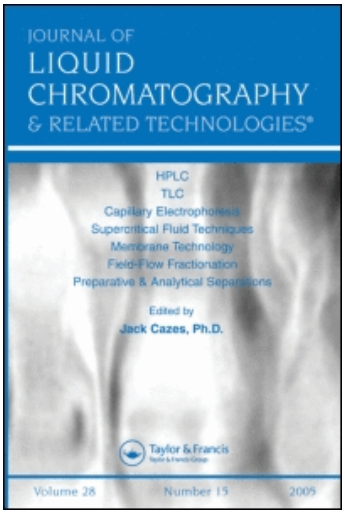

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DETERMINATION OF AMINACRINE HYDROCHLORIDE IN DRUG PREPARATIONS BY TLC WITH FLUORESCENCE DENSITOMETRY

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ABSTRACT

A densitometric TLC method was developed for quantification of aminacrine hydrochloride in creams, jellies, and suppositories. Aminacrine was extracted into acidic ethanol, diluted to a known volume, and separated by high performance silica gel TLC. The fluorescence of aminacrine zones in samples and standards was compared by in situ scanning. Recoveries of aminacrine from authentic samples ranged from 95.4 to 103.4%.

INTRODUCTION

Aminacrine hydrochloride (9-aminoacridine hydrochloride) is an antiseptic ingredient in pharmaceutical preparations used to treat vaginal infections. A fluorescence spectrometric method for the analysis of these preparations was developed (1), but subsequent study (2) of this method showed that it could not be used for reliable quantification because of self-absorption of the emitted fluorescence by aminacrine at higher concentrations,

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coupled with the tendency to form fluorescence quenching complexes with the additional ingredients sulfanilamide and sulfisoxazole and an apparent random extraction bias. A visible spectrometric method was then developed to quantify aminacrine hydrochloride, and a qualitative TLC identification test was also described (3). We have found that the fluorescence properties of aminacrine can provide an accurate and precise assay by combining the TLC procedure with in situ fluorescence densitometry. This paper reports the quantitative TLC procedure and the results obtained in the analysis of five creams, one jelly, and two suppositories containing aminacrine as an active ingredient.

EXPERIMENTAL

Standard Solutions

Aminacrine hydrochloride was purchased from Sigma Chemical Co. (No. A-1135). A stock standard solution of aminacrine was prepared by dissolving 0.500 g in 100 ml of ethanol-concentrated HCl (99:1 v/v). A 50.0 ng/µl standard solution was prepared by dilution of 1.00 ml of the stock solution to 100 ml with ethanol-concentrated HCl (99:1 v/v). This solution was quantitatively diluted 1:1 to give a standard solution containing 25.0 ng/µl.

Sample Preparation

Creams and jellies. About 2.5 g of sample was accurately weighed into a 150 ml beaker, 40 ml of acidic ethanol was added, and the beaker was heated at low temperature on a hot plate for 10 minutes with occasional stirring. After cooling to room temperature, the solution was quantitatively transferred to a 100 ml volumetric flask, which was filled to the line with acidic ethanol. Cloudy solutions were filtered while being transferred into the volumetric flask. For a sample of exactly 2.5 g containing 0.2% aminacrine, the theoretical concentration of the final solution is 50.0 ng/µl.

Suppositories. Gelatin encapsulated suppositories were slit along the seam with a razor and the contents extruded as completely

as possible into a 250 ml beaker. The capsule was placed in the beaker, and 100 ml of acidic ethanol was added. The sample was dissolved by heating at low temperature for 10 minutes with occasional stirring. The solution was filtered into a 250 ml volumetric flask, and the capsule was examined for completeness of content extraction. The theoretical concentration of the solution was 48 ng/µl for the 12 mg suppository and 56 ng/µl for the 14 mg suppository when acidic ethanol was added to the line. Molded suppositories were treated by the same general procedure, except that extrusion of the sample material was not required because it slipped easily out of its casing into the 250 ml beaker. The final solution was filtered into a 100 ml volumetric flask, making the theoretical concentration 60.0 ng/µl for the 6 mg suppository.

TLC Determination

Whatman LKHPD high performance silica gel plates (10 x 10 cm) were used for the analysis. These plates are scored into 9 lanes of 8 mm width and contain a 2 cm high preadsorbent spotting strip at the bottom. Plates were precleaned by development with methanol-methylene chloride (1:1 v/v) followed by air drying. Duplicate 2.0 µl aliquots of final sample solution and the 50 ng/µl standard solution were applied to the preadsorbent area of four adjacent lanes using Drummond disposable microcap micropipets. Chromatograms were developed with ethyl acetate-concentrated ammonium hydroxide (17:2 v/v) to within 1.5 cm of the top of the plate in a paper lined, vapor saturated metal HPTLC chamber (Analtech). The mobile phase was removed by drying in air. Aminacrine zones were scanned with a Kontes Chromaflex fiber optics densitometer (K-495000) in the single beam, transmission mode using the 5 mm scanning head and the longwave UV source, which was covered with a blue cobalt glass to filter out visible wavelengths. The scanner was equipped with a Kontes baseline corrector and Bausch and Lomb VOM 6 recorder. Attenuation settings were typically 100 or 200, and the scan speed was 4 cm/minute. The areas of the peaks were measured

by hand from the recorder chart using the formula height x width at half height or directly with an electronic integrator attached to the densitometer. The ng of aminacrine in the spotted sample was calculated using the equation: (average area standard peaks/ng standard spotted) = (average area sample peaks/ng aminacrine in 2 µl aliquot). Based on the label value of aminacrine in each product, the sample size, and the volume of the flask containing the final, spotted solution, the theoretical value and percentage recovery were calculated.

RESULTS AND DISCUSSION

Aminacrine was detected at low ng levels as a compact brilliantly fluorescent elliptical zone with an R_F value of 0.57 after development with ethyl acetate-NH_4OH (17:2 v/v) on a preadsorbent high performance silica gel layer. The time required for the mobile phase to advance 6.5 cm beyond the silica gel-preadsorbent interface was approximately 10 minutes.

Linearity of fluorescence intensity with concentration was established by spotting 25, 50, 100, 150, and 200 ng of aminacrine using the 25.0 ng/µl and 50.0 ng/µl standard solutions and 1,2,3, and 4 µl micropipets. The linearity correlation coefficient (R) was always above 0.98 and usually above 0.99 for the 25-200 ng range when the resultant peak areas were plotted against ng/zone. Figure 1 illustrates scans of zones containing 50, 100, and 150 ng of aminacrine. It is apparent that the self quenching that was noted during studies of the fluorescence assay in solution (2) is not a problem at the low ng levels used in the TLC method.

A study was made of the reproducibility of the TLC procedures by spotting seven 250 ng aminacrine samples on a single plate (each 5 µl of the 50.0 ng/µl standard). The zones were scanned after development, and the relative standard deviation (CV) of the peak areas was 1.9%.

A study was also made of the variation of fluorescence with time by scanning a single zone 15 minutes after removing

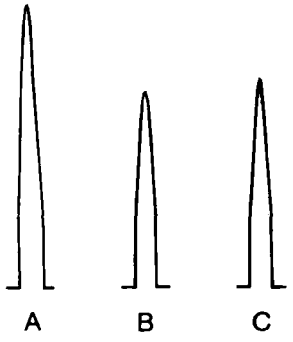

Figure 1. Scans of a chromatogram containing 150 ng (A), 100 ng (B), and 50.0 ng (C) zones of aminacrine standard using the Kontes densitometer with the longwave UV source. The attenuation was X200 for A and B and X100 for C.

the plate from the chamber and repeating the scan every 15 minutes for two hours. No significant change in fluorescence intensity was noted over this time, and visual observation of old chromatograms indicates that fluorescence is stable over a period of many months.

Samples of commercial products were analyzed using the proposed TLC method. Excluding Sample 3, results ranged from 95.4 to 103.4% of the labeled amount of aminacrine hydrochloride (Table 1), which compared favorably with those reported by Bunch (3) for the spectrophotometric method. Recovery was calculated using the average of the areas of the two sample aliquots applied, which almost always differed by less than 10% and often by less than 5%. Figure 2 illustrates typical scans of duplicate sample and standard aminacrine zones.

Sample 2 (Table 1) yielded very high results unless a freshly prepared light yellow-orange solution was spotted. The solution changed to a darker orange-red with time, apparently indicating decomposition of the sample. Analysis of a solution after it changed color yielded a 161% recovery by TLC. This sample was also analyzed by the spectrophotometric method of

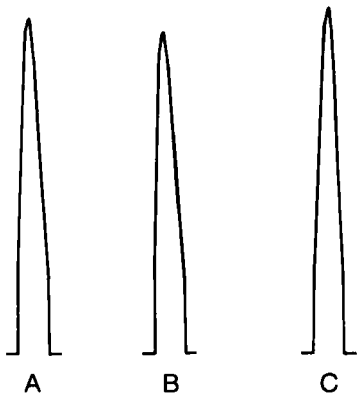

Figure 2. Scans of duplicate 2 µl aliquots of Sample 1 (A and C) and 100 ng of aminacrine standard at attenuation 100.

TABLE 1

Analysis of Commercial Products for Aminacrine Hydrochloride

Sample No.	Dosage form	Dosage level	% Label claim
1	cream	0.2%	99.4
2	cream	0.2%	101.0
3	cream	0.2%	120.2
4	jelly	0.2%	96.6
5	molded suppository	6 mg	103.4
6*	cream	0.2%	97.5
7	gelatin-encapsulated suppository	14 mg	100.7
8	gelatin-encapsulated suppository	12 mg	95.4

* cream sample mixed by E. Bunch

Bunch (3) and gave a recovery of 515%. Analysis of the freshly prepared solution of Sample 2, which gave 101.0% recovery by TLC, assayed at 111.8% by the spectrophotometric method. Our ability to correctly perform the spectrophotometric method was checked by analyzing Sample 1, which gave no evidence of breakdown on storage in the refrigerator, and a recovery of 97.8% was obtained.

Sample 3 gave consistently high results by the TLC method. Analysis of it by the spectrophotometric method gave 111.1% recovery, indicating some problem with the sample we had obtained rather than with the method. This sample contained the 4-hexylresorcinol salt of 9-aminacridine instead of the hydrochloride salt in the other samples, and calculation of results required use of a correction factor (1.68) representing the ratio of molecular weights of the two salts.

Four samples of cream Number 1 were assayed by four different analysts. In addition to the 99.4% recovery listed in Table 1, 102.7, 98.6, 99.5, and 99.2% recovery was obtained. These results indicate the adequate precision and simplicity of the method.

All but one of the samples analyzed contained one or more ingredients in addition to aminacrine, including allantoin, sulfanilamide, sulfisoxazole, oxyquinoline benzoate, and polyoxyethylene nonyl phenol. None of these compounds was detected as a fluorescent zone under longwave UV light. When chromatographed on the same silica gel HPTLC plates containing fluorescent indicator, only sulfanilamide was detected as a dark (quenched) zone under shortwave UV light. This zone had an R_F value of 0.47 and was completely resolved from aminacrine.

The proposed quantitative fluorometric TLC method proved to be accurate, reproducible, and selective for the determination of aminacrine in combination with other drugs in the limited number of commercial products tested. The ability to spot multiple samples along with standards on the same plate allows high sample throughput to be achieved.

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REFERENCES

1. Official Methods of Analysis (1975), 12th Ed., AOAC, Arlington, VA, Sections 38.109-38.115.

2. Bunch, E. A., J. Assoc. Off. Anal. Chem., 66, 145 (1983).

3. Bunch, E. A., J. Assoc. Off. Anal. Chem., 66, 140 (1983).