Development and Validation of RP-HPLC Method for Determination of Famotidine and its Application in Quality Control of Different Pharmaceutical Dosage Forms

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Summary: A precise and fast novel high-performance liquid chromatography method was developed and validated for the quantitative determination of Famotidine (FMT) in commercially available pharmaceutical dosage forms. An Agilent 1200 Series High Performance Liquid Chromatography (HPLC) system having the column C_{18} (5µm particle size, 150×4.6 mm) was used in this study and detection (diode array detector) was made at 280 nm. The mobile phase was acetonitrile, distilled water, triethylamine and phosphoric acid (49.9:49.9:0.1:0.1, v/v), isocratic elution under ambient temperature at flow rate of 1.5 mL min^{-1} with injection volume 5µL. In this method, the retention times for FMT pure, tablets and suspension were 0.787 min, 0.789 min and 0.839 minutes respectively. The new method was validated by different validation parameters. The procedure provided a linear response over the concentration range of 0.1-1.0 mg mL^{-1} (r^2 =0.998) and equation was y=3902.6+18.651. The mean % recovery for inter-day (96.56%) and intra-day (97.36%) assuring a good precision and accuracy was 96-98%. The method was found to be very rapid and the overall assay time was less than 2 minutes and the results obtained were accurate, precise and selective enough to allow the determination of FMT in the presence of certain excipients.

Keywords: Famotidine (FMT), HPLC, Pharmaceutical dosage form.

Introduction

Famotidine (Fig. 1) is a histamine H_2 receptor inhibitor which blocks the production of the acid secreted by the stomach and used for the treatment of peptic ulcer disease, gastro esophageal reflux disease and Zollinger-Ellison syndrome. It was rapidly and orally well absorbed. It is white or yellowish-white, polycrystalline powder or crystals and molecular formula is $C_8H_{15}N_7O_2S_3$. It was very slightly soluble in water and anhydrous ethanol, freely soluble in glacial acetic acid and insoluble in ethyl acetate. It competitively and reversibly blocks the action of histamine on H_2 receptors present in the stomach and heart. This action is relatively specific for the histamine H_2 receptors and finds greatest use as particularly effective inhibitors of gastric acid secretion [1]. Although H_2 receptors are present in many tissues, including vascular and bronchial smooth muscle, it interfere very little with physiological functions other than gastric secretion [2, 3]. It also blocks the secretion of acid which is initiated by gastrin and to a negligible extent, by muscarinic agonists [4].

It is mostly excreted in the urine but are not metabolized during this process of excretion [5]. The cytochrome P450 enzyme system is not affected by the first H_2 receptor antagonist FMT and have not interaction with other drugs. FMT replaced the imidazole-ring of cimetidine with a 2-guanidinothiazole ring. Pioneer representatives of this group, such as burimamide [6] and cimetidine retain the imidazole ring of histamine. This ring is replaceded in recently developed compounds by a furan (ranitidine) or a thiazole (famotidine). FMT is thirty times more potent than cimetidine [7]. It was regularly marketed for the first time in 1981. In 1999 orally-disintegrating tablets were released and generic preparations came in the market in 2001.

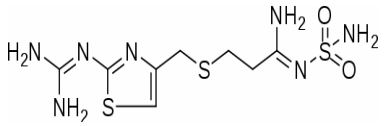

Fig. 1: N'- (aminosulfonyl) -3- [[[2-[(diaminomethylene) amino] -4-thiazolyl] methyl] thio] propanimidamide.

Many previously published methods for estimation of FMT were developed using different

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analytical techniques which includes RP-HPLC method [8-14], Spectrophotometry [15], HPTLC [16], capillary zone Electrophoresis [17], flow injection analysis [18] and LC-MS method [19].

There is an assay of FMT in British Pharmacopeia (B.P.) 2011, include non-aqueous titration, UV spectrophotometric method and HPLC method. The existing methods were lengthy, expensive or involving costly equipments and tedious.This research work aims to make a contribution to the assessment of FMT and its tablet, suspension dosage forms available in market. Therefore, it is important to develop a novel, less expensive and fast method for the determination of FMT in pure and different pharmaceutical dosage forms.

Results and Discussion

Several methods have been reported for the determination of FMT which includes spectrophotometry HPLC, flow injection analysis and HPTLC etc. The titration by using the perchloric acid as titrant and crystal violet as indicator (0.5% in glacial acetic acid) is the classical method for the determination of FMT, in pharmaceutical dosage forms. However, this procedure is slow, tedious and consumes great amount of reagents and is time consuming method.

Although direct spectrophotometric determination is widely used in pharmaceutical analysis and is cheaper than HPLC, but most of the spectrophotometric methods have been noted to suffer from the disadvantages like narrow range of determination and long time for the reaction to complete. These spectrophotometric procedures lack specificity, accuracy and precision which is required for the analytical determination of the drugs like FMT in pharmaceutical dosage forms. Therefore, the study was undertaken using the HPLC technique, which can accurately and precisely determine the quantities of FMT in different pharmaceutical dosage forms.

In order to develop the new method by using HPLC technique for the determination of FMT in commercially available different pharmaceutical dosage forms, Agilent 1200 HPLC series system was used. The mixture of acetonitrile, water, triethylamine and phosphoric acid in proportion of (49.9:49.9:0.1:0.1, v/v) proved to be better than the mixture of methanol and water for the separation, since chromatographic peaks were better defined and resolved and almost free from tailing effect. Among several flow rates tested (0.5-2 mL min^{-1}), the flow rate of 1.5 mL min^{-1} was the best with respect to location and resolution of analytical peaks. Using the diode array detector at 280 nm, the above described chromatographic conditions allow a resolution of FMT in a very short time that is 0.787 minutes. Our method has been validated successfully as shown in the six different brands of tablet as well as suspension dosage forms. The validation of the developed method was studied according to specification for FMT in B.P 2011 (95.0–105.0%) and U.S.P 2009 (90.0–110.0%) in pure and tablet dosage forms, statistical data (Table 1-6) shows that the developed method is reproducible, specific, precise and accurate.

This method has an importance in the quality assurance of the pharmaceutical dosage forms especially tablets and suspensions in the drug testing laboratories. In this method, mobile phase, wave length, retension time and flow rate were different from the already published HPLC methods of FMT. On comparison of this work with already developed RP-HPLC methods, this concludes that the developed method has short analysis time (< 1 minute) and work was only performed regarding estimation of FMT in different pharmaceutical dosage form. The developed HPLC method has advantages of less sample volume (5µL) and minimum time of analysis over the already existing methods.

Experimental

Reagents

FMT pure was kindly provided by Wilson's Pharmaceuticals Pakistan Limited, Islamabad. All kinds of chemicals and reagents were of analytical grade. Acetonitrile was of HPLC grade (Sigma Alrich). Triethylamine and phosphoric acid were of analytical grade.

Different Pharmaceutical Dosage Forms of Different Manufacturer

The six tablet dosage forms brands of FMT Polypep tablets (Wilson), Ulcofin Tablets (Pharmix), Ulfarid Tablets (Davis), Famot Tablets (Shaigan), Famotid Tablets (Amson) and Feptid Tablets were used in this study.

The six suspension dosage forms brands of FMT Polypep Suspension (Wilson), Suspension Ulcofin (Pharmix), Suspension Peptiban (Werrick), Suspension Acicon (Barrett Hodgson), Suspension

Afomit (Alliance) and Suspension Gimed (Albro) were used in this study.

Method Development

A direct method was developed for the determination of FMT in pure and different pharmaceutical dosage forms. The drug was eluted through Agilent 1200 Series HPLC system having diode array detector and the column C_{18} (5µm particle size, 150×4.6 mm), detection was made at 280 nm. The mobile phase was acetonitrile, distilled water, triethylamine and phosphoric acid (49.9:49.9:0.1:0.1, v/v) at flow rate of 1.5 mL min^{-1} with injection volume 5µL. In this method, the retention times for FMT raw material, FMT tablets and suspension were 0.786 min, 0.798 min and 0.793 min respectively, without interference of excepients.

Preparation of Standard Solution

100 mg of FMT was weighed accurately and was taken in a 100 mL volumetric flask. Added 80 mL of mobile phase and dissolved completely and degassed for 15 minutes by ultrasonic bath. The volume was made to 100 mL with the mobile phase. It was filtered through 0.45 micron membrane filter. The concentration of the above solution was 1.0 mg mL^{-1}. Standard solutions of different concentrations were prepared from this solution.

Preparation of Sample Solution

Five tablets of each brands of FMT were powdered. A portion of powdered tablets equivalent to 100 mg of FMT was weighed accurately and dissolved in 80 mL of mobile phase in 100 mL volumemetric flask and degassed for 15 minutes by ultrasonic bath. The volume was made up to 100 mL with the mobile phase. It was filtered through 0.45 micron membrane filter. The concentration of the above solution was 1.0 mg mL^{-1}. Sample solutions of different concentrations were prepared from this solution.

Analytical Procedure

Separately equal volume of assay preparation and standard preparation in the HPLC vials were kept in auto sampler compartment in six replicate. Recording the chromatogram and measured the major peak response of FMT in an assay preparation and standard preparation. The relative standard deviation of standard preparation replicate should not be more than 2 %.

Validation of the Method

The HPLC developed method for determination of FMT in pure and different pharmaceutical dosage forms was validated according to specification lay down in B.P. 2011 and U.S.P. 2009.

Linearity and Range

The linear response of the method was evaluated by plotting the different concentrations of FMT from 0.1-1.0 mg mL^{-1} versus their respective peak areas (Table-1). A good determination coefficient (r^2=0.998) was obtained and calibration equation was (y=3902.6+18.651) and the calibration curve is shown in the Fig. 2.

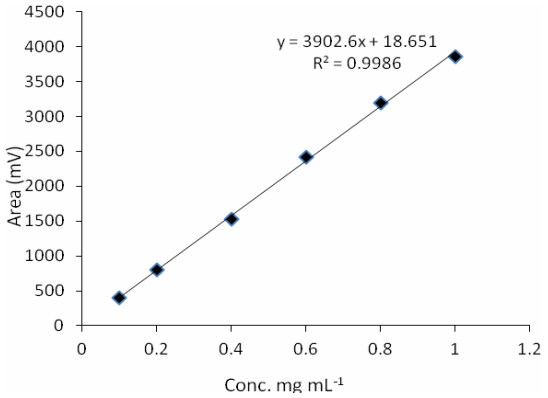

Fig. 2: Calibration curve of Famotidine at different Concs. (mg mL^{-1}).

Table-1: Calibration Standards of FMT at different Concentrations.

FMT (mg mL^{-1})	Peak Area (mV)
0.1	405.97
0.2	799.52
0.4	1523.93
0.6	2422.22
0.8	3195.77
1.0	3862.51

Accuracy

The accuracy of an analytical method is defined as the similarity of the results obtained by the analytical method to the true value. Accuracy was assessed using a minimum of 9 determinations over a minimum of 3 concentration levels (low, medium and high) covering the specified range (3 concentrations / 3 replicate each of the total analytical procedure), the accuracy of FMT were 97.63%, 97.66% and 95.95%, respectively (Table-2).

Table-2: Accuracy of the proposed HPLC method for the determination of FMT.

S. No.	Conc. Of FMT takenmg mL^{-1}	Conc. Of FMT Found mg mL^{-1}	Accuracy (%age recovery)	Mean of %age recovery
1	0.1	0.0978	97.8%	
2	0.1	0.0976	97.6%	97.63%
3	0.1	0.0975	97.5%	
4	0.2	0.197	98.5%	
5	0.2	0.195	97.5%	97.66%
6	0.2	0.194	97.0%	
7	0.4	0.383	95.84%	
8	0.4	0.383	95.86%	95.95%
9	0.4	0.385	96.15%	

Precision

The precision of an analytical method is defined as the degree of the similarity of the results obtained by the analytical method. Precision of this developed method was 95.93% (n=6) and the values within the range of specifications (Table-3).

Table-3: Precision of the proposed HPLC method for the determination of FMT.

S. No.	Conc. Of FMT Taken mg mL^{-1}	Conc. Of FMT Found mg mL^{-1}	%age recovery
1	0.4	0.383	95.80%
2	0.4	0.383	95.86%
3	0.4	0.384	96.10%
4	0.4	0.383	95.84%
5	0.4	0.383	95.86%
6	0.4	0.385	96.15%
Mean		0.383	95.93%

Intra-Day and Inter-Day Variations of the Method

The intra-day and inter-day variations of the method were determined using three replicate injection of three different concentrations of FMT in commercial samples which were prepared and analysed on the same day and on three different days over a period of two weeks (Table-4 and 5). The results for intra-day 97.36% and inter-day 96.56% show a considerable degree of precision and reproducibility of the proposed method.

Table-4: Intra-day precision of FMT.

Serial No.	Concof FMT used (mg mL^{-1})	Conc. of FMT found (mg mL^{-1})	%age recovery	Mean %age recovery
1	0.1	0.097	97.00%	
2	0.2	0.197	98.50%	97.36%
3	0.4	0.383	95.75%	
4	0.5	0.491	98.20%	

Table-5: Inter-day precision of FMT.

S. No.	Concof FMT used (mg mL^{-1})	Conc. of FMT found (mg mL^{-1})	%age recovery	Mean %age recovery
1	0.1	0.095	95.00%	
2	0.2	0.196	98.00%	96.56%
3	0.4	0.381	95.25%	
4	0.5	0.490	98.00%	

Specificity

In validation process, specificity test is a parameter used for the determination of impurity, degradants or excipients. The chromatogram of FMT (Fig. 3) indicating that good separation was achieved at 280 nm and the retention time of FMT was 0.787 minutes using the chromatographic conditions described in the experimental portion. The run time was two minutes that allowed analysis of large number of samples in a very short period of time. The peak area responses of FMT were compared between standard solutions and FMT pharmaceutical dosage forms (tablets and suspension) containing excipients. The peak area responses and retention time of FMT from the two solutions were not significantly different. Thus the presence of excipients in the solution did not interfere with the determination of FMT under these HPLC conditions.

Sensitivity

The limit of detection (LOD) and Limit of quantification (LOQ) were 25 µg mL^{-1} and 0.1 mg mL^{-1}, respectively (Table-6).

Table-6: Validation and sensitivity parameters of the developed method of FMT.

Parameter	Value
λ_{max}	280 nm
Range	0.1-1.0 mg mL^{-1}
LOD	25 µg mL^{-1}
LOQ	0.1 mg mL^{-1}
r^2	0.998
Equation	y=3902.6+18.651
Accuracy	95.95-97.66%
Precision (mean % recovery)	Intra-day = 97.36% Inter-day = 96.56%

Stability Studies

In the case of unexpected delay during analysis, it is important to have information about the stability of the solution to be analyzed. It is the merit of this method that solution of FMT is already made in the mixture which is also used as mobile phase and even extraction of tablets and suspensions was done using the same mobile phase. So there is no need of any other reagent for making sample solution and extraction. The analysis of Afomit suspension was done by both, making extraction and centrifuging with 0.01N HCl and with mobile phase, there was no difference in retention time and in the peak area response. This is the advantage of this novel technique over existing methods of HPLC used in laboratories.

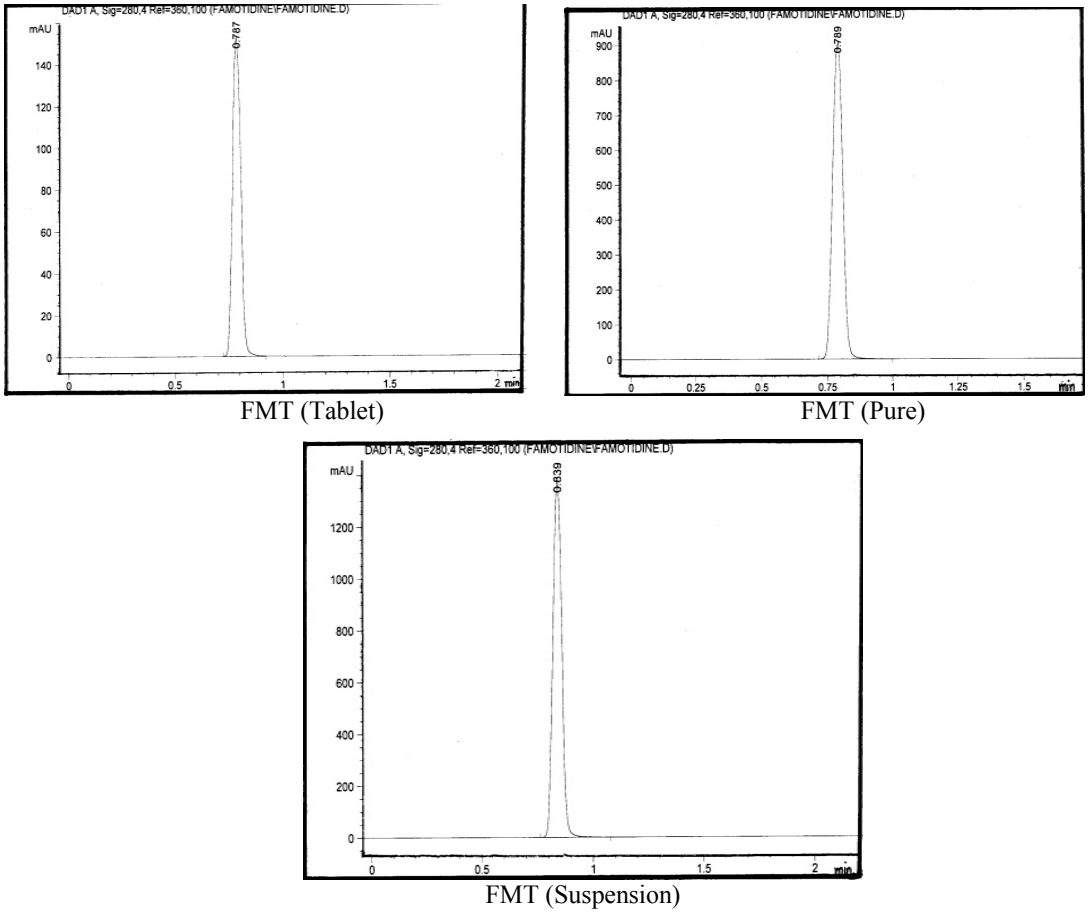

Fig. 3: Chromatograms of FMT pure, tablet and suspension.

Application of Method

The developed HPLC method was applied for the determination of FMT in commercially available pharmaceutical dosage forms, tablets and suspensions. The differences between the amount claimed and those measured were very low and results were within the acceptable windows mentioned in the pharmacopoeias. The mean percentage recoveries obtained after six repeated experiments were within B.P 2011 (95.0 – 105.0%) and U.S.P 2009 (90.0 – 110.0%) specification for FMT indicating that results are accurate and there is no interference from the excipients. After validation of the newly developed method the applicability of this HPLC method for the assay of FMT was tested by analyzing six different brands of tablet dosage forms, results are given in Table-7 and results for six different brands of suspension dosage forms are given in Table-8. The analysis showed that the results are consistent with the label claim of the formulations. The differences between the amount claimed and those measured were very low and showing that results are accurate and there is no interference from the excipients and preservatives.

Table-7: Percentage purities of different commercial brands of FMT tablet dosage forms.

Serial No.	Name of the sample	Approx. retention time(min)	Conc. of FMT used (mg mL^{-1})	Peak areas of FMT tablets	Conc. of FMT found. (mg mL^{-1})	%age recovery
1	Tablet Polypep	0.798	0.4	1506.64	0.382	95.50%
2	Tablet Ulcofin	0.798	0.4	1580.49	0.397	99.47%
3	Tablet Ulfarid	0.798	0.4	1562.63	0.398	98.33%
4	Tablet Famot	0.798	0.4	1572.73	0.395	98.97%
5	Tablet Famotid	0.798	0.4	1567.08	0.394	98.61%
6	Tablet Feptid	0.798	0.4	1529.89	0.385	96.60%

Table-8: Percentage purities of different commercial brands of FMT suspension dosage forms.

Serial No.	Name of the sample	Approx. retention time(min)	Conc. of FMT used (mg mL^{-1})	Peak areas of FMT suspension	Conc. of FMT found. (mg mL^{-1})	%age recovery
1	Suspension Polypep	0.793	0.4	1561.15	0.393	98.25%
2	Suspension Ulcofin	0.793	0.4	1566.09	0.394	98.50%
3	Suspension Peptiban	0.793	0.4	1530.44	0.385	96.60%
4	Suspension Acicon	0.793	0.4	1594.55	0.401	100.25%
5	Suspension Afomit	0.793	0.4	1511.056	0.382	95.50%
6	Suspension Gimed	0.793	0.4	1505.21	0.380	95.00%

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