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Pharmacokinetic Study of Sulphoxide Prodrug of Famotidine by LC-MS/MS Method in Rabbit Plasma

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Sulphoxide prodrug of famotidine is a water soluble and biologically inactive derivative of famotidine, a H_2 receptor antagonist to treat ulcer. As the sulphoxide prodrug is a new entity so far no methods has been reported for its estimation in bulk and biological fluids. A rapid liquid chromatography tandem mass spectrometry (LC-MS-MS) method has been optimized for analysis of sulphoxide prodrug of famotidine in rabbit plasma using clopidogrel as internal standard. Following turboionspray ionization, the analytes were quantified on a triple-quadrupole mass spectrometer in multiple-reaction-monitoring positive ion mode. Sample preparation involved a simple one-step protein precipitation with methanol, followed by centrifugation and evaporation of the organic solvent. The residue was re-dissolved in mobile phase and analyzed by LC-MS/MS. A Symmetry C18, 50 × 4.6, 5 µ, a mobile phase composed of acetonitrile: water:triethyl amine:orthophosporic acid (49.9:49.9:0.1:0.1 % v/v/v/v) and a flow rate of 0.6 mL/min were employed and the total run time was 3 min. The method was validated for accuracy, precision, linearity, selectivity, lower limit of quantification (LLOQ), recovery and matrix effect. The method was found to be linear in the range of 15 to 600 ng/mL. Lower limit of quantification was found to be 3.6 ng/mL. All validation parameters met the acceptance criteria according to regulatory guidelines. This method was successfully applied to pharmacokinetic study of the prodrug in rabbit through oral administration.

Keywords: Sulphoxide prodrug, Famotidine, LCMS/MS method, Pharmacokinetic study.

INTRODUCTION

Sulphoxide prodrug of famotidine, chemically 3-([2-(diamino methyleneamino)thiazol-4-yl]methylsulfinyl)-N'-sulfamoylpropimidamide is a synthetic derivative of famotidine (Fig. 1). Famotidine has been indicated to treat peptic ulcer disease, hypersecretory syndromes and gastroesophageal reflux disease (GERD) [1]. Famotidine sulphoxide is an inactive prodrug of famotidine which shows better solubility compared to existing drug. In the literature, methods have been reported for the quantification of famotidine in biological fluids. Methods are reported for the quantification of famotidine from biological matrix by spectrofluorimetric method [2]. Methods are also reported for estimation of famotidine in low volume human plasma using liquid chromatography/tandem mass spectroscopy [3]. Other methods were reported with estimation of famotidine in plasma by LC ESI-MS [4], HPLC method [5-8], capillary electrophoresis [9]. As famotidine sulphoxide is a newly reported prodrug of famotidine so far no bio-analytical methods were developed to estimate famotidine sulphoxide. Hence the current research paper describes rapid and sensitive analytical method to determine famotidine sulphoxide concentrations in rabbit plasma. The plasma assay utilized protein precipitation for sample preparation. LC/MS/MS was employed to separate further and detect the analytes in the positive ionization mode using electro spray ionization (ESI) and monitoring their precursor-product ion combination in the multiple-reaction monitoring (MRM) mode. The method could be applied to pharmacokinetic study of the prodrugs.

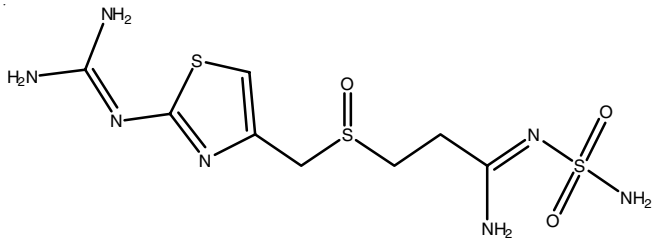

Fig. 1. Sulphoxide prodrug of famotidine

EXPERIMENTAL

Famotidine sulphoxide was synthesized and purified, clopidogrel (internal standard) was procured from Yarrow Chem laboratories, Mumbai. HPLC grade acetonitrile, methanol and water are purchased from Merck India Pvt. Ltd. All the other chemical used for the study were of analytical grade.

LCMS/MS conditions: Chromatographic separation was performed on a symmetry C_{18}, (50 × 4.6, 5 μ) column using Shimadzu LC system equipped with degasser, binary pump along with auto sampler. An isocratic mobile phase of acetonitrile:water:triethyl amine:orthophosporic acid (49.9: 49.9:0.1: 0.1 % v/v/v/v) was applied at a flow rate of 0.6 mL/ min. The column temperature was kept at 30 °C. The total analytical run time was 3 min for each sample. The eluent flow was led into the MS/MS starting 0.5 min after injection by switching the MS inlet valve.

The mass spectrometer was run in positive ion ESI-APCI combined mode equipped with turboion spray using multiple reaction monitoring (MRM) to monitor the mass transitions. The multiple reaction monitoring transitions were chosen to be *m/z* 336.2 → *m/z* 239.4 for famotidine, *m/z* 352.4 → *m/z* 274.0 for famotidine sulphoxide and *m/z* 322.1 → *m/z* 212 for clopidogrel (IS). Instrument parameters optimized were collision activated dissociation gas (CAD): 5 psi; curtain (CUR) gas: 15 psi; nebulizer gas (gas1): 65 psi; heater gas (gas 2): 40 psi; ion spray voltage: 4500 V; source temperature: 500 °C. Compound dependent parameters collision energy (CE) was set at 15 V for famotidine and famotidine sulphoxide and 20 V for IS. Declustering potential (DP), entrance potential (EP) and cell exit potential (CXP) for famotidine, famotidine sulphoxide and IS were kept at 50, 10 and 6 V, respectively. Quadrupole 1 and quadrupole 3 were maintained at unit resolution and dwell time was set at 100 ms. System control and data analysis were performed by AB Sciex Analyst soft-ware (version 1.5.2).

Synthesis of famotidine sulphoxide: One mole of famotidine and 2 equivalents of urea hydrogen peroxide adduct (equimolar quantities of urea and hydrogen peroxide) are transformed into the beaker, the temperature at 85 °C was maintained in electric water-bath for 30 min. During heating, for each 10 min TLC analysis was performed to check the completion of the reaction [10].

Animals: Albino rabbits (male and female) weighing 2.5 kg were used for oral bioavailability studies. All animal experiments were approved by Institutional Animal Ethical Committee (CPCSEA No: 930/PO/a/2006/CPCSEA). All the rabbits were fasted for 12 h before the experiments but had free access to water.

Preparation of standard stock solution of sulphoxide prodrug of famotidine (FM1): Accurately weighed 10 mg of sulphoxide prodrug of famotidine was transferred into a 10 mL volumetric flask and the volume was made up to the mark with mobile phase [acetonitrile:water:triethyl amine:orthophosporic acid (49.9:49.9:0.1:0.1 % v/v/v/v)] to give 1 mg/mL (1000 μg/mL) solution. From this stock solution, 10 mL of 100 μg/mL solution was prepared.

Preparation of standard stock solution of famotidine (FM): Accurately weighed 10 mg of famotidine was transferred into a 10 mL volumetric flask and the volume was made up to the mark with mobile phase [acetonitrile:water:triethyl amine:orthophosporic acid (49.9:49.9:0.1:0.1 % v/v/v/v)] to give 1 mg/mL (1000 μg/mL) solution. From this stock solution, 10 mL of 100 μg/mL solution was prepared.

Preparation of blank plasma: Blank plasma (0.5 mL) was transferred into 2 mL centrifuge tube and 0.1 mL of mobile phase and 0.3 mL of precipitating agent (10 % perchloric acid) were added. The resulting solution was vortexed for 5 min and centrifuged at 4000 rpm for 4 min. The supernatant layer was separated and analyzed.

Drug administration and plasma sample collection: In the pharmacokinetic study, 18 rabbits were randomly divided into three groups (n = 6 per group). According to the principle of parallelism, famotidine and famotidine sulphoxide were dissolved in 0.3 % w/v sodium carboxy methyl cellulose. The rabbits of the first group were treated with blank, the rabbits of the 2nd groups were orally administered with the dose of 2.8 mg (calculated based on human dose of 40 mg, conversion factor = 0.07) of famotidine, the rabbits of the third group were orally administered with the dose of 2.8 mg of famotidine sulphoxide. 0.5 mL of blood samples were collected by marginal ear vein puncture at 0, 1, 2, 4, 6, 7, 8, 9.5, 10, 10.5, 12, 18 and 24 h after dose administration. Blood samples were collected into eppendorf tubes containing 0.3 mL of anticoagulant (citrate) solution and centrifuged at 4000 rpm for 4 min. The plasma was separated and stored at -20 °C until further analysis.

Preparation of plasma samples: Plasma samples (0.5 mL) obtained from study subjects was transferred into 2 mL centrifuge tube and 0.3 mL of precipitating agent was added. The resulting solution was vortexed for 5 min and centrifuged at 4000 rpm for 4 min. The supernatant layer was then evaporated to dryness at 40 °C under nitrogen. The resulting extract was dissolved in 1 mL of mobile phase and vortex mixed for 3 min. After centrifugation at 4000 rpm for 10 min, an aliquot of 10 μL sample was then injected into the LC-MS.

Method validation: Linearity was analyzed by regression equation obtained by plotting peak area ratios of famotidine and famotidine sulphoxide to IS *versus* concentrations. The accuracy and precision were determined by analyzing plasma samples at three concentration levels on three validation run in three different days. Within-run and between-run precision was expressed as coefficient of variation (CV, %) and accuracy was estimated (expressed as coefficient of variation, CV (%)) for the same samples by comparing concentration measured with the nominal value. The assay accuracy was calculated as (observed concentration – spiked concentration)/(spiked concentration) × 100 %.

Sensitivity of the method was determined by limit of quantitation by analyzing six replicates of LLOQ that can be measured with acceptable accuracy and precision. Analyte stability was evaluated at two levels (LQC and HQC) in matrix and in sample extracts placed in auto sampler (8 °C). For room temperature stability, long-term stability (≤ 15 °C) and five freeze-thaw cycles stability test, the concentration of each analyte after storage was compared to its nominal concentration. To evaluate the matrix effect in the experiment, chromatographic peak areas of each analyte from the spike after extraction

samples, at low, medium and high concentration levels, were compared to those for the clean standard solutions at the same concentrations. Short term, post preparative and freeze thaw stability studies were performed [11-13].

Application to pharmacokinetic study: The LC-MS/MS procedure developed here was used to investigate the plasma profiles of famotidine and famotidine sulphoxide after oral administration. All the data were subsequently processed by Analyst 1.4.1 software and PK1 and PK2 kinetic software. The non-compartmental pharmacokinetic parameters of C_{max}, T_{max}, half-life ($T_{1/2}$), area under the plasma concentration time curve (AUC_{0-t} and $AUC_{0-\infty}$) and elimination rate constant (K_e), were calculated based on moment methods. Data were shown as mean ± standard deviation (mean ± SD).

RESULTS AND DISCUSSION

Linearity of calibration curves and lower limit of quantification (LLOQ): The standard calibration curve for spiked rabbit plasma containing famotidine and famotidine sulphoxide were linear over the range 15-600 ng/mL with a correlation coefficient $r^2 > 0.998$, 0.997, respectively. The results indicated that the calibration curve was linear, accurate and precise over the range of the method Figs. 2 and 3.

Sensitivity: The developed method was sensitive. The lowest reliable detection was set at the concentration of LOD and the lowest reliable quantification was set at the concentration of LLOQ for all the analytes (Table-1).

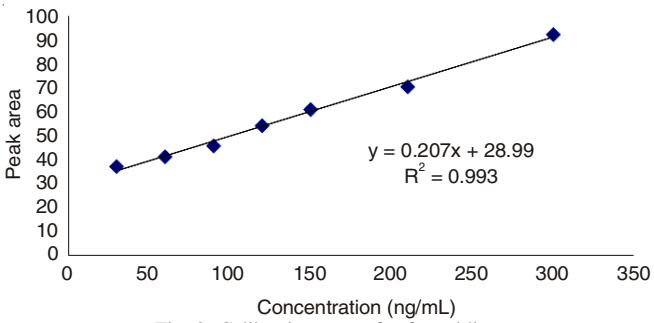

Fig. 2. Calibration curve for famotidine

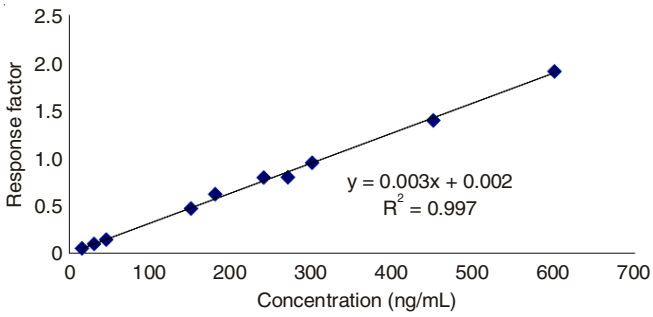

Fig. 3. Calibration curve for sulphoxide prodrug of famotidine

TABLE-1
LLOD AND LLOQ DATAS OF FAMOTIDINE AND SULPHOXIDE PRODRUG OF FAMOTIDINE

Analytes	LLOD (ng/mL)	LLOQ (ng/mL)
Famotidine	1.0	3.0
Sulphoxide prodrug of famotidine	1.2	3.6

Assay precision and accuracy: The intra-day and inter-day precision and accuracy were shown in Tables 2 and 3. The accuracy and precision of within-run and between-run should be within 15 % of the nominal values, except for the LLOQ which should be within 20 % of the nominal value. This indicated that the method was accurate and precise over the range of the assay (Figs. 4-6).

Analyte stability: The freeze-thaw stability was determined after three freeze-thaw cycles at -20 °C with a minimal interval of 24 h. The short-term stability was assessed after keeping the samples at ambient condition for 2 h. Post-preparative stability was evaluated by analyzing samples kept in the auto sampler at ambient condition for 12 h. The results of the stability study are presented in Table-4, which confirm the

TABLE-2
PRECISION STUDIES OF FAMOTIDINE AND SULPHOXIDE PRODRUG

Drugs	Concentration (ng/mL)	Intra-day precision			Inter-day precision		
		Mean[a]	SD	C.V. (%)	Mean[a]	SD	C.V. (%)
Famotidine	15	14.98	0.12	0.80	13.99	0.15	0.93
	180	178.62	0.81	0.45	179.52	0.24	0.13
	600	597.85	0.94	0.16	598.43	1.45	0.24
Sulphoxide prodrug of famotidine	15	14.12	0.14	0.99	14.57	0.11	0.75
	180	179.25	0.74	0.41	177.94	1.54	0.86
	600	594.24	1.24	0.21	597.24	0.36	0.06

[a]Mean of six readings n = 6

TABLE-3
ACCURACY STUDIES OF FAMOTIDINE AND SULPHOXIDE PRODRUG

Drug	Concentration (ng/mL)		Measured concentration (ng/mL)	Nominal (%)	Mean[a]	SD	C.V. (%)
	Actual	Added					
Famotidine	15	20	34.84	99.54	99.54	0.23	1.2
	180	20	198.47	99.23			1.5
	600	20	617.62	99.61			1.0
Sulphoxide prodrug	15	20	34.97	99.91	99.49	0.54	1.5
	180	20	197.42	98.71			1.5
	600	20	619.15	99.86			2.0

[a]Mean of three readings n = 3

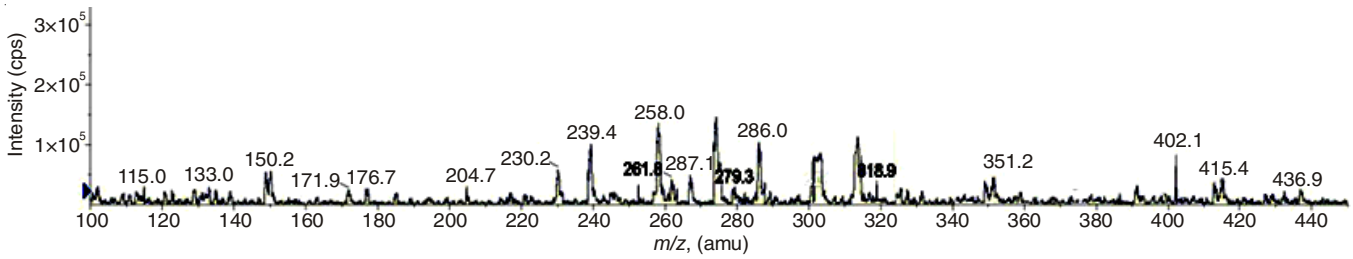

Fig. 4. LC-MS-MS chromatogram of blank plasma

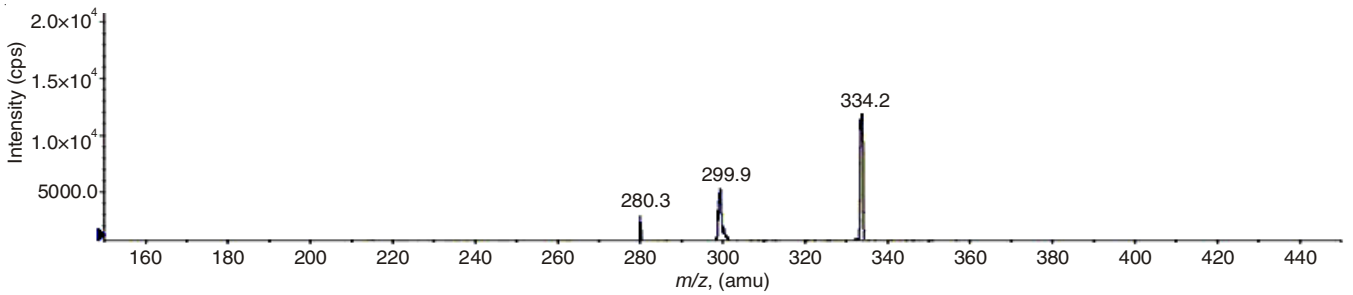

Fig. 5. LC-MS-MS chromatogram of famotidine

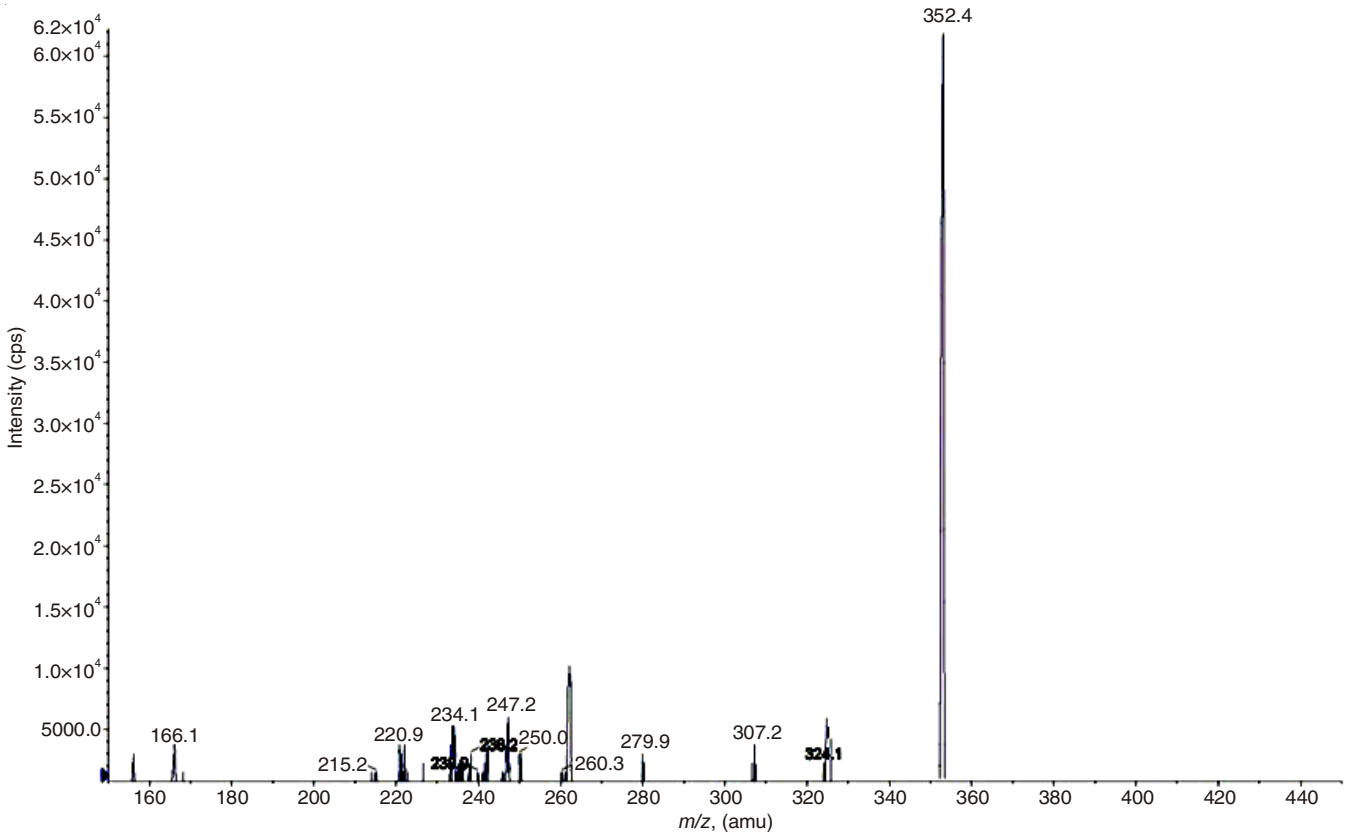

Fig. 6. LC-MS-MS chromatogram of sulphoxide prodrug of famotidine

high stability of famotidine and famotidine sulphoxide throughout the determination.

Application of the analytical method to pharmacokinetic studies: The LC-MS/MS method was successfully applied to the determination of famotidine and famotidne sulphoxide plasma concentration levels in rabbits following oral administration of a single dose. In the study we compared the pharmacokinetic parameters of rabbits which were administered famotidine and famotidne sulphoxide. The main pharmacokinetic parameters calculated using non-compartmental analysis was shown in Table-5. Mean plasma concentration-time curves (n = 6) were presented in Figs. 7 and 8. Significant differences of T_{max}, C_{max}, $AUC_{(0-t)}$, $AUC_{(0-\infty)}$ and K_e were observed between famotidine and famotidne sulphoxide treated groups after administration of equal dose.

Conclusion

The developed LC-MS/MS method for famotidine and its prodrug famotidine sulphoxide is selective, rapid and suitable for routine analysis of subject samples. This method has signi-

TABLE-4
STABILITY OF FAMOTIDINE AND FAMOTIDINE S OXIDE UNDER A VARIETY OF STORAGE CONDITIONS

Conditions	Famotidine Accuracy (average ± SD; ng/mL)			Famotidine S oxide Accuracy (average ± SD; ng/mL)		
	15	180	600	15	180	600
Short-term stability (at ambient condition for 2 h)	14.87±0.11	179.91±0.23	599.98±0.14	14.91±0.16	179.86±0.19	599.14±0.10
Post preparative stability (in the auto sampler at ambient condition for 12 h)	14.92±0.13	179.82±0.18	599.88±0.14	15.04±0.11	179.96±0.15	601.04±0.10
Freeze-thaw stability (three cycles)	15.08±0.21	179.88±0.17	599.88±0.14	14.92±0.13	179.76±0.11	599.84±0.10

TABLE-5
PHARMACOKINETIC PARAMETERS OF FAMOTIDINE AND FAMOTIDINE S OXIDE

Parameters	Famotidine	Famotidine S oxide
C_{max} (µg/mL)	137.0567±14.61608	492.2167±14.5596
T_{max} (h)	3	2.5
AUC_{0-t}	788.0063±30.6626	1945.006±162.819
T1/2 (h)	2.458951±0.09562	2.4458±0.2272
K_e	0.28225±0.01121	0.2854±0.02712
$AUC_{0-\infty}$	789.372±30.634	1950.291±163.645

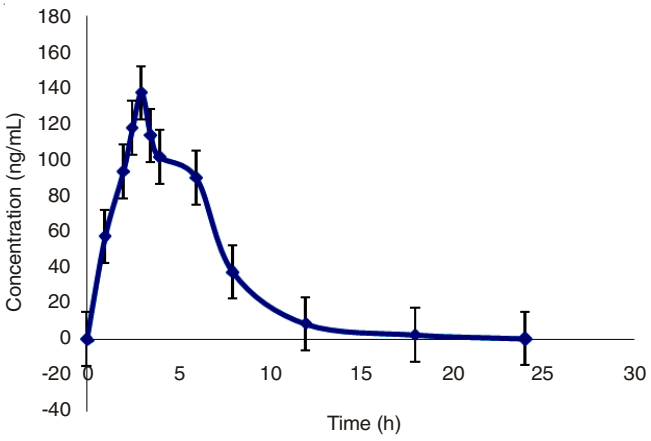

Fig. 7. Mean plasma concentration *vs.* time curve for famotidine

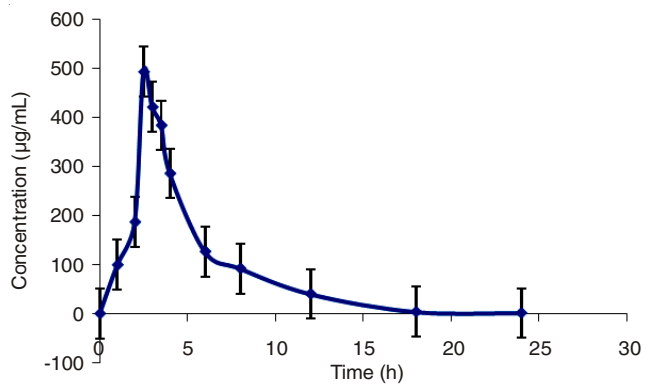

Fig. 8. Mean plasma concentration *vs.* time graph for sulphoxide prodrug of famotidine

ficant advantages in terms of simple one step protein precipitation procedure and a shorter chromatographic run time (3 min). The validation was performed according to regulatory guidelines. In addition, to our best of knowledge, this is the first validated LC-MS/MS method for estimation of famotidine sulphoxide in rabbit plasma. The established LLOQ is sufficient to conduct a pharmacokinetic study for the famotidine sulphoxide. Increment in C_{max} value of sulphoxide prodrug of famotidine was 3.59 folds compared to that of famotidine which revealed the possibility of enhancement in bio-availability of synthesized prodrugs. This increase may be due to enhancement in solubility of famotidine sulphoxide. The results suggest synthesized prodrug is better than existing drug in terms of solubility and bio-availability.

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