ELSEVIER

Journal of Chromatography A, 846 (1999) 193–198

JOURNAL OF CHROMATOGRAPHY A

Short communication

High-performance thin-layer chromatography for the determination of ranitidine hydrochloride and famotidine in pharmaceuticals

J. Novaković*

PRO.MED.CS, Telčká 1, 141 00 Prague 4, Czech Republic

Abstract

The H_2 receptor agonist pharmaceuticals containing ranitidine hydrochloride and famotidine are widely used to inhibit gastric acid secretion. A high-performance thin-layer chromatographic method was developed for their in-process control and content uniformity testing. HPTLC separation was performed on silica precoated plates using the USP 23 mobile phase for famotidine and toluene–methanol–diethylamine (9:1:1, v/v) for ranitidine. The samples were applied on a HPTLC plate automatically. Quantification was done by densitometry at in situ UV absorption maxima of ranitidine hydrochloride and famotidine at 320 nm and 276 nm, respectively. The method was validated in terms of selectivity (related compounds and placebo effect), system suitability, range (30 to 230 ng for ranitidine hydrochloride and 80 to 580 ng for famotidine), accuracy, precision, ruggedness and analyte stability. A large number of analyses were performed simultaneously with a low solvent consumption. The method is fast, accurate and cost-effective.

Keywords: Pharmaceutical analysis; Validation; Famotidine; Ranitidine

1. Introduction

The H_2 receptor antagonist pharmaceuticals containing ranitidine hydrochloride and famotidine are widely used to inhibit gastric acid secretion [1]. Both Eur Ph 97 [2] and USP 23 [3] prescribe their purity control by thin-layer chromatography (TLC). Purity control of these compounds by using instrumental high-performance TLC (HPTLC) was reported by Sethi [4]. A HPTLC procedure was developed for the estimation of total ranitidine in plasma [5]. Potentiometric titrations and high-performance liquid chromatographic methods are prescribed according to Eur Ph 97 and USP 23 for quantitative analysis of pharmaceuticals containing ranitidine hydrochloride and famotidine [2,3].

Due to recent progress in plate technology and instrumentation, modern TLC is comparable in terms of accuracy, precision and sensitivity to other chromatographic techniques and can be performed in full compliance with good laboratory practice (GLP). Eur Ph 97, Supplement 1999 [6] prescribes instrumental TLC as an official method for quantitative analysis. Advantages of the technique are high sample throughput, simplicity and speed [7,8].

The aim of this work was to develop and validate instrumental HPTLC method for determination of ranitidine hydrochloride and famotidine in pharmaceuticals, which is applicable to their in-process control and content uniformity testing.

2. Experimental

2.1. Material and reagents

Ranitidine hydrochloride, ranitidine-related com-

*Fax: +420-2-6121-8147.

0021-9673/99/$ – see front matter
PII: S0021-9673(99)00510-5

pound A, ranitidine-related compound B and famotidine were USP 23 chemical reference compounds (CRCs). Famotidine-related compound A and famotidine-related compound B were Ph Eur 97 CRCs. Ranisan coated tablets, Famosan 20 and Famosan 40 coated tablets, containing 168 mg of ranitidine hydrochloride, and 20 mg or 40 mg of famotidine, respectively, were products of PRO.MED.CS (Prague, Czech Republic). Methanol, toluene, ethylacetate, ammonium hydroxide 25%, diethylamine, isopropanol and glacial acetic acid were analytical grade purity (Merck, Darmstadt, Germany).

2.2. Sample preparation

2.2.1. Samples containing ranitidine hydrochloride

An accurately weighed amount of sample containing 168.0 mg of ranitidine hydrochloride was transferred to a 50-ml volumetric flask, dissolved in water, sonicated for 10 min, diluted to the volume, filtered through filter paper and diluted 1:10 with methanol. A 300-nl aliquot was automatically applied to the plate.

2.2.2. Samples containing famotidine

An accurately weighed amount of sample containing 20.0 mg of famotidine was transferred to a 25-ml volumetric flask and dissolved in methanol–glacial acetic acid (9:1, v/v), sonicated for 10 min, diluted to the volume and filtered through filter paper. A 300-nl aliquot was automatically applied to the plate.

2.3. Chemical reference compounds solutions

2.3.1. Ranitidine hydrochloride

168.0 mg of ranitidine hydrochloride CRC was accurately weighed and transferred to a 50-ml volumetric flask. Further procedure as described in Section 2.2.1.

2.3.2. Famotidine

20.0 mg of famotidine CRC was accurately weighed and transferred to a 25-ml volumetric flask. Further procedure as described in Section 2.2.2.

2.4. Chromatography

A Camag TLC system composed of an automatic TLC sampler III, TLC Scanner 3 and CATS4 software (Camag, Muttenz, Switzerland) was used for sample application and quantitative evaluation. Chromatography was performed on HPTLC precoated silica gel $60F_{254}$ plates 20×10 cm (Merck, Darmstadt, Germany) using toluene–methanol–diethylamine (9:1:1, v/v) and ethylacetate–methanol–toluene–ammonium hydroxide (8:5:4:0.4, v/v) as mobile phases for ranitidine hydrochloride and famotidine, respectively. Total volume of each solvent mixture was 25 ml. Samples were band applied (2 mm length), at 5 mm intervals under nitrogen stream. Position of starting line was 12 mm. Number of applications per plate was 36. Ascending development to a distance of 50 mm was performed in a saturated TLC tween-trough developing chamber 20×10 cm (Camag). Chromatograms were evaluated via peak area after scanning in absorbance mode at 320 nm for ranitidine hydrochloride and 276 nm for famotidine, respectively.

3. Results

3.1. Linearity

The linearity of the method was tested using solutions of ranitidine hydrochloride CRC and famotidine CRC, prepared as described in Sections 2.3.1 and 2.3.2, respectively. Aliquots of 100 nl, 200 nl, 300 nl, 400 nl, 500 nl, 600 nl and 700 nl of the solutions were applied to the plate. The calibration dependance was polynomial from 30 to 230 ng and 80 to 580 ng of in situ amount of ranitidine hydrochloride and famotidine, respectively. Statistical evaluation of linear part of the calibration dependance of ranitidine hydrochloride and famotidine are given in Table 1.

3.2. Accuracy (trueness), range and placebo analysis

3.2.1. Ranitidine hydrochloride

Model samples were prepared by mixing Ranisan placebo and an amount of ranitidine hydrochloride in

Table 1
Statistical evaluation of the linear part of calibration dependance of ranitidine hydrochloride and famotidine

Compound	Range (ng)	Intercept	Slope	Coefficient of correlation (*r*)	RSD (%)
Ranitidine hydrochloride	65–163	2250.72	29.53	0.9976	1.9
Famotidine	165–413	2336.42	14.20	0.9969	2.3

the same ratio as declared (six samples), about 80% (two samples) and 120% (two samples) of declared amount. Accuracy was determined by calculation of the percentage recovery of ranitidine hydrochloride.

3.2.2. Famotidine

Model samples of Famosan 20 and Famosan 40 coated tablets were prepared by mixing placebo and amount of famotidine in the same ratio as declared (six samples), about 150% and 50% of declared ratio. Accuracy was determined by calculation of percentage recovery.

The results of ranitidine hydrochloride and famotidine recoveries are summarized in Table 2.

3.3. Precision and range

The analytical method procedure was applied repeatedly by sampling 10 times different amounts (six times 100%, two times 120%, and two times 80% of the average coated tablet mass) of one homogenous sample of Ranisan and Famosan 20 coated tablets, respectively. The results are presented in Table 3.

Table 2
Recoveries of ranitidine hydrochloride and famotidine spiked into placebo matrix

Compound	Recovery (%)
Ranitidine hydrochloride	98.52–101.55
Famotidine	98.09–102.66

3.4. System suitability

3.4.1. Resolution

3.4.1.1. Ranitidine hydrochloride

A mixture containing 0.2 mg ml^{-1} of ranitidine hydrochloride, and 0.1 mg ml^{-1} of ranitidine hydrochloride-related compound A and 0.01 mg ml^{-1} of ranitidine-related compound B in methanol was separated in toluene–methanol–diethylamine (9:1:1, v/v) as well as in the USP 23 solvent mixture consisting of ethyl acetate–isopropanol–ammonium hydroxide–water (25:15:5:1, v/v). The applied volume was 600 nl. Densitograms scanned at 230 nm are given in Figs. 1 and 2. In situ absorption spectra of ranitidine-related compound A and ranitidine-related compound B exhibit maxima at 230 nm and 230 and 320 nm, respectively.

3.4.1.2. Famotidine

A densitogram of a mixture containing 0.8 mg ml^{-1} of famotidine CRC and 0.04 mg ml^{-1} of famotidine impurity A CRC and 0.04 mg ml^{-1} of famotidine impurity B CRC in methanol–glacial acetic acid (9:1, v/v) scanned at 276 nm is presented in Fig. 3. The applied volume was 600 nl.

3.4.2. Peak symmetry

The peaks of ranitidine hydrochloride and famotidine and their symmetry factors are given in Fig. 4.

Table 3
Precision of ranitidine hydrochloride and famotidine determination

Compound	Found amount (mg $tablet^{-1}$)	% of declared amount	% of average amount
Ranitidine hydrochloride	168.171–174.983	100.10–104.16	98.44–102.43[a]
Famotidine	19.129–19.765	95.64–98.83	98.57–101.85[b]

[a] Average amount per tablet calculated from samples 1–6 is 170.83 mg, RSD=0.57%.
[b] Average amount per tablet calculated from samples 1–6 is 19.407 mg, RSD=0.93%.

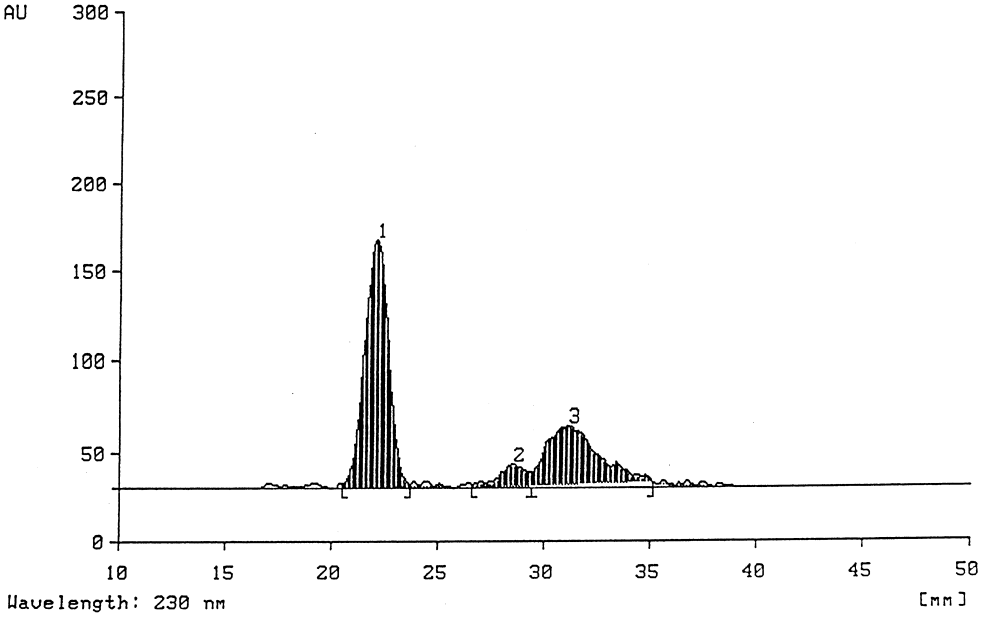

Fig. 1. Resolution of ranitidine hydrochloride (0.2 mg ml^{-1}) and its related compound A (0.1 mg ml^{-1}) and B (0.01 mg ml^{-1}) on silica gel $60F_{254}$ in the mobile phase toluene–methanol–diethylamine (9:1:1, v/v). Applied volume was 600 nl. Densitogram was scanned at 230 nm. Peak identification: 1=ranitidine hydrochloride, 2=ranitidine-related compound B, 3=ranitidine-related compound A.

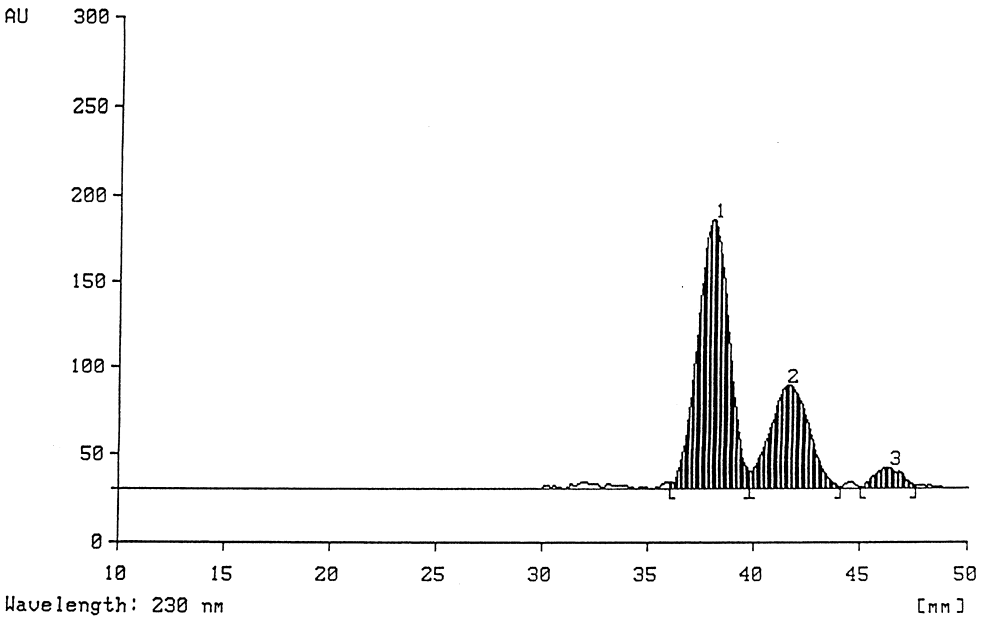

Fig. 2. Resolution of ranitidine hydrochloride and its related compounds A and B in the USP 23 mobile phase. Peak identification: 1=ranitidine hydrochloride, 2=ranitidine-related compound A, 3=ranitidine-related compound B. Other conditions as in Fig. 1.

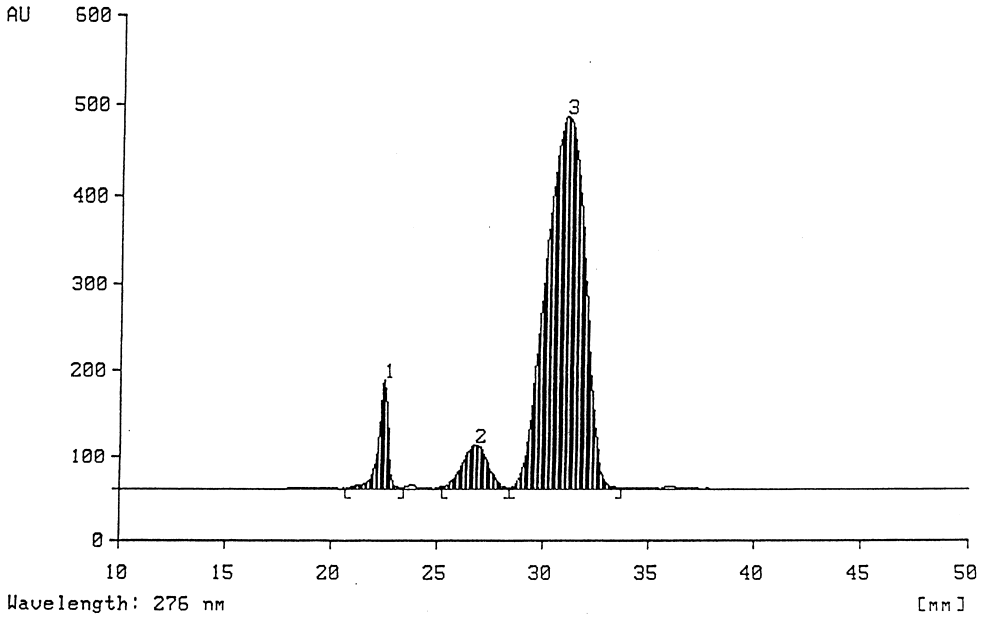

Fig. 3. Resolution of famotidine (0.8 mg ml^{-1}) and its impurities A (0.04 mg ml^{-1}) and B (0.04 mg ml^{-1}) on silica gel $60F_{254}$ in the USP 23 mobile phase. Applied volume was 600 nl. Densitogram was scanned at 276 nm. Peak identification: 1=famotidine impurity A, 2=famotidine impurity B, 3=famotidine.

3.4.3. System repeatability

Ten replicate injections on a HPTLC plate of ranitidine hydrochloride CRC and famotidine CRC solutions prepared as described in Sections 2.3.1 and 2.3.2, respectively, were performed. The relative standard deviations (RSDs) of the results were 0.59% and 0.74% for ranitidine hydrochloride and famotidine, respectively.

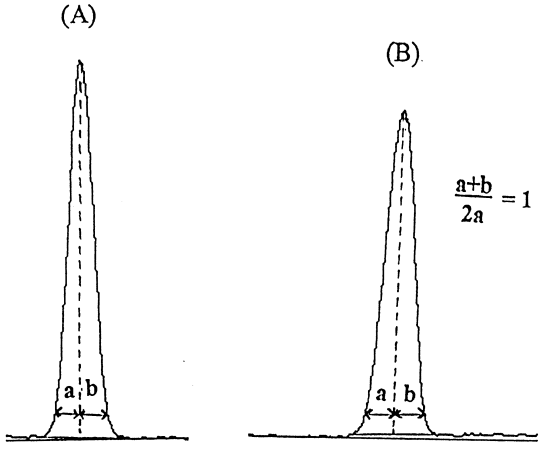

Fig. 4. Symmetry of ranitidine hydrochloride peak (A) and famotidine peak (B) measured at 5% of peak height.

3.5. Ruggedness

3.5.1. Wavelength optimization

''In situ'' UV spectra of ranitidine hydrochloride and famotidine measured from 190 nm to 360 nm exhibit maxima at 320 nm and 276 nm, respectively.

3.5.2. Sample stability

Samples were protected from light during preparation, application to the HPTLC plate and scanning. After development residues of the mobile phase were evaporated and the plates were scanned immediately.

4. Discussion and conclusions

The HPTLC method for determination of ranitidine hydrochloride and famotidine in pharmaceuticals was validated in terms of selectivity, precision, accuracy (trueness), linearity (range), system suitability and ruggedness. Calibration dependance

of ranitidine hydrochloride and famotidine was linear from 60 to 160 ng per spot and from 165 to 415 ng per spot, respectively. Sample application on the plate, the most critical step of the method, was performed automatically and therefore a good precision (RSD<1.0%, $n=6$) and recoveries (98–102%) were obtained. Resolution of ranitidine hydrochloride and its related compounds A and B was measured at 230 nm due to in situ spectra properties of the compounds. Baseline separation of ranitidine hydrochloride from its related compounds A and B was achieved using a mixture of toluene–methanol–diethylamine (9:1:1, v/v). Baseline separation of famotidine and its related compound was achieved in the USP 23 mobile phase. For both ranitidine hydrochloride and famotidine no interference with placebo (matrix) was observed. Method was applied on the process parametric assessment, in-process control and content uniformity testing of Ranisan, Famosan 20 and Famosan 40 coated tablets. Large number of samples was analyzed simultaneously (10 samples for 50 min including sample preparation). The results were evaluated automatically using both ''single level'' (for content uniformity testing) and ''multi level'' calibration (for in-process control of different manufacturing steps).

Acknowledgements

I appreciate very much the technical assistance of Mr. T. Durst

References

[1] Goodman and Gilman's, in: The Pharmacological Basis of Therapeutics, 9th ed., McGraw-Hill, New York, 1996, pp. 904–907.
[2] European Pharmacopeia, Council of Europe, Strasbourg, 3rd ed., 1997, pp. 841–842 and pp. 1434–1435.
[3] US Pharmacopeia 23, National Formulary 18, United States Pharmacopeial Convention, Rockville, MD, 1995, pp. 651–652 and pp. 1360–1364.
[4] P.D. Sethi, in: High-Performance Thin-Layer Chromatography – Quantitative Analysis of Pharmaceutical Formulations, CBS Publishers and Distributors, New Delhi, 1996, p. 48.
[5] V.D. Mody, M.C. Satia, T.P. Gandti, I.A. Modi, B.K. Chakravarthy, J. Chromatogr. B 676 (1996) 175.
[6] European Pharmacopeia, Council of Europe, Strasbourg, 3rd ed., 1998, Suppl. 1999, pp. 5–7.
[7] J. Sherma, B. Fried (Eds.), Handbook of Thin-Layer Chromatography, Marcel Dekker, New York, 2nd ed., 1996, pp. 129–148 and pp. 273–306.
[8] B. Renger, J. AOAC Int. 76 (1993) 7.