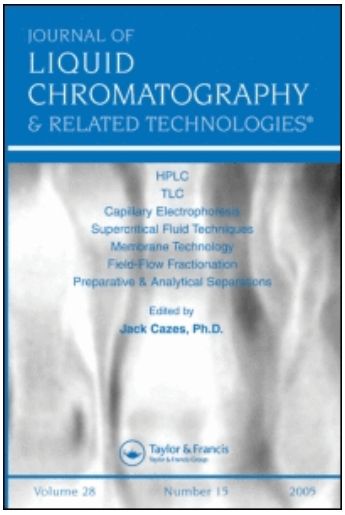

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Determination of Allantoin in Drug Preparations by Quantitative High Performance TLC

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DETERMINATION OF ALLANTOIN IN DRUG PREPARATIONS BY QUANTITATIVE HIGH PERFORMANCE TLC

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ABSTRACT

A densitometric TLC method was developed for the quantification of allantoin in creams and suppositories used to treat vaginal infections. Allantoin was extracted with water at elevated temperature, diluted to a known volume, and separated by HP silica gel TLC. Allantoin was detected by spraying with p-dimethylaminobenzaldehyde reagent. The absorption of standards and samples was compared by in situ scanning. Recoveries of allantoin from authentic samples ranged from 96-102%, except for one sample that assayed high. The accuracy of the method was demonstrated by standard addition analysis of this sample.

INTRODUCTION

In earlier papers we reported quantitative TLC methods based on fluorescence and fluorescence quench densitometry for the determination of aminacrine hydrochloride (1) and the sulfa drugs sulfanilamide and sulfisoxazole (2) in pharmaceutical preparations used to treat vaginal infections. All of these products also contain

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allantoin [2,5-dioxo-4-imidazolidinyl urea] as a therapeutic component that promotes healing.

Most analyses for allantoin have been based on nonspecific solution colorimetry after hydrolysis (e.g., 3-5). Determination by HPLC in cosmetics and pharmaceutical products (6-9) and in biological fluids (10,11) has also been reported.

This paper describes the determination of allantoin in vaginal products by scanning the yellow zones produced from samples and standards with the detection reagent p-dimethylaminobenzaldehyde on preadsorbent high performance silica gel layers. The method is adapted from a previously published TLC procedure (12) for estimation of allantoin in serum, lymph, and urine.

EXPERIMENTAL

Standard Solution

Allantoin standard was purchased from Aldrich (No. A2,839-2). Preparation of a 1.00 µg/µl distilled water solution in a 25 ml volumetric flask required gentle heating on a hotplate to affect complete dissolving.

Sample Preparation

Creams. About 1.25 g of sample was accurately weighed into a 25 ml beaker, 15 ml of distilled water was added, and the beaker was heated at low temperature on a hotplate for 15 minutes with occasional stirring. After cooling to room temperature, the solution was transferred quantitatively to a 25 ml volumetric

flask, which was filled to the line with distilled water. Cloudy solutions were filtered hot while being transferred into the volumetric flask. For a sample of exactly 1.25 g containing 2.0% of allantoin, the theoretical concentration of the final solution is 1.00 µg/µl.

Suppositories. Samples of suppositories were prepared as previously described (1), except that water was used instead of acidic ethanol and samples were heated for 15 minutes rather than 10. The theoretical concentrations of the final solutions for suppositories containing 140 mg and 120 mg of allantoin were 0.560 and 0.480 µg/µl, respectively.

TLC Determination

TLC was carried out on 10 X 20 cm Whatman high performance silica gel plates containing a preadsorbent spotting area and scored into 8 mm wide lanes using procedures described earlier (1,2). Duplicate aliquots of the final sample solution (5 µl for creams and 9 µl for suppositories) and 3.00, 5.00, and 7.00 µg standards (3, 5, and 7 µl of the 1.00 µg/µl solution) were applied to adjacent lanes using a 10 µl Drummond microdispenser. The initial zones were dried thoroughly with a hairdrier and the plates developed in a saturated TLC chamber with methyl ethyl ketone-acetone-formic acid-water (40:2:1:6 v/v). The plate was air-dried in a fume hood, sprayed heavily (but not soaking) with p-dimethylaminobenzaldehyde detection solution (0.25 g reagent in 38 ml methanol and 12 ml conc. HCl), and heated in an oven at 100°C for 5 minutes. Allantoin zones were measured with a Kontes

Chromaflex fiber optics scanner as described earlier (1,2). A calibration equation was computer calculated from the scan areas of the standards, the amounts of allantoin in the sample aliquots were interpolated and averaged, and contents of the preparations were calculated and compared to the label values.

RESULTS AND DISCUSSION

p-Dimethylaminobenzaldehyde reagent detected allantoin as a compact yellow zone with an R_F value of 0.35. Development with the mobile phase for a distance of 7 cm beyond the preadsorbent-HP silica gel junction required 30 minutes. The linearity correlation coefficient for the scan areas of the three standards versus micrograms spotted was usually 0.995 or higher.

Eleven different samples were analyed by the TLC procedure and the results are shown in Table 1. The samples represent different brands and different lots of the same brand, with three combinations of active ingredients. As can be seen in Table 1, results ranged from 96 to 102%, except for Sample 5. Calculations for each trial were based on average areas of duplicate sample aliquots, which had a percentage difference that was always below 6% and usually less than 2%.

Triplicate analysis of cream Sample 5 yielded consistently high results. Figure 1 shows scans of the three standards and duplicate sample aliquots for the first analysis of this product shown in Table 1. A standard addition analysis was carried out

TABLE I

Analyses of Commercial Vaginal Creams and Suppositories for Allantoin

Sample No.	Dosage form	Dosage level	% Label claim[a]
1	cream[b]	2.0%	100 96.0
2	cream[c]	2.0%	105
3	cream[b]	2.0%	100
4	cream[b]		100
5	cream[c]	2.0%	115 119 115
6	cream[d]	2.0%	101
7	suppository[b]	140 mg	100
8	suppository[d]	120 mg	102 102
9	suppository[d]	120 mg	96.0 99.5
10	suppository[b]	140 mg	97.0 99.3
11	suppository[b]	140 mg	102 100

[a] Replicate analyses of certain samples are given in this column.

[b] Also contained sulfanilamide and aminacrine.

[c] Also contained sulfanilamide, aminacrine, and dienestrol.

[d] Also contained sulfisoxazole and aminacrine.

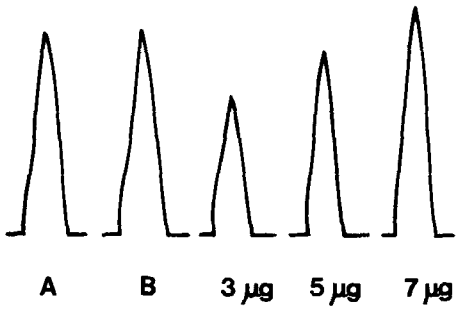

Figure 1. Scans of 3.00, 5.00, and 7.00 µg standard zones and duplicate aliquots of cream Sample 5 (Table 1) representing 114% (A) and 116% (B) compared to the label value. The attenuation setting on the Kontes densitometer was X50.

by weighing two 1.25 g portions of Sample 5, adding 25.0 mg of allantoin standard to one portion to double the label value, and analyzing the samples in an identical manner except that 2.5 µl of the fortified sample and the usual 5 µl of the unfortified sample were spotted for TLC. The difference in assay values represented 99.5% of the added standard, confirming the accuracy of the method and the unexplained high results obtained for Sample 5.

In addition to allantoin, the pharmaceutical products tested contained a variety of the ingredients listed in Table 1 and Reference 2. The only compounds that are detected with the p-dimethylaminobenzaldehyde reagent are the sulfa drugs ($R_f > 0.8$) and aminacrine ($R_F < 0.1$). The sulfas become yellow immediately upon spraying, while aminacrine requires brief heating. None of these spots interfere with measurement of allantoin zones.

The described quantitative TLC method proved to be accurate, reproducible, and selective for the determination of allantoin in a

number of commercial drug formulations. The ability to analyze multiple samples using a common series of standards on a single plate allows high sample throughput to be achieved.

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REFERENCES

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

2. Sherma, J. and Duncan, M., J. Liq. Chromatogr., in press.

3. Nirmala, J. and Sastry, K. S., Anal. Biochem. 47, 218 (1972).

4. Vrbaski, M. M., Grujic-Injac, B., and Gajic, D., Anal. Biochem. 91, 304 (1978).

5. Pelegrino, E. and Covalschi, E., Farmacia (Bucharest) 31, 179 (1983).

6. Carroll, M. A., White, E. R., and Zerembo, J. E., Anal. Chem. 53, 1111A (1981).

7. Kawase, J., Ueno, H., and Tsuji, K., J. Chromatogr. 253, 237 (1982).

8. Nakao, K., Honda, K., and Yoneya, T., J. Assoc. Off. Anal. Chem. 65, 1362 (1982).

9. Zaidi, Z. R., Sena, F. J., and Basilio, C. P., J. Pharm. Sci. 71, 997 (1982).

10. Tiemeyer, W. and Griesecke, D., Anal. Biochem. 123, 11 (1982).

11. Hirota, K., Kawase, M., Ohmori, S., and Kishie, T., J. Chromatogr. 277, 165 (1983).

12. Abraham, J., Simeone, F. A., and Hopkins, R. W., Anal. Biochem. 70, 377 (1976).