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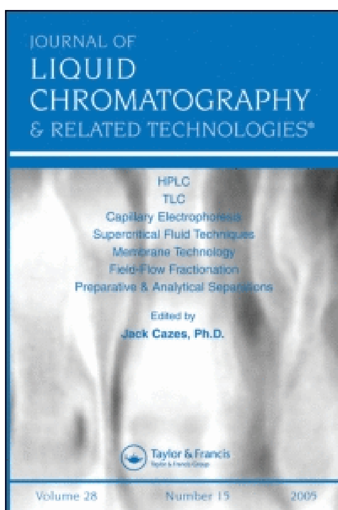

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QUANTITATIVE HPTLC DETERMINATION OF DIPHENHYDRAMINE HYDROCHLORIDE IN TABLET, GELCAP, AND CAPSULE ANTIHISTAMINE PHARMACEUTICALS

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QUANTITATIVE HPTLC DETERMINATION OF DIPHENHYDRAMINE HYDROCHLORIDE IN TABLET, GELCAP, AND CAPSULE ANTIHISTAMINE PHARMACEUTICALS

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ABSTRACT

A quantitative method involving high-performance thin-layer chromatography (HPTLC) with automated sample application and UV-absorption scanning densitometry was developed for the determination of diphenhydramine hydrochloride in tablets, gelcaps, and capsules. Separation was performed on high performance silica gel plates containing fluorescent indicator, and the analyte was detected as fluorescence-quenched zones under short-wave UV light. Four different pharmaceutical products containing diphenhydramine hydrochloride as an active ingredient were analyzed to test the applicability of the new method. Precision was validated by replicate analysis of samples and accuracy by analysis of spiked samples. The percent diphenhydramine hydrochloride in the tested pharmaceutical samples ranged from 86.8% to 111% compared to label values, precision ranged from 1.7% to 1.9% relative standard deviation, and the errors in the two spiking experiments were 0.00% and 0.81% compared to the fortification levels.

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INTRODUCTION

In previous papers, we reported the quantitative determination of the active ingredients pseudoephedrine hydrochloride (PE) and guaifenesin in decongestant tablets[1] and of phenylephedrine hydrochloride, chlorpheniramine maleate, brompheniramine maleate, and phenylpropanolamine hydrochloride in antihistamine and decongestant tablets, gelcaps, and capsules[2] by high performance thin layer chromatography (HPTLC) using automated spray-on application of samples and standards as bands and measurement of separated, fluorescence-quenched zones by scanning densitometry. This paper describes the use of similar HPTLC procedures for the simple, rapid, quantitative analysis of antihistamine tablets, gelcaps, and capsules containing diphenhydramine hydrochloride (abbreviated DPH below) as an active ingredient.

Current methods for determining DPH in pharmaceutical drug substance and dosage forms are based on high performance liquid chromatography (HPLC) using a nitrile-bonded silica gel column and 254 nm UV detector.[3] A silica gel TLC method with acetonitrile-methylene chloride-n-propylamine (56:40:4) mobile phase has been reported for the qualitative identification of DPH in capsules containing PE.[3] The quantitative HPTLC method described below, for which excellent accuracy and precision are demonstrated, is less complex and faster than HPLC.

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EXPERIMENTAL

Preparation of Standard Solutions

Standard solutions for TLC analysis (1.00 mg/mL) and spiking experiments (10.0 mg/mL) were prepared by dissolving DPH reference standard (2-diphenylmethoxy-N,N-dimethylethanamine hydrochloride, CAS # 147-24-0, Sigma, St. Louis, MO) in absolute ethanol.

Preparation of Sample Solutions

Tablets, gelcaps, and capsules with a label value of 25 mg of DPH were purchased at a drug store. Tablets were ground into a fine powder by use of a mortar and pestle, capsules were crushed open with a mortar and pestle to release the white powder, and gelcaps were cut open with a razor. The powder or gel was rinsed completely with 65 mL of ethanol into a 100 mL volumetric flask through a funnel, the solution was stirred magnetically for 1 hr, the flask

was filled to the line with ethanol and shaken, and the solution was allowed to sit overnight so that undissolved material settled to the bottom of the flask and would not be applied to the layer.

HPTLC Analysis

Analyses were performed on Merck 20 x 10 cm silica gel 60 F_{254} GLP plates (No. 5613/6, EM Separations Technology, Gibbstown, NJ). Plates were pre-cleaned by development to the top with dichloromethane-methanol (1:1) and air-dried. Using a Camag (Wilmington, NC) Linomat IV automated applicator, single 2.00 and 8.00 μL and duplicate 4.00 μL aliquots of the 1.00 μg/μL standard and duplicate 16.00 μL aliquots of sample were spotted on the plate. The Linomat IV was equipped with a 100 μL syringe and was programmed with the following settings: band length 6 mm, application rate 4 sec/μL, table speed 10 mm/sec, and distances of 4 mm between bands, 20.7 mm from the plate edge, and 1.5 cm from the bottom of the plate.

Plates were developed 6.0 cm past the origin using ethyl acetate-methanol-concentrated ammonium hydroxide (85:10:5) as the mobile phase in a paper-lined Camag HPTLC twin-trough chamber that had been allowed to equilibrate for approximately 15 min prior to insertion of the spotted plate. The development time was 13-15 min, after which the plate was removed and allowed to air dry in a fume hood. Sample and standard zones were scanned with a Camag TLC Scanner II at 260 nm, which had been predetermined to be the wavelength of maximum absorbance by measuring the UV absorption spectrum of a standard zone using the spectral-scanning mode of the densitometer and the deuterium source.

Scanning parameters were set as follows for area measurements: slit length 3, slit width 4, and scanning rate 4 mm/sec. By means of the CATS-3 software, a calibration curve relating the weights and scan areas of standard bands was produced. To determine the amount of DPH in the sample, the weight of the middle standard (4.00 μg) was multiplied by the ratio of the average sample zone peak area to the average standard zone peak area. Percent recovery was calculated by dividing this weight by the theoretical weight based on the label value and multiplying by 100.

Spiking experiments were performed to validate the accuracy of the method. A tablet was prepared and analyzed for DPH as described above. A 10.0 mL aliquot of the sample solution was pipetted into a vial and enough of the spiking standard solution was added, using a 1000 μL Drummond (Broomall, PA) digital dispenser, to exactly double the concentration of DPH in

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the sample based on the analysis. This spiked sample was analyzed by spotting the same standards with duplicate 8.00 μL aliquots of the sample. After separation and scanning, the percent difference between the results of the first and second analyses, which theoretically should have been identical, was calculated.

RESULTS AND DISCUSSION

Four different pharmaceuticals were analyzed; three of the products, a tablet, a gelcap, and a capsule, contained 25 mg of diphenhydramine hydrochloride as the only active ingredient, while the fourth, another tablet, also contained 60 mg of PE. The capsules and gelcaps contained a powder and a gel, respectively, enclosed by a plastic-like coating. This coating was transferred to the flask to minimize loss of any active ingredient that might adhere to it.

When viewed under 254 nm UV light, DPH (and PE) appeared as dark bands against a green background due to the fluorescent phosphor in the layer. Ethyl acetate-methanol-ammonium hydroxide (85:10:5) was chosen as the mobile phase after testing many different layer-solvent mixture combinations. It produced compact bands near the optimum central region of the plate for DPH, and it completely separated DPH from PE so that overlap did not occur during scanning of chromatograms of samples having both of these ingredients. R_f values of DPH varied between 0.50 and 0.60, depending on the temperature and humidity conditions in the laboratory on any particular day, but sample and standard zones always lined up exactly on each plate. PE had an R_f value of 0.15.

The calibration curves given by the CATS-3 software had linear correlation coefficients (R values) between 0.998 and 1.00 for the three applied standards, covering the range of 50%-200% of the theoretical (label) value represented by the middle standard. Typical slope and intercept values of the calibration curves obtained on each plate were 232.82 area counts/μg and 153.71 area counts, respectively.

As described earlier,[4,5] a high degree of linearity, which was obtained in this study because of automated spotting and scanning, enables quantitative analysis to be performed reliably by comparing the average scan area of duplicate sample zones with the average area of a closely matching standard zone within the linear region. This quantification method was applied in the present study rather than using the complete calibration curve.

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Analysis of two antihistamine tablets containing 25 mg of DPH gave percent recovery values of 94.3% and 99.3%, respectively, in relation to the label value. Four samples of a capsule antihistamine product were each analyzed once, yielding recoveries of 111%, 101%, 100%, and 100% relative to the 25 mg DPH label value. A fifth capsule was analyzed four times, and the percent recovery was 100 ± 1.9% (mean ± RSD). The third product analyzed was a tablet containing 60 mg of PE in addition to 25 mg of DPH; recoveries of 94.0%, 86.8%, and 96.3% were obtained for three samples. The analysis of a fourth tablet was replicated four times, and the recovery was 93.3 ± 1.7%.

Lastly, four individual gelcaps with a label DPH value of 25 mg were analyzed with recoveries of 92.8%, 96.3%, 95.8%, and 94.8%. All except two of the samples assayed within the 90-110% specification range stated in the USP 23/NF[3] for DPH antihistamine pharmaceuticals, with or without PE. In addition to the RSD values given above, reproducibility was evaluated by calculating the percent difference between the scan areas of duplicate 16.00 μL sample zones applied for each analysis; the difference was found to vary between 0.12% and 4.8%, with a mean of 1.9%.

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The accuracy of the proposed method was validated by determining recoveries from capsule and gelcap solutions that were pre-analyzed, spiked to exactly double the concentration, and then re-analyzed by spotting one-half of the original sample volume. Comparison of the unspiked and spiked samples yielded agreement of the weights obtained within 0.81% for the gelcap, while no difference was found for the capsule (0% error).

The quantitative HPTLC method described should also be applicable to other pharmaceutical formulations containing DPH. For example, some tablets containing DPH also contain acetaminophen (Tylenol®) as an active ingredient. These compounds can be separated using 1-butanol-deionized water-glacial acetic acid (70:20:10) mobile phase, with respective R_f values of 0.17 and 0.73. Chewable tablets containing DPH also have aspartame (NutraSweet®) as an inactive ingredient. Aspartame does not quench fluorescence and will not interfere with the detection and scanning of DPH zones.

Densitometric HPTLC methods for the quantification of acetaminophen[6] and aspartame[7] were described earlier and could be adapted for the determination of these ingredients in antihistamine tablets containing DPH.

The results demonstrated for accuracy and precision are equivalent or superior to those obtained in many previously published TLC and HPLC analyses of drugs in pharmaceuticals. For example, recoveries from fortified

samples ranged from 98.9-101.3% for lamotrigine in tablets[8] and from 97-104% for miconazole and ketoconazole added to azole-containing creams and ointments[9] using TLC-densitometry. Diphenoxylate hydrochloride and atropine sulfate were recovered from fortified combination drug formulations at 98.7-102.5% levels[10] and nalidixic acid from tablets and suspensions at 99.4-101.3%[11] using HPLC.

In addition to excellent accuracy and precision, advantages of the new method in comparison to HPLC include the ability to analyze up to eight samples on a single plate because 19 lanes are available for spotting duplicate aliquots of a single standard along with duplicate aliquots of each sample. This leads to cost-efficient, rapid analyses that are simple in procedure with low solvent usage on a per-sample basis.

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