HPLC Method for Determination of Famotidine in Human Plasma and its Application in Bioequivalence Studies

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(Received 12th February, 2006, revised 21st February, 2007)

Summary: A sensitive, accurate and rapid reverse phase HPLC method was developed to quantify plasma levels of famotidine in order to conduct a comparative bioavailability study. Famotidine was extracted by liquid-liquid extraction method and analyzed on a Hypersil ODS C_{18} (150 × 4.6 mm) column, with disodium hydrogen phosphate (20 mM), sodium dodecyl sulphate (50 mM) in distilled water (pH 3.0) and acetonitrile in the ratio of 70: 30 with UV-detector at 267 nm. The standard curve covering 0.093-1.5 μg/ mL concentration range was linear (r^2 = 0.9986), CV ranged 1.34-5.83 for intra-day and 1.10-6.92 for inter-day variation. The method was applied for the determination of comparative bioavailability of the two formulations coated with different coating agents. There was a statistical significant difference ($p < 0.05$) between area under concentration vs time curve of formulation 1 (F1) and formulation 2 (F2) and their values were 3.97 ± 1.61 μg.h/ mL and 10.07 ± 0.21μg.h/ mL, respectively.

Introduction

Famotidine, [3-(((2-((aminoaminemethyl)-4-thiozolyl)-methyl)thio)-N-(amionosulfonyl) propan-amidamine] is a histamine H_2 receptor antagonist (H_2RA) that potently inhibits gastric acid secretions and is commonly used in the treatment and prevention of the gastric and duodenal ulcer [1]. It is a relatively new H_2RA that is 7 and 20 times more potent than ranitidine and cimitidine, respectively, in inhibiting basal and pentagestrin-stimulated gastric acid secretion in adults [2]. Famotidine, like the other H_2RAs, is useful for both prophylaxis and treatment of gastric acid reflux and ulcers in children. It offers the advantages of having relatively few adverse effects and no significant drug interactions. The ideal dosing regimen is still debated, with most investigators choosing either an every 8 or 12 h interval. Additional clinical trials, particularly in infants and young children, are needed to better establish the safety and efficacy of current dosing strategies [3-5].

Several HPLC methods for the determination of famotidine in human plasma have been reported [6-8]. These methods either utilized solid phase extraction (SPE), or liquid phase extraction techniques. Limitations of these methods include the requirement to extract large volumes of plasma. We have developed simple and reproducible liquid-liquid extraction method having high sensitivity and selectivity for famotidine. Previous studies on the effects of various coating materials on the bioavailability of famotidine in plasma are lacking in literature. Therefore, the newly modified HPLC method is applied to study the effect in the new formulations. It is shown that the given method is rapid, sensitive, accurate and can be used in the bioavailability studies.

Results and Discussion

Standard curve of famotidine in plasma shows the concentration *vs* response (peak area) that is linear in the range of 0.093-1.5 μg/mL (Fig 1). The r^2 value is 0.9986. The slope, intercept and CV values are given in Table-1. The CV within-day and between-days variability for 0.093 μg/ mL is 1.34 and 1.10 respectively. These values for 1.5 μg/mL are 5.83 and 6.92 respectively (Table-2).

Percent extraction yield for each dilution was calculated by dividing extracted serum samples with serum free dilutions. The average recovery for

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Table-1: Mean ± standard deviation, slope, intercept and correlation coefficient of calibration curve equations. (n = 5).

Parameters	Mean	S.D.
Slope	0.0043	0.00054
Intercept	0.0057	0.00067
Correlation coefficient	0.9986	0.00086

Table-2: Within-day and between-days variability of the assay (n = 5).

Conc.	Within-day Variability			Between-day Variability		
(μg/ mL)	Mean	S.D.	CV	Mean	S.D.	CV
0.093	0.094	0.07	1.34	0.096	0.087	1.10
0.187	0.188	0.089	2.11	0.189	0.090	2.10
0.375	0.372	0.096	3.87	0.376	0.085	4.42
0.750	0.755	0.153	5.03	0.748	0.114	6.56
1.5	1.495	0.256	5.83	1.489	0.215	6.92

all samples was 90.25 ± 3.5 % (Table-3). Fig. 2 exhibits typical chromatogram of serum famotidine samples. The bioavailability and pharmacokinetic parameters of famotidine include C_{max}, T_{max} and AUC of both the formulations. For F1, C_{max}, T_{max} and AUC are 0.97 ± 0.47 μg/ mL, 1.68 ± 0.37 h and 3.97 ± 0.61 μg/ h/ mL respectively. For F2, these values are 1.64 ± 1.02 μg/ h/ mL, 1.50 ± 0.46 h and 10.07 ± 0.21 μg h/ mL respectively (Table- 4). A standard curve of famotidine in plasma was drawn and the

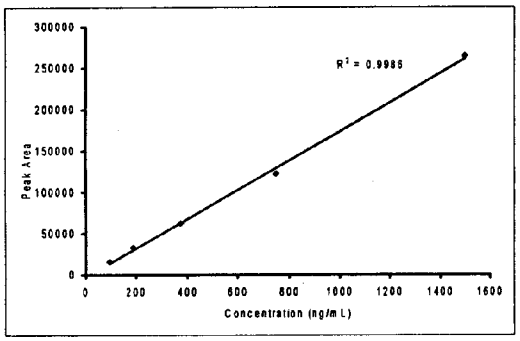

Fig. 1: Standard curve of famotidine in plasma.

method showed a r^2 value of 0.9986, reflecting good linearity over the entire concentration range of the standard curve (Fig. 1). The extraction efficiency of the method was 88-92 % (Table- 3). All these results were obtained with modified mobile phase (30:70 v/v ratio of acetonitrile and water containing 20 mM disodium hydrogen phosphate and 50 mM sodium dodecyl sulphate, pH 3.0). The run time was also reduced from 10 min to 7.5 min that reduced the sample analysis time.

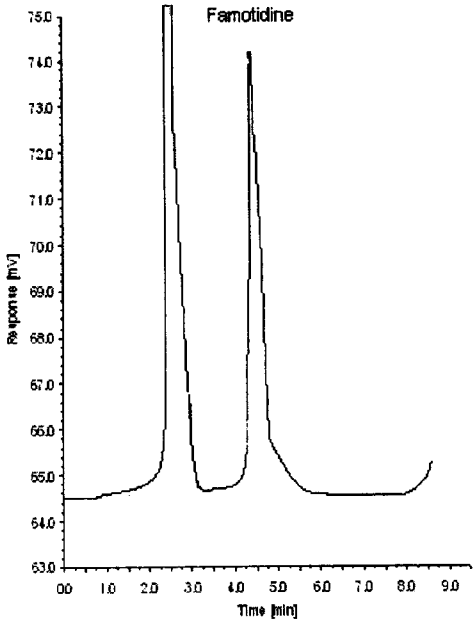

Fig. 2: Chromatographic peaks of famotidine after separation on C_{18} column.

Table-3: Percentage recovery of famotidine calculated in three standard dilutions. (n = 5).

Concentration (μg/ mL)	(%) recovery	S.D.
0.187	90.25	4.2
0.750	88.36	3.8
1.50	92.16	2.5
mean	90.25	3.5

The applicability of the present method was demonstrated by the determination of famotidine in plasma samples on sixteen subjects receiving oral doses of famotidine in a comparative bioavailability study. F1 and F2 formulations were prepared with different coating agents hydroxypropyl methyl cellulose and opadry white and were evaluated in healthy subjects. Plasma samples at different time intervals were collected and analyzed.

A typical chromatogram is shown in Fig. 2. Plasma concentration *vs* time profile of each subject was calculated and plotted on graph (Fig. 3). Bioavailability and pharmacokinetic parameters were determined by non-compartmental model. The values of C_{max}, T_{max} and AUC of F1 were compared with F2. The value of C_{max} for F1 was 0.97 ± 0.47μg/ mL and for F2 was 1.64 ± 1.02 μg/ mL. There was a statistically significant difference ($p < 0.05$) between

Table-4: Comparison of the bioavailability and pharmacokinetic parameters of famotidine administered in the oral dose of 40 mg in human subjects (n = 8).

Parameters	F1	F2
C_{max} (μg/ mL)	0.97 ± 0.47	1.64 ± 1.02
T_{max} (h)	1.68 ± 0.37	1.50 ± 0.46
AUC (μg/h/mL)	3.97 ± 0.61	10.07 ± 0.21

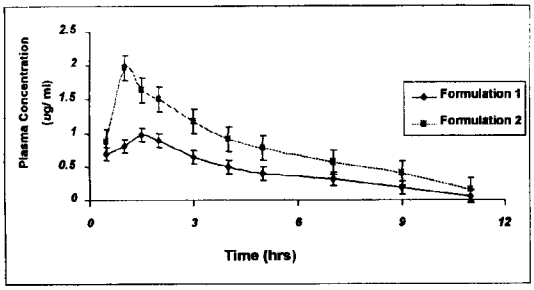

Fig. 3: Comparison of plasma concentration vs time profile of F1 and F2 administered in the oral dose of 40 mg in human subjects.

the two formulations. In a pervious study conducted on children, C_{max} was 0.6466 ± 0.2566 μg/ mL [7]. The reported mean (± SD) C_{max} for adults was 0.5276 ± 0.0282 μg/ mL [8]. In normal volunteers C_{max} of 0.104 ± 0.039 μg/ mL has been reported [9]. The values reported here in the present work are higher than those reported in the literature [7-9]. These differences might be due to change in the race and population and changes in the body composition of different individuals and partly due to the changes in the coating materials used in the formulation development. The C_{max} values for F1 are less than of F2 which reflects that F2 may have better pharmacological effects than F1. In the present studies, T_{max} of F1 was in the range of 1.0-2.0 h with a mean 1.68 ± 0.37 h and T_{max} of F2 was in the range of 1.0-2.0 h with mean 1.50 ± 0.46 h (Table-4). There was a statistically non-significant difference ($p > 0.05$) between the two formulations. These two values were lower than those reported in the previous studies [7-9]. A T_{max} of 2.3 ± 1 h is previously reported in normal volunteers [9]. Similarly, in another study conducted on patients, T_{max} was 3.6 ± 1.9 h while its control had 2.3 ± 1.05 h [10].

The values for AUC of F1 and F2 are 3.97±0.61 μg.h/ mL and 10.07 ± 0.21μg.h/mL respectively (Table-1). There was a statistically significant difference ($p < 0.05$) between the two formulations. In published work on the pharmacokinetics of famotidine in patients with cirrhosis and ascites, AUC of patients was 837± 300 ng.h/ mL and AUC in control subjects was 630 ± 204 ng.h/ mL [10] and 744 ± 372 ng.h/ mL [9].

Experimental

Chemicals and Reagents

Famotidine was kindly given as a gift by Merck Laboratories Pakistan. Disodium hydrogen phosphate and HPLC grade acetonitrile were purchased from Merck (Germany). All other chemicals and solvents were of analytical grade.

Solutions and Mobile Phase

Stock solution of famotidine was prepared by dissolving 100 mg drug in 100 mL of acetonitrile to give a final concentration of 1000 μg/ mL. Standard solution were obtained by diluting with acetonitrile in the range of 0.093-1.5 μg/ mL. The mobile phase consisted of disodium hydrogen phosphate (20 mM), sodium dodecyl sulphate (50 mM) in distilled water with pH 3.0 and acetonitrile in the ratio of 70:30 was prepared, degassed and delivered at a rate of 1.0 mL/ min.

Chromatographic Conditions

Analysis was performed by using Perkin Elmer Liquid Chromatography 200 Series, with a pump, Perkin Elmer 200 Series UV-visible detector at 267 nm. A reverse phase system was used which consisted of a 5-μm Hypersil ODS C_{18} column (250 mm x 4.6 mm) preceded by 5-μm Hypersil ODS C_{18} cartridge guard (10 mm x 4.6 mm) column.

Preparation of Standards and Extraction Procedure

Standard dilutions were made from the stock solution in the range of 0.093-1.5 μg/ mL comprising of 0.093, 0.187, 0.375, 0.750 and 1.5 μg/ mL. 1 mL of blank serum and 1 mL of standard dilution, were mixed and centrifuged at 4000 x g for 10 min. The supernatant layer was separated and evaporated to dryness under the constant stream of nitrogen at 40 °C. The residue was reconstituted with 100 μL of mobile phase and 20 μL was injected into HPLC system.

Quantification

Calibration curves were drawn by plotting the measured peak area of famotidine vs concentration of standard dilutions. The intra-day (within-run) and inter-day (between-run) accuracy and precision were determined in quadruplicates on three separate days.

Bioequivalence Studies

Bioavailability studies of famotidine in healthy human subjects were carried out with their written consent. Eight healthy human subjects were scheduled to participate in the study and were selected according to the FDA and ICH (International Conference for Harmonization) criteria for inclusion and exclusion for healthy human selection.

Experimental Design

The study consisted of a single oral dose of two treatments, two-way crossover design. This study employed the subjects to receive single oral dose of 40 mg of famotidine tablets coated with hydroxypropyl methylcellulose nonaqueous formulation (F1) and opadry white (Hydropropyl methylcellulose, titanium dioxide and polyethylene glycol 400) aqueous formulation (F2). The % age of weight increase in both the formulations after coating was 2.98 %. This single dose drug regimen (F1 and F2) was administered on an empty stomach; the subjects were selected randomly for both of the formulations and housed at the study center from one hour before to 12 hour after giving dose. Each subject was instructed to fast overnight prior to the treatment visit. The subjects were allowed to drink water *ad libitum*. The standardized breakfast and lunch were given to the subjects according to the study protocols. One week of wash out period was given before second treatment.

Sample Collection

A 20-gauge venous catheter was inserted into a fore arm of each subject for the collection of blood samples and 11 samples each of 3 mL blood were collected by syringe before the subject was dosed (zero time) and at 0.5-11.0 hours after giving dose of the famotidine. Blood samples were centrifuged at 4000 rpm for 10 min. The plasma was harvested and frozen at -20 °C until assay was performed.

Pharmacokinetic Analysis

Pharmacokinetic analysis was performed using MS Excel. Using non-compartmental model, maximum concentration of famotidine in serum (C_{max}) and times corresponding to these concentrations (T_{max}) were determined by visual inspection of plasma concentration time profiles. The area under the concentration time curve from 0 hour-infinity (AUC) was calculated by the linear trapezoidal rule using the AUC from 0 hour to last measured concentration (C_{last}) plus C_{last}/ K_{el} where C_{last} is the measured concentration at last sampling time and (K_{el}) is the terminal elimination rate constant. Other pharmacokinetic parameters were calculated and are given in the results.

Statistical Analysis

Statistical analysis was performed using (SPSS) v.12. Paired t-test was used to check the difference between the two formulations.

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