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Analysis of the Active Ingredient Cimetidine in Acid Reduction Tablets By High Performance Thin Layer Chromatography with Ultraviolet Absorption Densitometry

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Abstract

A quantitative method using silica gel HPTLC plates with fluorescent indicator, automated sample application, and UV absorption densitometry was developed for the determination of cimetidine in acid reduction tablets used to treat ulcers and other hyperacidity stomach disorders. Four pharmaceutical tablet products containing cimetidine as the active ingredient were analyzed to test the applicability of the new method. Precision was evaluated by replicate analyses of samples and accuracy by simultaneous analysis of unfortified and fortified samples on a single plate (standard addition). The percent cimetidine in the tablets analyzed ranged from 102% to 109% compared to label values, precision ranged from 1.2% to 2.2% relative standard deviation, and the error in the standard addition analysis was 0.985% compared to the fortification level. These validation levels are within the guidelines of the International Conference on Harmonization for pharmaceutical analysis.

Keywords: thin layer chromatography, cimetidine, densitometry.

1. Introduction

Cimetidine is a histamine H_2—receptor antagonist that markedly inhibits gastric secretion. It is widely prescribed throughout the world in tablet form as a therapeutic agent to reduce stomach acidity and treat ulcers. The standard method for determining cimetidine pharmaceutical drug substance and tablets involves high performance liquid chromatography (HPLC) using a C-18 chemically bonded silica gel column; a mobile phase composed of methanol, phosphoric acid,1-hexanesulfonate, and water; and UV absorption detection at 220 nm [1]. A computer-based literature search of Chemical Abstracts located 19 papers describing thin layer chromatography (TLC) analysis of cimetidine, including separation and study of UV spectra [2]; detection on layers by chemical derivatization [3], qualitative screening [4,5], qualitative identification [6], determination of drug release from tablets [7], and detection by TLC/fast atom bombardment-mass spectrometry [8]. No previous publications on the quantitative analysis of cimetidine in pharmaceutical dosage forms were found. The new quantitative high performance thin layer chromatography (HPTLC) method described below, for which excellent accuracy and precision are demonstrated, is faster and more convenient and uses less solvent compared to HPLC.

2. Experimental

Preparation of Standard Solutions

The TLC standard solution of cimetidine (Sigma, St. Louis, MO, USA; catalog no. C 4522; CAS registry no.51481-61-9) was prepared at a concentration of 2.50 mg/ml in absolute ethanol. A stock solution for fortification in the standard addition analysis was prepared at a concentration of 20.0 mg/ml in absolute ethanol.

Preparation of Sample Solutions

Four brands of cimetidine tablets with label values of 200 mg were obtained from pharmacies. Test solutions were prepared by grinding a tablet into a fine powder with a mortar and pestle ; the powder was quantitatively transferred through a funnel into a 100-mL volumetric flask by washing with absolute ethanol. The solution was stirred magnetically at high setting for 30 min, after which

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the stir bar was removed by use of a magnetic rod. The solution was diluted to volume with absolute ethanol and shaken to mix thoroughly. The undissolved solid excipients were allowed to settle for 60 minutes prior to analysis. The theoretical concentration of each tablet test solution was 2.00 mg/ml based on the label declarations.

Thin Layer Chromatographic Analysis

Analyses were performed on Merck 20 cm × 10 cm high performance silica gel $60F_{254}$ GLP plates (EM Separations Technology, Gibbstown, NJ, no. 5613/6). 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 s/µl, table speed 10 mm/s, distance between bands 4 mm, distance from the plate edge 0.7 cm, and distance from the bottom of the plate 1.5 cm. The volumes applied for each analysis were 4.00 µl, duplicate 8.00 µl, and 16.00 µl of the TLC standard (10.0-40.0 µg) of cimetidine and duplicate 10.00 µl aliquots of the sample solutions (20.0 µg theoretical content).

Plates were developed to a distance of 6 cm beyond the origin with ethyl acetate-methanol-conc. ammonium hydroxide (75 : 20 : 5) in a vapor-equilibrated Camag HPTLC twin trough chamber lined with a saturation pad (Analtech, Newark, DE, no. 81-12). The development time was 17 min. After development, the plates were air-dried for 5 min in a fume hood, and sample and standard zones were quantified by linear scanning at 254 nm with a Camag TLC Scanner I with a deuterium source, slit dimension settings of length 4 and width 4, and a scanning rate of 4.0 mm/s. The wavelength used was 254 nm. The CATS-3 software produced a linear regression calibration curve relating standard zone weights to their optimized scan areas. The analyte weights in the sample zones were determined from their areas by automatic interpolation from the calibration curve. The percent recovery was calculated for each tablet analysis by comparing the theoretical weight predicted by the label value to the mean experimental weight of the duplicate sample zones.

The accuracy of the method was validated by a standard addition analysis. A tablet test solution was prepared according to the procedure described above. An 800 µl aliquot of this solution was mixed with 80.0 µl of the stock solution to double the concentration of cimetidine based on the label value. Volumes were measured with 1000 µl and 100 µl Drummond (Broomall, PA, USA) microdispensers, respectively. The original and fortified sample solutions were analyzed on the same plate by application of duplicate 10.0 µl and 5.00 µl volumes, respectively, and the four standards described above. The difference between the mean experimental weights and the added weight was calculated to determine the accuracy of the method.

3. Results and Discussion

Development with the mobile phase described above on the HPTLC silica gel layers containing fluorescent indicator produced compact, flat, dark fluorescence-quenched bands of cimetidine (R_f 0.43) against a bright green background when viewed under a 254 nm UV light. The excipients in the tablets analyzed included cornstarch, cellulose, magnesium stearate, polyethylene glycol, polysorbate 80, povidone, sodium lauryl sulfate, sodium starch glycolate, and titanium dioxide. No additional zones representing these excipients were detected in chromatograms. Calibration curves had linearity correlation coefficient (r) values of 0.997-0.999 for 10.0 to 40.0 µg of cimetidine.

Four brands of tablets (a name brand and three generic brands) were analyzed by the described procedure. The recoveries compared to the label value of 200 mg are shown in Table 1. To assess precision, two tablets of different brands were analyzed four times each, and recoveries were 106%±2.2% and 106%±1.8%.One tablet was analyzed six times, and the recovery was 108%±1.2%. As an additional measure of reproducibility, in addition to the RSD of duplicate analyses, the percent difference between the scan areas for duplicate sample aliquots was calculated for each analysis, and the range was 0.90-2.9%. All tablets analyzed throughout the method development and validation studies assayed within the 90-110% specification range in the USP 24/NF 19 for cimetidine tablets [1].

Table 1. Recoveries of Cimetidine from Tablets

Sample	Recovery
Brand 1	
Tablet 1	105%
Tablet 2	107%
Tablet 3	109%
Brand 2	
Tablet 1	106%
Tablet 2	102%
Tablet 3	106%
Brand 3	
Tablet 1	104%
Tablet 2	102%
Tablet 3	105%
Brand 4	
Tablet 1	109%
Tablet 2	107%
Tablet 3	108%

The accuracy of the new method was validated by a standard addition method in which unfortified and fortified sample solutions were analyzed on the same plate. The analysis of the unfortified sample yielded a 98.0% recovery relative to the label value. The theoretical fortification weight was 17.9 µg of cimetidine, and the analysis of the fortified sample yielded 18.0 µg, representing a percent error of 0.985% or a percent recovery of 101%.

It has been shown that the new HPTLC method achieved recoveries as a percentage of tablet label value, precision for replicate analyses, and accuracy of analyte analysis from a fortified standard addition sample that compare favorably with those reported regularly in the literature for HPTLC and HPLC pharmaceutical dosage forms. The results also meet the guidelines of the International Conference on Harmonization (ICH) for validation of pharmaceutical assays of drug products, which are a precision of 2-3% RSD and recovery (accuracy) of 95-105% [9]. Previous papers describe the overall advantages of quantitative HPTLC relative to HPLC for assay of pharmaceutical dosage forms [10-12].

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