100 ml volumetric flask. Consecutive 10 to 50 and 20 to 50 dilutions were made to give a solution with a 400 ng/µl theoretical concentration.

TLC Determination

TLC was carried out on 10x10 cm Whatman LKHPDF laned high performance silica gel plates with preadsorbent spotting area using procedures described earlier (1). Duplicate 1.0 µl aliquots of the final sample solutions and the 400 ng/µl standard were applied to adjacent lanes with Drummond micropipets. Plates were developed with ethyl acetate-concentrated ammonium hydroxide (99:1 v/v) for

determination of sulfanilamide and ethyl acetate-chloroform-methanol (25:25:5 v/v) for sulfisoxazole. Mobile phase was removed by forced cool air from a hair dryer. Sulfa zones were scanned with a Kontes Chromaflex fiber optics densitometer in the single beam, transmission mode using the 5 mm light beam and shortwave (254 nm) UV source. See Reference 1 for the details of zone scanning, peak measurement, and calculation of the drug content.

RESULTS AND DISCUSSION

Sulfanilamide and sulfisoxazole appeared as dark, flat bands on a bright fluorescent background under 254 nm UV light. The minumum level for visual detection of both compounds was about 50 ng, but 200 ng was the lowest amount that could be scanned reliably. The respective R_F values of sulfanilamide and sulfisoxazole in the ethyl acetate-NH_3 solvent were 0.43 and 0.0. In the ethyl acetate-chloroform-methanol solvent, both compounds moved with R_F 0.43. None of the products analyzed contained both of the sulfas, but a product that did contain both compounds could be analyzed by development first with the ammonia-containing solvent, scanning of sulfanilamide samples and standards, followed by development with the second solvent and scanning of sulfisoxazole. Sulfanilamide would move to the top of the plate in the second development.

Linearity of absorption by the quenched zones was established by spotting 200 to 800 ng amounts of the sulfas using variable

volumes of the two working standard solutions. Correlation coefficients (R) were greater than 0.995 for both compounds for linear regression plots of peak area against nanograms per zone. Figure 1 shows typical scans for a series of standard zones used to establish linearity of response for sulfanilamide. The degree of quenching (scan areas) and the calibration curve were very similar for sulfisoxazole.

Samples of various brands of commercial products with a variety of ingredients were analyzed by the TLC procedure. As seen in Table 1, results ranged from 96.0 to 105% of the label values. Average areas of duplicate sample and standard aliquots were used for calculations. Areas of duplicates almost always differed by less than 5% (Figure 2). Entries 7a and 7b in Table 1 were for two different tubes of the same brand of cream. Creams 4 and 5 were the same brand and had the same formula, except that 4 also contained dienestrol. Three different suppositories containing sulfanilamide and one suppository containg sulfisoxazole assayed between 44 and 88%. In each case, the products displayed 1981 or 1982 expiration dates. Sample 9 was not a vaginal product but a generic sulfisoxazole tablet used by physicians and veterinarians as an antibacterial and antimicrobial agent and to treat infections.

Additional ingredients in the vaginal products included aminacrine, allantoin, polyoxyethylene nonyl phenol, acrisorcin, and oxyquinoline benzoate. None of these interfered with the determination of the sulfa drugs. The ability to spot multiple

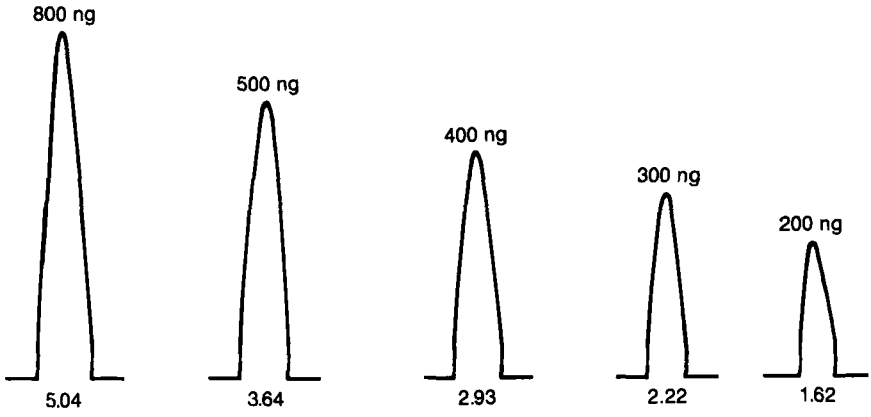

Figure 1. Scans of a plate containing 200 to 800 ng of sulfanilamide using the Kontes densitometer with baseline corrector, shortwave UV source, and attenuation X100. Areas (cm^2) are shown below the peaks.

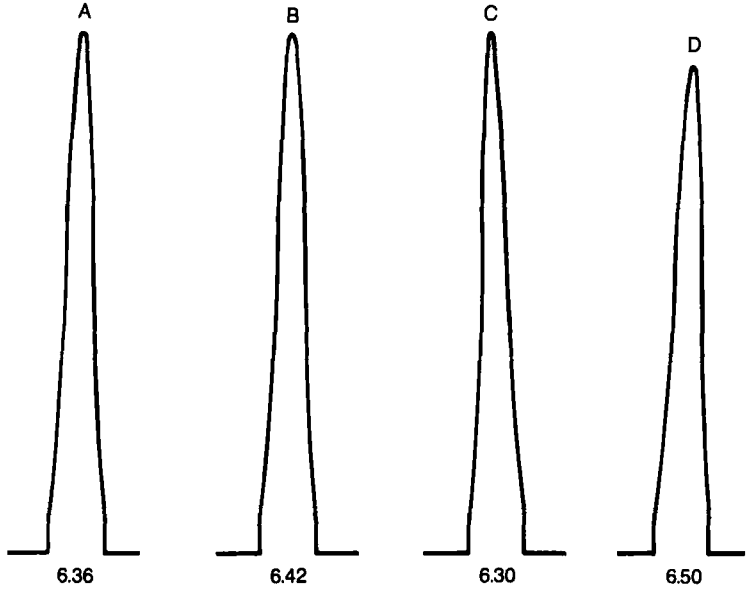

Figure 2. Scans of duplicate aliquots of cream Sample 2 (Table 1) (A and B) and 400 ng of sulfanilamide standard (C and D) at attenuation 100. The areas shown beneath the peaks resulted in an analysis that represented 100% of the label value.

TABLE 1

Analysis of Commercial Vaginal Creams and

Gelatin-Encapsulated Suppositories for Sulfa Drugs

Sample No.	Dosage form	Dosage level (sulfa)	%Label claim
1	cream	15% (A)	99.3
2	cream	15% (A)	100
3	cream	15% (A)	102
4	cream	15% (A)	98.7
5	cream	15% (A)	96.0
6	suppository	1.05g (A)	99.3
7a	cream	10% (B)	104
7b	cream	10% (B)	98.8
8	suppository	600 mg (B)	105
9	tablet	500 mg (B)	104

A = sulfanilamide

B = sulfisoxazole

samples along with standards on the same plate allows the HPTLC assay procedure to be accurate and precise and to have high sample throughput.

To confirm the identity of the sulfa drugs in the sample solutions, fluorescamine and/or the Bratton-Marshall reagent can be used. Fluorescamine derivatization was accomplished by dipping the

dried plate in a solution containing 30 mg of reagent in 250 ml of acetone. After air drying for 15-30 minutes, fluorescent zones were visible for both sulfas under longwave (365 nm) UV light. Red zones of the sulfas were produced by exposing the dried plate to nitrogen oxide vapors generated when solid $NaNO_2$ is added to dilute H_3PO_4 in a closed chamber, then air drying, and spraying with 0.3% N-1-(Naphthyl)ethylenediamine dihydrochloride in methanol. The sensitivity limit of both reagents was below 50 ng, and quantification could be accomplished by scanning the detected zones at appropriate wavelengths.

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REFERENCE

1. Dittamo, M., Kraus, L., Lee, A., and Sherma, J., J. Liq. Chromatogr., 8, 1247 (1985).