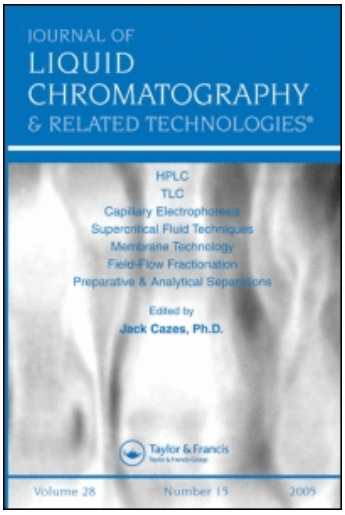

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Determination of Famotidine in Acid Reduction Tablets by HPTLC and Videodensitometry of Fluorescence Quenched Zones

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ABSTRACT

A quantitative method using silica gel high performance thin layer chromatography (HPTLC) plates with fluorescent indicator, automated sample application, and ultraviolet (UV) absorption videodensitometry was developed for the determination of famotidine tablets, which are widely used to inhibit gastric acid secretion. Three pharmaceutical tablet products containing famotidine as the active ingredient were analyzed to test the applicability of the new method. Precision was evaluated by replicate analyses of the samples and accuracy by analysis of a sample, fortification with standard, and reanalysis (standard addition). The percent famotidine in the tablets ranged from 92.5% to 140% compared to label values, precision from 1.25% to 2.55% relative standard deviation, and the error in the standard addition analysis was 1.76% compared to the

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fortification level. These validation results are within the guidelines of the International Conference on Harmonization for pharmaceutical analysis.

Key Words: High performance thin layer chromatography; Determination of famotidine; Videodensitometry; Pharmaceutical analysis.

INTRODUCTION

In a previous paper,[1] a method was described for assay of the active ingredient cimetidine in acid reduction tablets using high performance thin layer chromatography (HPTLC) and slit scanning densitometry. Like cimetidine, famotidine is a histamine H_2-receptor antagonist that markedly inhibits gastric acid secretion. It is widely prescribed throughout the world in tablet form as a therapeutic agent to reduce stomach acidity and treat ulcers. The standard USP[2] method for determining famotidine involves high performance liquid chromatography (HPLC) using a 5–10 μm porous silica gel column, a mobile phase composed of methanol, water, and aqueous potassium phosphate solution adjusted to pH 5.0, and ultraviolet (UV) absorption detection at 254 nm. The USP[2] contains qualitative thin layer chromatography (TLC) methods using silica gel layers and detection under 254 nm UV light for determining purity of famotidine drug substance and identification testing of famotidine tablets, and Gyeresi et al.[3] described the TLC separation and qualitative identification of famotidine from cimetidine, nizatidine, and ranitidine. The latter two compounds are structurally related H_2-recptor antagonists.

Video-imaging (videodensitometry), home-built and commercial instruments, have been used for about 17 years for documentation and quantification of TLC and HPTLC results based on total irradiation of the plate with a light source, photography of the image with the CCD camera, and rapid image processing via a digitizing board (frame grabber) and personal computer system. The current specialized and sophisticated software in commercial instruments for in situ quantitative analysis allows video-scanning of standard and sample zones separated in adjacent chromatogram tracks on thin layers, and quantitative evaluation of the captured images via peak areas or heights and linear or polynomial regression calibration curves. A computer-based literature search using the ISI Web of Science, Camag Bibliography Service (CBS) CD-ROM (volumes 51–84), and Medline located a number of papers on quantitative determinations by videodensitometry, including amino acid transforming enzymes in human tissue homogenates;[4] industrial amino acids in culture liquids used for their manufacture;[5] flavonoids in plant extracts;[6] persilben in extracts from herbs and rhizomes of Polygonum L;[7] *L*-tryptophan in fermentation broth;[8] pesticides on silica gel after detection by biological or

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biochemical methods;[9] food dyes in batch-certification tests;[10] and the pesticide atrazine by measurement of fluorescence quenching on silica gel 60 layers.[11] Two other papers reported the only applications of videodensitometry that were found for the determination of drug active ingredients in pharmaceutical tablets, i.e., nadolol and pindolol,[12] and fleroxacin, sparfloxacin, and cinoxacin.[13] In the latter two drug analysis papers, the Desaga videodensitometer was used to measure quenched zones at 254 nm on layers containing a fluorescent indicator.

The purpose of this study was to develop and validate a quantitative HPTLC-videodensitometry method for assay of famotidine in acid-reduction tablets using the other videodensitometer in wide use today, the Camag Reprostar 3 with Videostore documentation/VideoScan densitometry software. It is shown that the developed method could be validated within the standard sets by the International Committee on Harmonization (ICH)[14] for assay of finished pharmaceutical products, and that it is, therefore, suitable for routine analysis in industry quality control and regulatory laboratories.

EXPERIMENTAL

Preparation of Standard Solutions

A 1.00 mg/mL stock standard solution, used directly for the standard addition analysis, was prepared by dissolving famotidine (*N′*-[aminosulfonyl]-3-[(2-[diaminomethyleneamino]-4-thiazolyl)methylthio]-propanamidine; Sigma, St. Louis, MO; catalog no. F-6889, >99% purity; CAS registry no. 76824-35-6) in methanol–glacial acetic acid (90 : 10). A 0.100 mg/mL HPTLC standard solution was prepared by 1 : 10 dilution of the stock solution with methanol–glacial acetic acid (90 : 10).

Preparation of Sample Solutions

Three store brands of famotidine tablets with label values of 10 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 10 mL of glacial acetic acid, and 60 mL of methanol was added. The solution was magnetically stirred for 30 min, sonicated for 10 min, diluted to volume with methanol, and shaken to mix thoroughly. To remove undissolved excipients prior to application of the sample solutions onto the HPTLC plate, about 5 mL of each solution was filtered through a Pall Gelman (Ann

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Arbor, MI) Acrodisc LC 13 mm syringe filter with 0.45 μm PVDF or Nylon membrane into a capped vial. The theoretical concentration of each tablet test solution was 0.100 mg/mL based on the label declarations.

High Performance Thin Layer Chromatography Analysis

Analyses were performed on 20 × 10 cm HPTLC silica gel 60 F_{254} plates (EM Science, Gibbstown, NJ, an affiliate of Merck KGaA, Darmstadt, Germany, catalog # 13 153) containing a concentrating zone, 19 channels, and fluorescent indicator. Layers were pre-cleaned by development to the top with dichloromethane–methanol (1 : 1) and dried in a fumehood. Sample and standard initial zones were applied as bands onto the concentrating zones of the respective channels by means of a Camag (Wilmington, NC) Linomat IV automated spray-on applicator, which was equipped with a 100-μL syringe and operated with the following settings: band length 6 mm, application rate 10 s/μL, table speed 10 mm/s, distance between bands 4 mm, distance from the plate edge 6.5 mm, 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 10.0 μL of the famotidine HPTLC standard solution (0.400–1.00 μg), and duplicate 8.00 μL aliquots of the sample solution (0.800 μg theoretical content).

Plates were developed to a distance of 6 cm beyond the concentrating zone–silica gel layer interface, using a mobile phase consisting of ethyl acetate–methanol–toluene–conc. ammonium hydroxide (40 : 25 : 20 : 2)[2] in a Camag HPTLC twin trough chamber lined with a saturation pad (Analtech, Newark, DE, no. 81-12), and equilibrated with the mobile phase for 15 min prior to inserting the plate. The development time was 15 min. After development, the plates were air-dried for 10 min in a fumehood. Using a Camag Reprostar 3 videodensitometer and VideoStore 2 software, an image of the plate under 254 nm light was obtained (Fig. 1). The contrast between the bright green background and the dark spots of the standard and sample zones was optimized by adjusting the integration time. Integration times between 0.433 and 0.500 sec were used. The aperture setting on the camera was 11. The VideoScan software was used to scan the sample and standard zones and produce a linear regression calibration curve relating standard zone weights to their scan areas. On the VideoScan software, the minimum peak width was set at five pixels, the minimum peak height was 100 pixels, and the minimum peak area was 300 pixels. The filter width was 5. 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.

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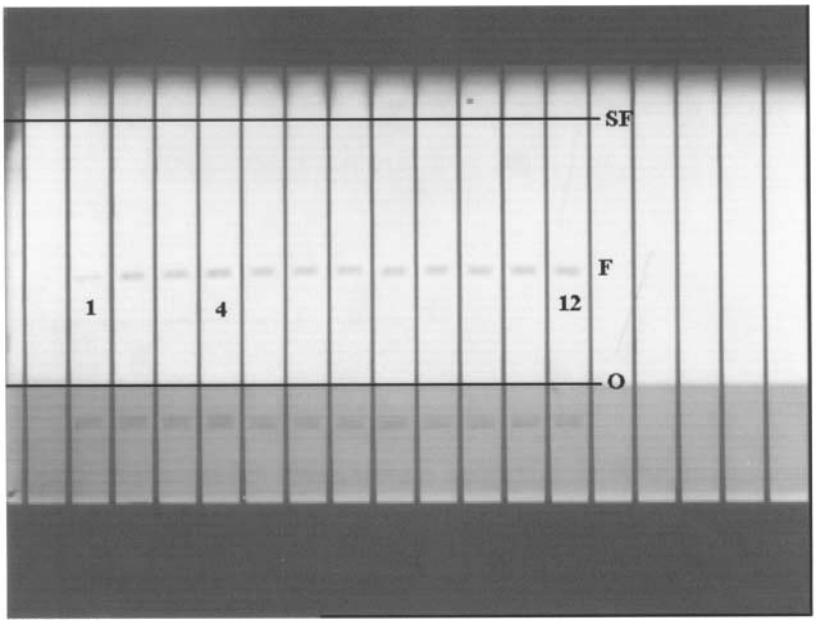

Figure 1. Chromatograms obtained in the duplicate analysis of four 10 mg famotidine tablets (Table 1, Brand 1, Tablets 1–4) on a channeled silica gel plate with concentrating zone by the described HPTLC-densitometry method. The plate was photographed under 254 nm UV light with a Camag VideoStore Image Documentation System. *Key:* SF, mobile phase front; O, origin; lanes 1–4: famotidine (F) standards; lanes 5–12: duplicate aliquots of the four sample extract solutions.

Validation

The accuracy of the method was validated by a standard addition analysis. A tablet test solution was prepared according to the procedure described above. A 1000 µL aliquot of this solution was mixed with 100 µL of the stock solution to double the concentration of famotidine based on the label value. Volumes were measured with 1000 and 100 µL Drummond (Broomall, PA) digital microdispensers, respectively. The original and fortified sample solutions were analyzed on the same plate by application of duplicate 8.00 and 4.00 µL volumes, respectively, and the four standards described above. The difference between the mean of the experimental weights and the added weight was calculated to determine the accuracy of the method.

Precision (repeatability) was validated by spotting six 8.00 µL aliquots of the standard solution and calculating the relative standard deviation (RSD)

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of the experimental weights. As another measure of precision, the percent difference between duplicate sample aliquots applied in each analysis was calculated.

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 famotidine (R_f 0.48) against a bright green background when viewed under a 254 nm UV light (Fig. 1). The excipients in all three brands of tablets were identical and included colloidal silicon dioxide, corn starch, hydroxypropyl cellulose, hydroxypropyl methylcellulose, indigo carmine aluminum lake FD&C blue no. 2, iron oxide red, iron oxide yellow, lactose monohydrate, magnesium stearate, microcrystalline cellulose, polyethylene glycol 4000, pregelatinized corn starch, and titanium dioxide. No additional zones representing these excipients were detected in chromatograms. Although the lowest weight of famotidine used for the calibration curve was 0.400 μg, the limit of visual detection was about 0.200 μg and the limit of quantification was about 0.100 μg.

As recommended by the ICH, a calibration curve was established using five analyte concentrations (2.00, 4.00, 6.00, 8.00, and 10.0 μL of the HPTLC standard, representing 0.200–1.00 μg of famotidine). The linear regression correlation coefficient (r value) of this curve was 0.996. For routine analytical procedures, a three point calibration curve within this range was used, produced by applying 4.00, 8.00, and 10.0 μL of the HPTLC standard on each plate. This calibration curve was repeated many times and was found to have a linear regression r value of 0.998. The concentration of the TLC standard was confirmed by weighing and diluting another portion of famotidine standard to prepare a second standard solution. The response (scan areas per standard weights spotted) for the two solutions agreed within 1.32%, which meets the requirements for a chromatographic check standard analysis.

The three brands of famotidine tablets were analyzed by the procedure described above with $n = 2$ or $n = 6$. The recoveries compared to the label value of 10 mg are shown in Table 1. The precision values meet the acceptance criterion for RSD of recovery in finished pharmaceutical product assay specified by the ICH.[14] The percentage difference between the scan areas for duplicate sample aliquots ranged from 1.0% to 7.6%, with a mean of 4.5%.

The accuracy of the new method was validated by a standard addition method in which unfortified and fortified sample solutions for a tablet of Brand 1 were analyzed on the same plate. The analysis of the unfortified sample yielded a 92.5% recovery relative to the label value. The analysis of the

Table 1. Recoveries of famotidine from tablets relative to the label value.

Sample	Recovery (%)	n[a]	RSD (%)[b]
Brand 1			
Tablet 1	103	2	
Tablet 2	94.6	2	
Tablet 3	96.0	2	
Tablet 4	108	2	
Tablet 5	110	2	
Tablet 6	104	6	1.25
Brand 2			
Tablet 1	107	2	
Tablet 2	119	2	
Tablet 3	100	2	
Brand 3			
Tablet 1	118	2	
Tablet 2	123	2	
Tablet 3	140	2	
Tablet 4	128	6	2.55

[a]Number of sample aliquots spotted on the layer.
[b]Relative standard deviation (coefficient of variation).

fortified sample yielded a 101.76% recovery, representing a percent error of 1.76%. This result easily meets the acceptance criterion of the ICH[14] for accuracy testing in the assay of finished pharmaceutical products. It can be seen in Table 1, that one tablet of Brand 2 and all of the Brand 3 tablets assayed above the 90–110% specification range for famotidine tablets declared in the USP.[2] The successful standard addition validation of the accuracy of the new method described above indicates that these high values represent the real content of the tablets. The high results were confirmed further by measuring the sample and standard chromatographic zones at 254 nm with a Camag TLC Scanner II slit scanning densitometer controlled by CATS-3 software using the instrumental parameters described earlier for the cimetidine analysis.[1] The famotidine values obtained with the slit scanner were consistent with those obtained using the videodenistometer.

The main advantages of using a videodensitometer are fast and efficient simultaneous multichannel data acquisition over an entire TLC plate, simple instrument design and absence of moving parts, visual control of the scanning process to minimize errors of track positioning, and a large number of digitized raw data (pixels) that can be processed with a computer. Although

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it is generally stated that slit-scanning densitometry is more accurate and precise than videodensitometry,[11] it is shown here that the results of the famotidine assay were adequate for validation according to ICH standards.[14] In addition, the results were similar to those obtained in an earlier determination of famotidine in pharmaceuticals by slit-scanning densitometry,[15] which included a calibration linearity of 0.997, quantification limit of 80 ng, precision of 0.93% RSD, and recovery of 98.1–102.7% from spiked samples. The new videodensitometry method allows simultaneous analysis of samples with low solvent consumption, and the method is fast and cost-effective compared to analysis by HPLC.

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