



October 2006

Volume 3 Issues 2-3

Trade Science Inc.

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 3(2-3), 2006 [94-98]

High Performance Liquid Chromatographic Assay Of Famotidine In Pharmaceuticals



Corresponding Author

K.Basavaiah
Department of Post Graduate Studies in
Chemistry, University of Mysore,
Manasagangotri, Mysore-570006 (INDIA)
E-mail: basavaiahk@yahoo.co.in

Received: 29th August, 2006

Accepted: 28th September, 2006

Web Publication Date : 11th October, 2006



Co-Authors

H.C.Prameela, U.Chandrashekar, B.C.Somashekar
Department of Post Graduate Studies in Chemistry,
University of Mysore, Manasagangotri,
Mysore-570006 (INDIA)

ABSTRACT

A rapid assay method for the determination of famotidine in pharmaceutical preparations has been developed for assessment of product quality utilising high-performance liquid chromatography (HPLC). The HPLC separation could be undertaken on a reversed phase Accurasil SS C₁₈ (5 µm) column (25 cm x 4.6 mm i.d.) by using a mobile phase consisting of acetonitrile- 0.1% phosphoric acid (pH 3.0) (80:20) at a flow rate 1.0 ml/min. The detector wavelength was at 268 nm with a sensitivity of 0.2 a.u.f.s. The calibration graph was linear from 5.0 to 330 µg/ml with the limits of detection (LOD) and quantification (LOQ) being 1.0 and 3.0 µg/ml, respectively. The method was validated according to the current ICH guidelines including assay of commercial tablets. Recoveries ranged from 96.28 to 102.21 %. The excipients present in the tablets did not interfere in the method.

© 2006 Trade Science Inc. - INDIA

KEYWORDS

Famotidine;
High-performance liquid
chromatography;
Pharmaceutical product;
Quality assessment.

INTRODUCTION

Famotidine (FMT), is chemically known as 3-[[[2-[(Aminoiminomethyl) amino]-4-thiazolyl] methyl] thio]-N-(aminosulfonyl) propanimidamide (Figure 1). FMT is a histamine H₂-receptor antagonist. It competitively inhibits the action of histamine on the H₂-receptors of parietal cells, reducing gastric acid secretion concentration under daytime and nocturnal

basal conditions and also when stimulated by food, histamine or pentagastrin. FMT is used in the treatment of duodenal ulcer, pathologic GI hypersecretory conditions and gastric ulcer. There are several reports available in the literature for the determination of FMT by techniques such as titrimetry^[1-5], UV-spectrophotometry^[6-8], visible spectrophotometry^[9-17], synchronous spectrofluorimetry^[18], polarography^[19] and differential pulse voltammetry^[20]. Perhaps, the most

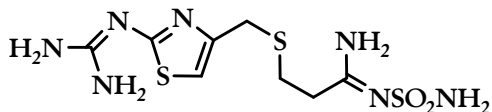


Figure 1: Structure of famotidine

widely used technique has been the high performance liquid chromatography (HPLC) using varying column and mobile phase combinations.

A reverse phase HPLC method using an ultra sphere Silica column and a Zorbax Silica column has been reported by Biffar and Mazzo^[21]. The mobile phase consisted of methanol-water and sodium dihydrogen phosphate and the UV-detection was at 254 nm. The method was applied to tablets with a recovery of 99.5 to 100.5 %.

A stability indicating HPLC analysis of FMT^[22] has been performed on a 25 cm column of Spherisorb ODS with a mobile phase (1.5 ml/min) of ammonium acetate buffer (pH 2.9)-acetonitrile (21:4), with UV detection at 254 nm. The calibration graph was rectilinear from 0.5 to 10 µg/ml of FMT. Detection limit was 0.4 µg/ml. Ficarra et al.^[23] have reported the determination of FMT by HPLC on a Perkin-Elmer high speed C18 column (5µm) at 35° with a gradient mobile phase of 3% acetic acid in acetonitrile (89 to 85% of 3% acetic acid over 2.5 min, then, 85 to 45% over 2 min). Detection was at 265 nm. Salicylic acid was used as the internal standard. The method was applied to tablets.

In a method reported by Salem et al.^[24], the separation and determination were caused on a column (25 cm × 4.69 mm i. d.) of LiChrosorb RP-18, 7µm) with 0.01 M phosphate buffer-acetonitrile – methanol (84:11:5) adjusted to pH 6.5 with phosphate as mobile phase (1.8 ml/ min) and UV-detection at 280 nm. Theophylline was used as the internal standard. Response was rectilinear from 5 to 25 µg/ml. HPLC analysis of FMT^[25] was also performed on a column (15 cm × 4.6 mm i.d.) of Spherisorb cyano bonded phase (3µm) with 0.05 M phosphate buffer (pH 7.0)-acetonitrile (17:3) as mobile phase (1.0 ml/min) and detection at 269 nm. Calibration graph was linear from 1.12 to 4.05 µg/ml.

FMT in its preparation was quantitated^[26] by HPLC by effecting separation and determination on

a column (25 cm × 4.6 mm) of Lichrosorb RP 18 (10 µm) by eluting with methanol-0.01 M phosphate buffer (pH 6.4)-acetonitrile (7:82:11) at 1.5 ml/min and detection at 280 nm. Theophylline was used as the internal standard. A good linearity was observed from 120 to 420 µg/ml FMT. The method was applied to tablets with recoveries of 98.9 to 100.4 %. Quantitative HPLC analysis of FMT in pharmaceutical dosage forms has been described by Cakir et al^[27]. The analysis was performed on a 5 µm Lichrosorb RP-18 column (25 cm × 4 mm i.d) with methanol-0.1M ammonium acetate buffer (3:7) as mobile phase (1 ml/min) and detection at 254 nm.

The present work describes a HPLC procedure for the determination of FMT using Accurasil SS C₁₈ column, mobile phase consisting of acetonitrile and H₃PO₄ with UV-detection at 268 nm and includes statistical evaluation of results.

EXPERIMENTAL

Apparatus

A high performance liquid chromatograph (Agilent 1100 series, Agilent Technologies Wallbrom, Germany), equipped with an in built solvent degasser quaternary pump and photo diode array detector with variable injector and auto sampler was used along with the reversed phase column (5 µm Accurasil SS C₁₈, 25 cm long and 4.6 i.d. Thernocil USA).

Reagents and materials

Analytical reagent grade phosphoric acid (Ranchem, India,) HPLC grade acetonitrile (E. Merck Ltd., Bombay, India) and distilled water filtered through 0.45 µm filter (Millipore) were used.

0.1% phosphoric acid (pH 3.0)

This was prepared by diluting 10 ml of orthophosphoric acid to 1 litre with water and the pH was adjusted with triethylamine.

Solvent system

Eluting solvent mixture of acetonitrile + 0.1% orthophosphoric acid (pH 3) in 80:20 proportion was kept isocratic mode at a rate of 1.0 ml/min.

Diluent

Full Paper

This was prepared by mixing acetonitrile and water in the ratio of 6:4

Standard FMT solution

Pharmaceutical grade FMT certified to be 99.7% pure was received as gift from Intas Pvt. Ltd., India and used as received. A stock standard solution containing 500 µg/ml FMT was prepared by dissolving 50 mg of pure drug in diluent solution and diluting to volume in a 100 ml calibrated flask.

Procedures

Chromatographic conditions

Chromatographic separation was performed at ambient temperature on a reversed phase Accurasil SS C₁₈ column (25 cm x 4.6 mm id) using a mobile phase consisting of acetonitrile- 0.1% phosphoric acid (pH 3.0) (80:20) at a flow rate 1.0 ml/min. The detector wavelength was at 268 nm with a sensitivity of 0.2 a.u.f.s.

Calibration graph

Working standard solutions equivalent to 5.0 – 330 µg/ml FMT were prepared by transferring 0.5 – 33 ml of stock solution (500 µg/ml) into separate 50 ml calibrated flasks and diluting to volume with the diluent solution. A 20 µl volume was injected automatically into the chromatograph, in duplicate and chromatographs were recorded. Calibration graph was constructed by plotting the mean peak area against FMT concentration.

Procedure for dosage forms

Twenty tablets containing FMT were chosen in random from a total number of 50. They were weighed accurately and ground into a fine powder. An amount of powder equivalent to approximately 50 mg of drug was weighed accurately and dissolved in the diluent solution in a beaker with stirring and the resulting mixture was transferred quantitatively to a 100 ml calibrated flask and made up to volume with the diluent solution and mixed well. A small portion of the solution was withdrawn and filtered through a 0.2 µm filter to ensure the absence of particulate matter. This filtered solution was appropriately diluted to get the final solution for analysis. An aliquot of FMT injection solution equivalent to 50 mg of FMT was diluted with the diluent solution in

a 100 ml standard flask and analysed after appropriate dilution with the solvent system.

RESULTS AND DISCUSSION

The chromatographic conditions used gave well-defined peak. A mobile phase consisting of acetonitrile-0.1% H₃PO₄ (pH 3) (80+20) was chosen after several trials with acetonitrile-water, methanol-water, acetonitrile-potassium dihydrogen phosphate and methanol- potassium dihydrogen phosphate combinations. The described chromatographic system gave the peak in a reasonable time of ~ 3.5 min. For quantitative determination, a linear calibration graph ($Y = 1.94 + 33.99 X$; $r = 0.9998$; $n = 6$; where Y = mean peak area and X = concentration in µg/ml) was obtained over the working concentration range 5.0- 333 µg/ml. The limits of detection (LOD) and quantification (LOQ) were 1.0 and 3.0 µg/ml, respectively.

Method validation

Accuracy and precision

The accuracy and precision of the method were established by performing seven replicate analyses on pure drug solution at three different concentration levels (within the linear range). The relative error (%), an indicator of accuracy, was around 1% and the intra-day precision expressed as relative standard deviation, RSD (%), was less than 1% indicating high accuracy and precision of the method. The results of this study are given in TABLE 1. The day-to-day precision was evaluated by performing replicate analyses on pure drug solution at three concentration levels over a period of five days by preparing all solutions afresh each day. The day-to-day RSD

TABLE 1: Evaluation of accuracy and intra-day precision

FMT taken, µg/ml	FMT found*, µg/ml	Range, µg/ml	RE, %	SD, µg/ml	RSD, %	ROE, %
50.00	50.50	0.52	1.00	0.19	0.38	± 0.37
100.00	101.20	0.13	1.20	0.06	0.06	± 0.05
150.00	149.21	0.34	0.53	0.41	0.27	± 0.26

* Average of seven determinations

RE. Relative error, SD. Standard deviation; RSD. Relative standard deviation; ROE, range of error at the 95% confidence for six degrees of freedom.

TABLE 2: Comparison of results of SBS determination by the proposed method with those of official method

Drug and formulation	Label claim, mg/ tablet or mg/ml	Found(% of label claim ± SD)		Student's t-value (2.78)*	F-value (6.39)*
		Proposed method	Official method		
Facid ^a Tablets	20	99.94±0.74	100.80±0.88	1.68	1.41
Topeid ^b Tablets	40	98.58±0.34	99.16±0.68	1.79	4.00
Facid ^a Injection	2	98.12±1.26	99.42±0.74	1.95	2.84

* Mean value of five determinations; Tabulated t - value at 95 % confidence level is 2.477; Tabulated F - value at 95% confidence level is 6.39
 **** Marketed by: ^a. Cipla; ^b. Torrent; India.

values were less than 4% reflecting the usefulness of the method in routine analysis.

Application to FMT tablets

Commercially available FMT tablets were analyzed by the described HPLC method. The results obtained are summarized in TABLE 2. As can be seen the results for tablets and injections were in agreement with the labeled amounts. The results obtained by the proposed method were compared with those obtained by the official method^[28] by applying Student's t-test and F-test at the 95% confidence level. The calculated t and F - values did not exceed the tabulated values of 2.77 and 6.39, respectively, indicating that the proposed method is as accurate and precise as the official method. In order to demonstrate the validity and applicability of the proposed method, recovery studies were performed via standard addition technique. To a fixed and known amount of drug in the pre analyzed tablet extract or injection solution, pure FMT (standard) was added at three different levels and the total amount was found by the proposed method. The experiment at each level was repeated three times. The percent recovery of the pure drug added which

is compiled in TABLE 3 reveals that the commonly added excipients such as lactose, talc, starch, gum acacia, sodium alginate and magnesium stearate did not interfere in the assay method.

CONCLUSIONS

A convenient method has been developed and appropriately validated for the assay of famotidine in its dosage forms for the purpose of product quality assessment. The method is rapid and selective besides being accurate and precise. A single chromatographic run took less than 5 min. The method does not involve extensive sample treatment and involves an HPLC system employing an inexpensive mobile phase. The UV detection was linear for the concentration studied. There was no interference from the matrix sources. The proposed method is applicable over a wide dynamic concentration range compared to many HPLC procedures reported earlier^[21-27], and is more sensitive than many existing HPLC procedures and the method employs no internal standard unlike most existing procedures. The method is suitable for regular assay and for checking the stability of its formulations.

TABLE 3: Results of recovery study by standard-addition method

Formulations	Amount of drug in formulation, µg/ml	Amount of pure drug added, µg /ml	Total found*, µg /ml	% Recovery of pure drug added
Topeid tablet (40 mg)	49.97	50.00	100.24	100.54
	49.97	100.00	148.58	98.61
	49.97	150.00	200.31	100.23
Facid injection (2 mg/ml)	49.06	50.00	98.89	99.66
	49.06	100.00	150.53	101.47
	49.06	150.00	198.98	99.95

* Average of three determinations

REFERENCES

- [1] J.Petkovic, D.Minic, Z.Koricanac, J.Jovanoric; *Pharmazie*, **53**, 163 (1998).
- [2] M.M.Ayad, S.Abdalla, H.E.Abdel Latef, H.M.Elsaid; *J.Pharm.Biomed.Anal.*, **29**, 247 (2002).
- [3] K.Basavaiah, H.C.Prameela; *Bulg.Chem.Commun.*, **35**, 37 (2003).
- [4] K.Basavaiah, H.C.Prameela; *ScienceAsia*, **29**, 147 (2003).
- [5] K.Basavaiah, H.C.Prameela; *Bulg.Chem.Industry*, **74**,

Full Paper

- 50 (2003).
- [6] S.Raghuveer, C.M.R.Srivatsva, D.K.Vatsa; Indian Drugs, **29**, 320 (1992).
- [7] H.Li, J.Xuan, Zhongguo; Yiyao Gonggong Zazhi, **24**, 319 (1993).
- [8] F.A.El-Yazbi; Spectrosc. Lett., **25**, 1011 (1992).
- [9] B.V.Kamath, K.Shivaram, S.Vangani; Anal.Lett., **2239**, 25 (1992).
- [10] B.V.Kamath, K.Shivaram, A.C.Shah; J.Pharm.Biomed. Anal., **12**, 343 (1994).
- [11] Y.K.Agarwal, K.Shivaramachandra, G.N.Singh, B.E. Rao; J.Pharm.Biomed.Anal., **10**, 521 (1992).
- [12] G.R.Rao, A.B.Avadhanulu, D.K.Vatsa; East-Pharm., **33**, 175 (1990).
- [13] B.Guvener, S.Ates; Acta Pharm.Turc., **32**, 67 (1998).
- [14] B.V.Kamath, K.Shivaram, S.Vangani; Anal.Lett., **26**, 2183 (1993).
- [15] Z.A.Ali, R.M.Shubietah, G.M.Badah; J.Pharm. Biomed.Anal., **21**, 459 (1999).
- [16] K.Basavaiah, H.C.Prameela; Indian Drugs, **39**, 546 (2002).
- [17] K.Basavaiah, H.C.Prameela; Indian Pharmacist, **3(20)**, 59 (2004).
- [18] A.El-Bayoumi, A.A.El-Shanawany, M.E.El-badek, A.Abdel Sattav; Spectrosc.Lett., **30**, 25 (1997).
- [19] J.A.Squella, G. Valencia, I. Lemus, L.J. Nunez Vergara; J.Assoc.Off.Anal.Chem., **72**, 549 (1989).
- [20] J.A.Squella, G.Rivera, I.Lemus, L.J.Nunez Vergara; Mikrochim.Acta, **I(5-6)**, 343 (1990).
- [21] S.E.Bifar, D.J.Mazzo; J.Chromatogr., **363**, 243 (1986).
- [22] M.S.Suleiman, H.Y.Muti, M.E.Abdel-Hamid, M. Hassan, Y.M.El-Sayed, N.M.Najib; Anal.Lett., **22**, 1499 (1989).
- [23] R.Ficarra, P.Ficarra, M.L.Calabro; Farmaco Ed.Prat., **42**, 307 (1987).
- [24] M.A.S.Salem, H.N.Alkaysi, A.A.Badwan; Anal.Lett., **22**, 2501 (1989).
- [25] N.Beaulieu, S.J.Graham, R.W.Sears, E.G.Loveering; J.Pharm.Biomed.Anal., **7**, 1705 (1989).
- [26] S.M.Zhang, W.L.Li, C.Zhang; Yaowu Fenxi Zazhi, **13**, 150 (1993).
- [27] B.Cakir, A.U.Tosun, M.F.Sahin; Pharm.Sci., **3**, 493 (1997).
- [28] United States Pharmacopoeia, 24, Fifteenth Revision, united States Pharmacopoeials Convention, Inc., Rock-Ville, M.D., 707 (1995).