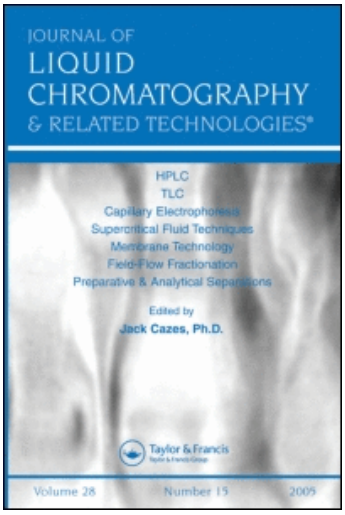

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ANALYSIS OF THE ACTIVE INGREDIENT, MECLIZINE, IN MOTION SICKNESS TABLETS BY HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY WITH DENSITOMETRIC MEASUREMENT OF FLUORESCENCE QUENCHING

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ANALYSIS OF THE ACTIVE INGREDIENT, MECLIZINE, IN MOTION SICKNESS TABLETS BY HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHY WITH DENSITOMETRIC MEASUREMENT OF FLUORESCENCE QUENCHING

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ABSTRACT

A quantitative method using silica gel HPTLC plates with fluorescent indicator, automated sample application, and UV absorption densitometry of the fluorescence quenching zones was developed for the determination of meclizine hydrochloride in motion sickness tablets. Samples of three brands of tablets assayed within 97.0-110% of the 25 mg label value. Precision (relative standard deviation) was 1.58 and 1.26% for replicate analyses of two tablets. The error of a standard addition analysis performed to evaluate accuracy was 0.506%. These validation data are within the guidelines of the International Conference on Harmonization for pharmaceutical analysis.

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INTRODUCTION

In an earlier paper,(1) the first quantitative high performance thin layer chromatography (HPTLC) method was described for analysis of motion sickness tablets containing the active ingredient dimenhydrinate. In this paper, adaptation of that method is reported for assay of tablet formulations containing another widely used motion sickness medication, meclizine hydrochloride (MH). A computer-based search of Chemical Abstracts found papers reporting the determination of MH by high performance liquid chromatography (HPLC) in tablets,(2) HPLC on manufacturing equipment surfaces,(3) spectrophotometry in tablets,(4) HPLC in serum,(5) mass spectrometry in plasma,(6) and gas chromatography in tablets.(7) The current official assay method for MH in tablets, involves HPLC using a strongly acidic cation exchange chemically bonded silica gel column and 230 nm UV absorption detector.(8) The USP contains a qualitative TLC identification test for MH in tablets using a silica gel layer, cyclohexane-toluene-diethylamine (15:3:2) mobile phase, and detection under short wave UV light..(8) No other qualitative or quantitative analysis of MH was found in the literature.

The new quantitative HPTLC method described below, involves densitometric measurement of the degree of fluorescence quenching of sample and standard zones of MH after instrumental application of initial zones in the form of bands and silica gel HPTLC separation. Excellent accuracy and precision are demonstrated for the method, which is more selective than spectrophotometry because of the TLC separation step and is simpler, faster, and more cost effective compared to HPLC.

EXPERIMENTAL

Preparation of Standard Solutions

A stock standard solution of the dihydrochloride salt of meclizine (1-[(4-chlorophenyl)phenylmethyl]-4-[(3-methylphenyl)methyl]piperazine); no. M 1637, Sigma, St. Louis, MO, USA; CAS registry no. 1104-22-9) was prepared at a concentration of 5.00 mg/mL in absolute ethanol. The TLC standard solution was prepared at 1.00 mg/mL by 1:5 dilution of the stock standard with absolute ethanol.

Preparation of Sample Solutions

Three brands of motion sickness tablets with label values of 25 mg MH were obtained from a local pharmacy. Test solutions were prepared by grinding a

tablet into a fine powder using a mortar and pestle, and quantitatively transferring the powder through a funnel into a 100 mL volumetric flask by washing with about 85 mL of absolute ethanol. The solution was magnetically stirred for 30 min, after which the stir bar was removed using a magnetic rod and the flask was filled to the line with absolute ethanol. The undissolved excipients were allowed to settle overnight prior to sample application onto the layer. The theoretical concentration of each tablet test solution was 0.250 mg/mL based on the label value of MH.

Thin Layer Chromatographic Analysis

Analyses were performed on Merck 20 x 10 cm high performance silica gel plates 60 F-254 GLP plates (No. 5613/6, EM Separations Technology, Gibbstown, NJ, USA). Sample and standard solutions were applied by means of a Camag (Wilmington, NC, USA) Linomat IV automated spray-on band applicator, equipped with a 100 µL syringe and operated 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, 0.7 cm from the plate edge, and 1.5 cm from the bottom of the plate. The volumes applied for each analysis were 2.00 µL, duplicate 4.00 µL, and 8.00 µL of the TLC standard (2.00-8.00 µL of MH) and duplicate 16.0 µL aliquots of the sample solution.

Plates were developed for a distance of 7 cm beyond the origin with 30 mL of n-butanol-deionized water-glacial acetic acid (85:10:5) in a vapor-equilibrated, Camag twin-trough chamber lined with a saturation pad (Analtech, Newark, DE, no. 81-12). After development, the plates were air dried in a fume hood until the odor of acetic acid was absent (ca. 15 min).

Sample and standard zones were quantified by linear scanning at 254 nm, the wavelength of maximum fluorescence of the phosphor in the layer, by use of a Camag TLC scanner II with a deuterium source, slit dimension settings of length 4 and width 4, and a scanning rate of 4.0 mm/sec. The CATS-3 software program controlling the densitometer produced a calibration curve, by linear regression of the scan areas of the standard zones vs micrograms spotted, and interpolated the weights of the sample zones based on their areas from the curve. For each tablet analysis, percent recovery was calculated by comparing the theoretical weight predicted by the label declaration (4.00 ug/16.0 µL initial zone) to the mean experimental weight of the duplicate sample zones.

Precision was evaluated by replicate analysis of two tablets with n=4 and 6 (Fig. 1). As another measure of reproducibility, the percentage differences between duplicate sample and standard zones applied in each analysis were calculated.

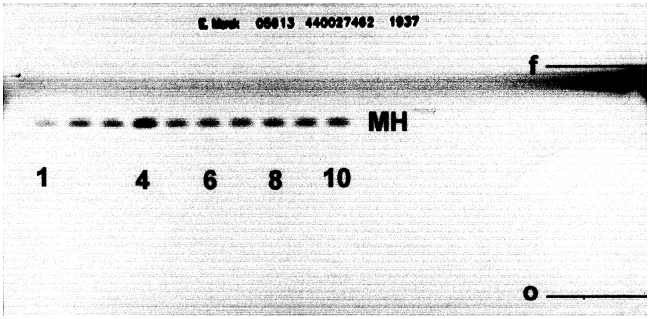

Figure 1. Chromatograms obtained in reproducibility study, photographed under 254 nm UV light with a Camag VideoStore Image Documentation System. f, mobile phase front; o, origin; lanes 1-4: 2.00, 4.00, 4.00, and 8.00 μg of meclizine (MH) standard, respectively; lanes 5-10: repeated 16.0 μL samples of Brand 3 MH tablet test solution.

Accuracy of the method was validated by standard addition analysis in which a tablet was prepared and analyzed as described above. A volume of MH stock standard solution was added to an aliquot of the original solution to increase the MH concentration by a known amount, and the unspiked and spiked solutions were analyzed on the same plate by applying duplicate 16.0 μL and 8.00 μL aliquots, respectively, next to the four standards specified above. The theoretical weight of MH in the spiked sample aliquot was calculated from the experimental weight found in the unspiked sample, plus the added weight. Accuracy was then calculated by comparing the theoretical and experimental weights of the spiked sample.

RESULTS AND DISCUSSION

Development on HPTLC silica gel layers containing fluorescent indicator using the butanol-water-acetic acid mobile phase produced dark, flat, compact zones of MH on a bright green background, when viewed under 254 nm UV light (Fig. 1). The R_f value of MH was 0.76. Inactive ingredients in the three products analyzed included food dyes, flavors, lactose, magnesium stearate, silicon dioxide, sodium saccharin, starch, talc, sodium croscarmellose, and cellulose. None of these ingredients produced detectable zones in any of the sample chromatograms. The calibration curves calculated on each plate by linear regression of the four MH standards, consistently had correlation coefficient (r) values of 0.999 with average slope and intercept values of 243 and 111, respectively. For confirmation of MH identity and qualitative purity testing in tablet preparations, an alternative, basic mobile phase consisting of ethyl acetate-methanol-conc. ammonium hydroxide (85:10:5) can be used (R_f of MH=0.80).

Two MH tablets of Brand 1 were analyzed, once each, by the new HPTLC method and gave results that were 109% and 107%, relative to the 25 mg/tablet label value. When also analyzed, once each, recoveries of 97.0% and 110% were found for two tablets of Brand 2 and 102% and 99.0% for two tablets of Brand 3, relative to the label value. One tablet of Brand 1 was analyzed four times on a single plate, and the recovery was 109%+/-1.26% (mean+/-relative standard deviation [RSD]). Six analyses of a tablet of Brand 3 on a single plate yielded 104%+/-1.58% (Fig. 1). Percent difference values between the scan areas of the duplicate sample and standard aliquots spotted in each analysis averaged 1.46% with a range of 0.0860%-3.13%. All tablets analyzed throughout the method development and validation phases of this study, assayed within the 90-110% content range specified in the USP 24/NF 19 for MH tablets.(8)

To prepare the spiked sample for standard addition validation of accuracy, 50.0 µL of the stock standard solution and 900 µL of a Brand 3 tablet test solution, measured with 100 µL and 1000 µL Drummond (Broomall, PA) digital microdispensers, respectively, were mixed in a 15 mL vial. The experimental mean weight of MH in the unspiked Brand 3 tablet test solution was 3.88 µg (representing 97.0% recovery), which led to a theoretical weight of 3.96 µg in the spiked solution. The analysis of the spiked solution gave 3.94 µg, representing 99.5% recovery of the spike and an error of 0.506%.

It has been shown that, the new HPTLC method achieved recoveries as a percentage of tablet label value, standard deviations for replicate analyses, percentage differences for the scans of duplicate samples, and recovery from a spiked standard addition sample, that compare favorably with those found regularly in the literature for HPTLC and HPLC analysis of pharmaceutical dosage forms. For example, Renger reported accuracy (recovery) of 101.3% (HPLC) and 98.5-102.8% (HPTLC) and repeatability or precision (RSD) of 0.9-5% (HPLC) and 1.2-2.8% (HPTLC), in a comparative study of pharmaceutical analytical results.(9) Our validation results meet the guidelines of the International Conference on Harmonization for pharmaceutical analysis as proposed for planar chromatographic procedures by Ferenczi-Fodor et al.(10)

Using the new method, up to 14 samples can be applied with the four standards on a single plate, leading to high sample throughput and low cost for solvent purchase and disposal. Other comparisons of HPLC and HPTLC, in terms of typical results and advantages and disadvantages resulting from methodological differences, have been presented earlier.(11)

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