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DEVELOPMENT AND VALIDATION OF BIOANALYTICAL METHOD FOR THE PHARMACOKINETIC STUDY OF FAMOTIDINE SNEDDS IN RATS USING RP-HPLC

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ABSTRACT: A selective and high throughput RP-HPLC method was developed to detect and quantify Famotidine in rat plasma using Ranitidine hydrochloride as an internal standard (IS). After liquid-liquid extraction, the analyte and the internal standard were chromatographed on Enable 18H (5µ, 250×4.6mm) C18 column using 20 µL injection volume with a run time of 15 minutes. An isocratic mobile phase consisting of phosphate buffer pH 6.8 and methanol (50:50 %v/v) was used to separate drug and internal standard. The method was validated according to USFDA guidelines over the range of 50-1200 ng/mL. The absolute recovery for analyte achieved from spiked plasma sample was consistent and reproducible. Precision and accuracy (%CV) across four validation runs (LLOQ, LQC, MQC and HQC) was less than 15. LLOQ was found to be 50 ng/mL. The validated RP-HPLC method was used successfully to study Famotidine SNEDDS pharmacokinetics in rats and also quantitative determination in Solid Self Nano Emulsifying Drug Delivery System (SNEDDS).

INTRODUCTION: Peptic ulcers take place in that portion of the gastrointestinal tract (G.I.T.) which is exposed to gastric acid and pepsin i.e. the stomach and duodenum. Various agents with different mechanisms are used to treat or ameliorate peptic ulcers or irritation of the gastrointestinal tract. Histamine H_2 Antagonists are the first class of highly effective drugs for acid-peptic diseases [1]. Famotidine belong to the class of H_2Antagonist and is described chemically as 3-[[2-(diaminomethyli-deneamino)-1, 3-thiazol- 4-yl] methylsulfanyl]- N'-sulfomylpropanimidamide with molecular mass of 337.435 g/mol. Its chemical formula is $C_8H_{15}N_7O_2S_3$, and it belongs to antiulcer category.

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40mg is the normal dose of the drug, and it is used in the treatment of gastric ulcers. It is white to pale yellow crystals freely soluble in glacial acetic acid, slightly soluble in methanol, very slightly soluble in water, and practically insoluble in ethanol, and solubility in water is 1000 mg/L (at 20 °C). It has a melting point of 163.5 °C and can be stored at room temperature and away from excess heat and moisture. Famotidine is absorbed incompletely. Bioavailability of oral doses is 40 to 45% [2].

The development of a sound bio-analytical method is of paramount importance during the process of drug discovery and development, culminating in a marketing approval. A bioanalytical method consists of two main components-Sample preparation (which is a technique used to clean up a sample before analysis) and/or to concentrate a sample to improve its detection [3]. The Self Nano Emulsifying Drug Delivery System (SNEDDS) is a Novel Drug Delivery System for the enhancement of water solubility of poorly water-soluble drugs.

It is isotropic mixture of oil, surfactant, co-surfactant molecules and it also contains a co-solvent molecule. This Drug delivery system is thermodynamically and kinetically stable. Famotidine is a BCS Class IV drug that exhibits poor solubility and permeability. Hence, it was selected for preparing the Self Nano Emulsifying Drug Delivery System (SNEDDS).

A wide-ranging literature survey was conducted to find out the bioanalytical methods for Famotidine. The literature reveals that many HPLC, UV and LC-MS but few bioanalytical methods have been developed for the estimation of Famotidine [4-20].

Thus the objective of the study is to develop a new RP-HPLC method for estimation of Famotidine in rat plasma and to validate the developed method as per USFDA guidelines and its application for the pharmacokinetic studies of Famotidine SNEDDS in rats. The structure of Famotidine is shown in **Fig. 1**.

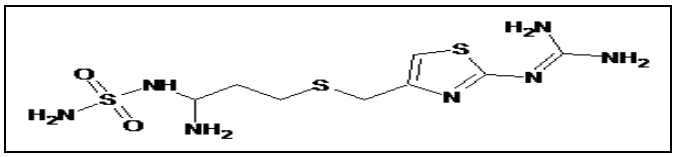

FIG. 1: STRUCTURE OF FAMOTIDINE

MATERIALS AND METHODS:

Materials: The sample Famotidine was obtained as a gift sample from Suven Life Sciences, and internal standard Ranitidine was obtained as a gift sample from Dr. Reddy's Laboratories, Hyderabad. Methanol and Potassium Dihydrogen orthophosphate were of HPLC Grade and were procured from SDFCL. HPLC grade water was used for the preparation of solutions. Oleic acid, tween 20, transcutol P and Neusilin US2 were of LR grade and were used for the preparation of SNEDDS. A gradient high-performance liquid Chromatography (SHIMADZU, HPLC), HPLC pump LC-20AT, with LC solutions software, UV-visible detector SPD-20A and Enable-18H C18column (250 × 4.6 mm, 5 µm) was used for Chromatographic analysis.

Experimental Animals: Male Albino Wistar rats (180-200 g) were obtained from National Institute of Nutrition, Hyderabad. Animals were maintained in standard cages under controlled laboratory conditions. All animals had free access to food (National Institute of Nutrition, Hyderabad) and water *ad libitium* during the quarantine period. The Institute of Animal Ethics Committee at G. Pulla Reddy College of Pharmacy, Hyderabad, India, has approved the experimental animal protocol. (Registration No: 320/CPCSEA; Date 03/01/2001, Date: 23/02/2019; GPRCP/IAEC/23/19/02/PCA/ AE-1-RATS-F/M-18.

Methods:

Preparation of Standard Stock Solution and Internal Standard Stock Solution: Appropriately, 10.0 mg of famotidine was weighed into a 10 mL graduated tube, volume was made up to the mark with HPLC Water. This gives 1 mg/mL or 1000 µg/mL solution. 1 mL of 1 mg/mL solution was transferred into 10 mL graduated tube, and the volume was made up to the mark with HPLC Water. This gives 100 µg/mL solution. 0.1 mL of 100 µg/mL was transferred into 10 mL, graduated tube and the volume was made up to the mark with HPLC Water. This gives 1 µg/mL solution.

Ranitidine solution of 1mg/mL concentration was prepared by taking 10 mg and dissolving it in HPLC Water and further making up to 10 mL using HPLC Water; further dilutions were made to make 1µg/mL of internal standard stock solution.

Chromatographic Conditions: C18 (250 mm x 4.6 mm), 5 µ column was used for analysis, mobile phase consisted of Phosphate buffer pH 6.8: Methanol (50:50) and flowrate was optimized at 0.8ml/min with runtime for 15 min. The volume of the injection loop was 20µL, and the detection wavelength at 267nm.

Preparation of Mobile Phase: Mobile phase consists of methanol and 0.05 M phosphate buffer (50:50). The pH of phosphate buffer was maintained to 6.8. The mobile phase was filtered through 0.2 µm cellulose acetate filters & degassed in sonicator prior to use.

Preparation of 0.05M Phosphate Buffer pH 6.8: 2 gm of potassium dihydrogen orthophosphate was dissolved in 300 mL of HPLC water to produce 0.05 M Solution. The pH of this solution was adjusted to 6.8 with sodium hydroxide solution. Samples of pooled plasma were used as both blank and for spiking throughout the validation studies.

Blank Plasma and Standard Preparation: The organic layer was separated from processed blank plasma and after filtration, it was directly injected into column without spiking with either sample or internal standard. Standard solutions of Famotidine 1 µg/mL and 10 µg/mL internal standard solutions were injected into the column and the chromatograms were recorded. A blank plasma chromatogram was also recorded.

Analytical Method Validation: All analytical procedures were validated according to USFDA guidelines 'Bioanalytical Method Validation'. Following parameters were performed for method validation

Selectivity and Sensitivity (LLOQ): Selectivity was performed to determine that no peak of impurity or any other substance is eluted, nearer to the retention time of Famotidine and Ranitidine. Sensitivity is the lower limit of quantification (LLOQ). The lowest concentration of the calibration curve where the analyte response was more than five times the blank response was selected as LLOQ.

System Suitability: System suitability was performed to measure that this specific method could generate results of acceptable accuracy and precision. Injections of Standard solutions of 1 µg/mL drug and 10 µg/mL internal standard were given for five times, and chromatograms were observed.

Linearity:

Preparation of Quality Control (QC) Standards in Plasma: Calibration samples were prepared by spiking with appropriate amounts of sample & internal standard into 80 µL of control plasma. From 1 mg/mL stock solution of drug 2.5, 5, 10, 20, 30, 40, 50, 60 µL samples were taken, and volume was made upto 5 mL with HPLC Water. From 1 mg/mL stock solution of the internal standard 50 µL of the sample was taken, and volume was made upto 5 mL with HPLC Water. From each concentration 10 µL of sample and 10 µl of internal standard (1µg/mL) were taken and spiked into 80 µL control plasma to get 50, 100, 200,400, 600, 800, 1000, 1200 ng/mL as shown in **Table 1**.

TABLE 1: CALIBRATION CURVE STANDARDS IN RAT PLASMA

S. no.		Volume taken from standard stock solution-I(µL)	Spiking volume (µL)	Volume of control plasma taken (µL)	Final volume (mL)	Final concentration after spiking (ng/mL)
1	Drug (Famotidine)	2.5	10	80	5	50
		5				100
		10				200
		20				400
		30				600
		40				800
		50				1000
		60				1200
2	Internal standard (Ranitidine)	50	10	80	5	1000

Precision: Samples were prepared by spiking with appropriate amounts of sample & internal standard into 80 µL of control plasma. From 1 mg/mL stock solution 3.75, 25, and 55 µL samples were taken and volume was made up to 5 mL with HPLC Water. From 1 mg/mL stock solution of the internal standard 250 µL of the sample was taken and volume was made up to 5 mL with HPLC Water. From each concentration 10 µL of sample and 10 µL of internal standard (1 µg/mL) were taken and spiked into 80 µL control plasma to get 75, 500 and 1100 ng/mL.

75 ng/mL- Lower Quality Control (LQC), 500 ng/mL –Middle Quality Control (MQC) and 1100 ng/mL- High Quality Control (HQC).

Variations of results within the same day (intraday) and variation of results between days (inter-day) were analyzed.

Accuracy: Samples were prepared by spiking with appropriate amounts of sample & internal standard into 80 µL of control plasma. From 1 mg/mL stock solution 3.75, 25, and 55 µL samples were taken

and volume was made up to 5 mL with HPLC Water. From 1 mg/mL stock solution of internal standard 250 uL of the sample was taken, and volume was made up to 5 mL with HPLC water. From each concentration 10 µL of sample and 10 µL of internal standard (1 µg/mL) were taken and spiked into 80 µL control plasma to get 75, 500, and 1100 ng/mL.

75 ng/mL- LQC, 500 ng/mL –MQC, 1100 ng/mL-HQC.

The three standard solutions, each five times were injected into the HPLC system and %CV was calculated.

Recovery Studies: Recovery was determined at LQC (75 ng/mL), MQC (500 ng/mL) and HQC (1100 ng/mL) levels. Concentrations were prepared by, spiking 3.75, 25 and 55µL of Famotidine from 1 mg/mL standard solution to get 75 ng/mL, 500 ng/mL and 1100 ng/mL respectively and 10µL of 5µg/mL internal standard solution to 80µL of control plasma and 10µL of 1µg/mL internal standard solution. Extraction of sample from plasma was done by liquid extraction technique using acetonitrile, and further dilutions to the desired extent were made with the same solvent.

Stability Studies (Plasma Samples): Three Types of Stability tests were conducted for prepared and stored plasma samples.

Freeze and Thaw Stability: Drug stability was determined after three freeze-thaw cycles. Three aliquots of each of higher (1200 ng) and lower concentrations (50 ng) were frozen for 24 h and then thawed unassisted at room temperature. When thawed completely, the same samples were refrozen for 24 h under the same conditions as before. Similarly, the freeze and thaw cycles were repeated twice more and then analyzed on the third cycle.

Short-Term Temperature Stability: Three aliquots of each of the low (50 ng) and high concentrations (1200 ng) were thawed at room temperature for 24 h and then analyzed.

Stock Solution Stability: The stability of stock solutions of both drug and internal standards were evaluated at room temperature for 6 h.

Formulation Details: Solid-self nano emulsifying drug delivery system (SNEDDS) of Famotidine will be prepared by first formulating Liquid SNEDDS and converting it into Solid SNEDDS and the formulation details are shown in **Table 2**.

TABLE 2: FORMULA FOR PREPARATION OF SOLID-SELF NANO EMULSIFYING DRUG DELIVERY SYSTEM OF FAMOTIDINE

S. no.	Excipient	Density	Volume	Mass
1	Oleic acid	0.895gm/ml	2ml	1.79gm
2	Tween 20	1.105gm/ml	6ml	6.630gm
3	Transcutol P	0.988gm/ml	2ml	1.976gm
4	Famotidine	100mg (0.1gm)		
5	Total (10ml of L-SNEDDS)	10.49gm		
6	Total (4ml of L-SNEDDS)	4.19gm		

Preparation of SNEDDS: About 100mg of pure famotidine was mixed with the co-surfactant transcutol-p in a beaker and heated upto 60 ºC. About 2ml of oleic acid is added to the beaker, and this was placed on a magnetic stirrer. Stirring was done at about 500rpm till the drug has dissolved completely. It was allowed to cool at room temperature. About 6ml of the surfactant Tween 20 was added dropwise to the cooled mixture with continuous stirring on a magnetic stirrer till a uniform Liquid SNEDDS is formed. This was converted into its Solid SNEDDS form by adding about 180mg of Neusilin US2 to about 0.4ml of Liquid SNEDDS.

***In-vivo* Studies - Pharmacokinetic Evaluation of Famotidine SNEDDS in Rats by Using RP-HPLC Method:** *In-vivo* studies were carried out in rats for the prepared famotidine SNEDDS. The developed RP-HPLC method was used for the analysis of rat plasma samples for the determination of concentrations of Famotidine.

Animals Used: Eighteen (18) male Wistar rats of 2- 3 months old, each Weighing 180 to 200 gms, were included in the study. The animals are divided into six groups (3 rats/group). The rats were caged 3 animals per cage and were labelled as subgroups T1 to T3 (for test groups of rats) and S1 to S3 (for

standard groups of rats) and acclimated overnight before dosing. The animals were assigned to each group randomly and the tails were marked with a permanent marker with an identification number. They were maintained on a 12-hour light/12-hour dark cycle. The room temperature was 65-72 °F. Animals were provided a standard diet and potable water during acclimatization. Animals were fasted overnight, and food was provided at 3 h after dosing. Study Design was planned for conducting the pharmacokinetic studies shown in **Table 3**.

TABLE 3: STUDY DESIGN

Group no.	Test Article	Route	No. of rats/Sex	Dose Volume (mL/185g)	Time Points (hr)
T_1	Prepared Famotidine SNEDDS	Oral	3/M	1	0, 1, 2.5, 6
T_2	Prepared Famotidine SNEDDS	Oral	3/M	1	0.25, 1.5, 3, 8
T_3	Prepared Famotidine SNEDDS	Oral	3/M	1	0.5, 2, 4. 24
S_1	Standard Famotidine	Oral	3/M	1	0, 1, 2.5, 6
S_2	Standard Famotidine	Oral	3/M	1	0.25, 1.5, 3, 8
S_3	Standard Famotidine	Oral	3/M	1	0.5, 2, 4. 24

Samples from both standard and test groups were collected until complete six hours for every half an hour in the first three hours. After three hours of study, all the rats were fed with food and water, and then the next three hours one-hour sampling was continued. All samples were processed and stored at -20 °C until analysis.

Samples were collected at zero time point from all the groups of test and standard is the control or blank sample.

Rat Dose Calculations:

Average Human adult weight: 50 kg
Adult dose of Famotidine: 40 mg

This adult dose can be converted to mg/kg by taking the ratio of the adult dose of the drug with average human adult weight. Therefore,

Adult dose in mg/kg = 40/50 = 0.8 mg/kg

Conversion of equivalent adult famotidine dose to rat dose was done using formula shown in **Table 4**.

TABLE 4: RAT DOSE CALCULATION

Drug	Adult dose mg/kg	Conversion factor- human to rat	Rat dose mg/kg	Rat dose µg/185g	Dose vol. Suspension µg/mL
Famotidine	0.8	6 (0.8 × 6)	4.8	880	880

Preparation of Rat Dose Formulation in 0.5% SCMC Suspension: Each formulation containing 40,000 µg of the drug was crushed and powder was transferred to a mortar, and 45ml of 0.5% SCMC suspension was added slowly with trituration to make a homogeneous suspension. This gave 880 µg/mL suspension. 1 mL of the above solution was orally fed to every rat in the test group with an oral feeding injection.

Preparation of Standard Drug Suspension: 40 mg of pure Famotidine was accurately weighed and dissolved in 45 mL of 0.5% CMC solution, agitated, sonicated, and then properly closed with an aluminium foil until administration to rats. 1ml of the above solution was orally fed to every rat in the Standard group with an oral feeding injection.

Plasma Sample Preparation: The blood samples were collected from retro-orbital puncture into disodium EDTA vials (20 mg disodium EDTA in 1 mL water, 1 mL of blood requires 50 µL of disodium EDTA). Plasma was separated from blood samples by centrifugation at 1400 rpm for 7 minutes. 100 µL of acetonitrile was added to 100 µL plasma sample. The mixture was vortexed for 20 min followed by centrifugation at 3000 rpm for 10 min at 4 °C. The organic layer was separated & filtered through a 0.2 µm cellulose acetate filter. The samples were stored at -20 °C until analysis.

Extraction from Plasma: The literature search revealed liquid-liquid extraction procedures for famotidine from rat plasma as it would facilitate analysis of famotidine in rat plasma in a large number of samples over a relatively short period of time and in a cost-effective manner.

Extraction Procedure: A pool of blank rat plasma was obtained and 90 mL of plasma sample in was

spiked with 10µl internal standard solution (1µg/mL) and 100 µL of methanol was added. The mixture was vortexed for 1 min and centrifuged at 3000 rpm for 10 minutes at 4 °C. The organic layer was separated & filtered through a 0.2 µm cellulose acetate filter. Later the organic layer was evaporated on a Savant vacuum evaporator at about 60 °C. The residue was reconstituted in 5 mL HPLC Water, and 20 µL was injected onto the HPLC column.

Pharmacokinetic Parameters: Different pharmacokinetic parameters like rate constant, biological half-life, C_{max}, T_{max}, the volume of distribution, AUC^{t}_{0}, AUC^{∞}_{t} were determined using the software PKPlus 2.0.

Calculation of Pharmacokinetic Parameters: The study was carried out for twenty-four hours. All the twelve processed samples were injected onto the HPLC column. Peak areas for these samples were obtained. These peak areas were used to calculate concentrations from the calibration curve

RESULTS AND DISCUSSION: A new RP-HPLC method was developed for estimation of famotidine in rat plasma and application of the method to pharmacokinetic studies using a gradient high-performance liquid Chromatography (SHIMADZU, HPLC), HPLC pump LC-20AT, with LC solutions software, UV-visible detector SPD-20A and Enable-18H C18 column (250 × 4.6 mm, 5 µm).

Method Development and Optimization: For optimum detection and quantification of famotidine and internal standard in rat plasma, it was necessary to develop an extraction method, which gives consistent and reproducible recovery of the analyte from plasma. The drug was extracted from plasma by solvent extraction technique using methanol. The high-performance liquid chromatography method was initiated under isocratic conditions to develop a separation process with short run time. Separation was tried using various combinations of phosphate buffer and methanol on C18column.

After completion of several trials with the same column and different mobile phases in varying compositions, chromatograms with good peak shape and retention time were obtained. The optimised conditions are shown in **Table 5** and standard chromatogram is shown in **Fig. 2**.

TABLE 5: OPTIMIZED RP-HPLC CONDITIONS

Parameters	Conditions
Stationary phase (column)	C18 (25 cm x 4.6 mm, 5 µ)
Mobile phase	Phosphate buffer pH6.8:
Flow rate	Methanol (50:50)
Run time	0.8 mL/min
Column temperature	15 min
Volume of injection loop	Ambient
Detection wavelength	20 µL
Drug retention time	267 nm
Internal standard retention	5.03 min
time	11.3min

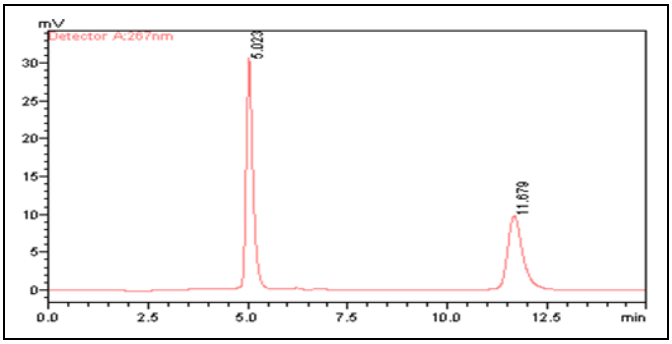

FIG. 2: CHROMATOGRAM FOR OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Selectivity and Sensitivity (LLOQ): There was no interference of peaks obtained in blank plasma, at the retention times of analyte peak and internal standard peak. The chromatograms of blank plasma, drug and internal standard demonstrated the selectivity results.

Sensitivity (LLOQ): LLOQ is the least concentration in the calibration curve, which is 50 ng/mL

Linearity Data and Chromatograms in Plasma: The working standard solutions of famotidine in plasma were prepared for checking the linearity in the concentration range of 50-1200ng/mL. Linear regression analysis was performed to check the linearity of the data obtained. The calibration curve data are shown in **Table 6** and the calibration curve is shown in **Fig. 3** and the overlay spectrum is shown in **Fig. 4**.

The response of the drug was found to be linear in the concentration range 50 ng-1200 ng. The linear regression equation for Famotidine was $y = 0.0016x + 0.1965$ and $r^2 = 0.9993$.

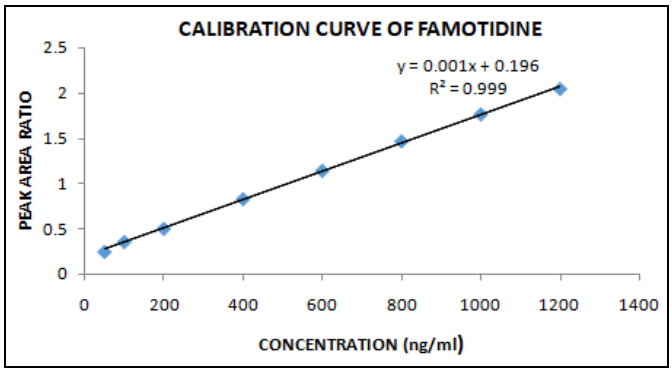

FIG. 3: CALIBRATION CURVE OF FAMOTIDINE IN PLASMA

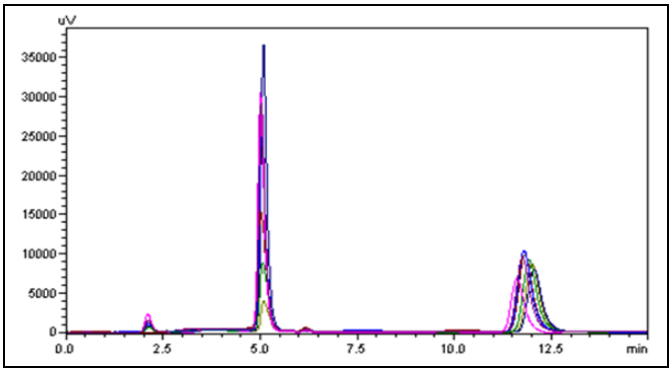

FIG. 4: LINEARITY-OVERLAY SPECTRUM OF FAMOTIDINE AND RANITIDINE (INTERNAL STANDARD)

TABLE 6: CALIBRATION CURVE DATA

S. no.	Concentration (ng/mL)	Peak area ratio[a]
1	50	0.25
2	100	0.36
3	200	0.50
4	400	0.83
5	600	1.14
6	800	1.46
7	1000	1.76
6	1200	2.04

[a] Average of three determination

Precision: Precision of the data was reported in terms of Repeatability, Intra-day, and Inter-day precision **Table 7**, **Table 8** and **Table 9,** respectively. The calculated % CV values were less than 15% which is very low, indicating that the method was precise. The overlay spectrum of precision studies is shown in **Fig. 5**.

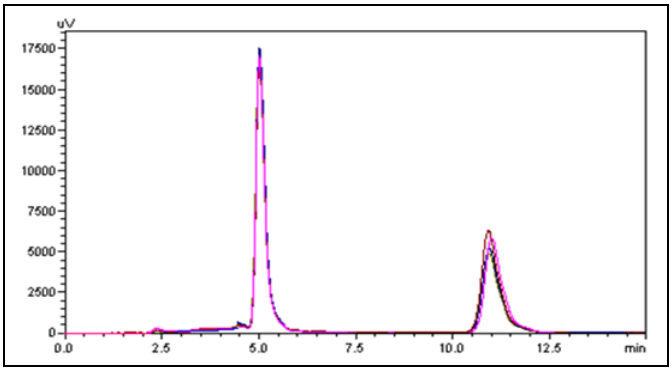

FIG. 5: PRECISION-OVERLAY SPECTRUM OF FAMOTIDINE AND RANITIDINE (INTERNAL STANDARD

TABLE 7: REPEATABILITY DATA

S. no.	Concentration (ng/mL)	Parameter		
		Peak area ratio	Mean[a]± Standard Deviation	%CV
1	600	1.201		
2	600	1.146		
3	600	1.279		
4	600	1.186	1.22666±0.05884	4.7937
5	600	1.299		
6	600	1.249		

[a] Average of six determinations

TABLE 8: INTRA-DAY PRECISION DATA

S. no.	Concentration (ng/mL)	Mean peak area ratio[a]		Mean[b]±Standard deviation	%CV
		Morning	Afternoon		
1	LLOQ (50)	0.280	0.252	0.266±0.019799	7.443
2	LQC (75)	0.336	0.306	0.321±0.021213	6.608
3	MQC (500)	0.925	0.880	0.9025±0.03182	3.525
4	HQC (1100)	1.865	1.789	1.827±0.05374	2.9414

[a, b] Average of five determinations

TABLE 9: INTER-DAY PRECISION DATA

S. no.	Concentration (ng/mL)	Mean peak area ratio[a]		Mean[b]±Standard deviation	%CV
		Day 1	Day 2		
1	LLOQ (50)	0.288	0.242	0.2651±0.0325	2.27
2	LQC (75)	0.356	0.315	0.3355±0.028991	8.64
3	MQC (500)	0.929	0.850	0.8895±0.056215	6.31
4	HQC (1100)	1.834	1.717	1.7755±0.82731	4.65

[a, b] Average of five determinations

Accuracy: The accuracy of the method was determined at four different concentration levels. Mean and %CV values were calculated in **Table 10**. As the %CV values were found to be less than 15% which are within the acceptable limits, the results indicate that the method was accurate.

TABLE 10: ACCURACY DATA

S. no.	Accuracy level	Concentration (ng/mL)	Mean Peak area ratio[a]	Mean Concentration From standard curve[b] (µg/mL)	Standard deviation	%CV
1	LLOQ	50	0.286	55.9375	4.9184	7.9267
2	LQC	75	0.302	69.0625	4.2002	5.831
3	MQC	500	0.939	464.0625	25.4118	5.2717
4	HQC	1100	1.88	1052	33.9411	3.1543

[a, b] Average of five determinations

Recovery Studies (Drug Product): It represents the extraction efficiency of a method. It was performed at LQC, MQC, and HQC. Six replicates at LQC, MQC and HQC levels were prepared for recording determination. The mean relative recovery found was 94.916. The data for relative recovery is given in **Table 11**. As the data was found to be within limits of 85% to 115%, the results indicate that extraction efficiency for the analyte was consistent and reproducible.

TABLE 11: RECOVERY DATA

S. no.	Concentration (ng/mL)	A		B		Relative recovery (%C)
		Peak area ratio[a]	Concentration[b]	Peak area ratio[a]	Concentration[b]	
1	LQC (75)	0.3125	72.5	0.3065	68.75	94.82
2	MQC (500)	0.9421	485.68	0.9208	452.68	93.20
3	HQC (1100)	1.912	1072.18	1.856	1037.18	96.73

[a, b] Average of five determinations

A: sample prepared by spiking externally in blank plasma; B: extracted samples; C: (B/A) ×100

Stability Studies (Plasma Samples): After completion of the storage times for different types of stabilities, the sample was tested by comparing the instrument response with that of freshly prepared solution. The freeze and thaw stability, short temperature stability, and stock solution stability is shown in **Table 12, Table 13** and **Table 14,** respectively.

After the completion of the storage times, plasma samples were found to stable as there was not much difference in the retention time and peak area.

TABLE 12: FREEZE AND THAW STABILITY DATA

S. no.	Concentration (ng/mL)	Time (hr)	Mean peak area ratio[a]	Mean Assay[b]±Standard deviation	%CV
1	LLOQ (50)	0	0.2680	42.562±5.3033	11.46
		72	0.2612		
2	HQC (1200)	0	1.995	1081.87±83.53	7.32
		72	1.860		

[a, b] Average of five determinations

TABLE 13: SHORT TERM TEMPERATURE STABILITY DATA

S. no.	Concentration (ng/mL)	Time (hr)	Mean peak area ratio[a]	Mean Assay[b]±Standard deviation	%CV
1	LLOQ (50)	0	0.2690	44.15±4.136	8.78
		24	0.2653		
2	HQC (1200)	0	1.999	1104.375±67.223	5.83
		24	1.926		

[a, b] Average of five determinations

TABLE 14: STOCK SOLUTION STABILITY DATA

S. no.	Concentration (ng/mL)	Time (hr)	Mean peak area ratio[a]	Mean Assay[b]±Standard deviation	%CV
1	LLOQ (50)	0	0.2586	47.812±3.1635	3.23
		6	0.2509		
2	HQC (1200)	0	2.03	1167.969±45.299	3.87
		6	1.998		

[a, b] Average of five determinations

Pharmacokinetic Detail: The developed method was applied to evaluate the pharmacokinetics of prepared solid self nano emulsifying drug delivery system of famotidine. In this study, rats received a single oral dose based on body weight via gavage, and blood samples were collected from retro-orbital plexus in a non-serial manner at various time points. The samples were analyzed by the developed RP-HPLC method. All pharmacokinetic parameters were derived from the mean plasma concentration-time data of famotidine. Pharmacokinetic parameters were estimated using a non-compartmental method of PKPlus 2.0.

Pharmacokinetic Data Analysis: Mean plasma concentration-time profile of the drug evaluated in this study exhibited both linear and non-linear patterns. The concentration-time data were pooled from different animals at each time point and mean plasma concentration versus time data was analyzed by a non-compartmental method to estimate the following pharmacokinetic parameters: rate constant (k), biological half-life, C_{max}, T_{max}, AUC^{t}_{0}, AUC^{∞}_{t}.

The mean concentration-time curve after oral dose of standard Famotidine suspension and Famotidine SNEDDS in rats **Fig. 6**. It was observed that at all time points the concentrations of famotidine in plasma were remarkably higher in rats fed with famotidine SNEDDS than with the standard famotidine suspension. Peak plasma concentration (C_{max}) for standard famotidine suspension was found to be 1.279ug/mL and AUC $_{0-\infty}$ was about 6.41 ug.hr/mL.

Peak plasma concentration (C_{max}) of famotidine SNEDDS was found to be 3.517ug/mL and AUC $_{0-\infty}$ was about 11.73 ug.hr/mL. The Concentration of standard famotidine and prepared famotidine SNEDDS in plasma at different time points are shown in **Table 15** and their pharmacokinetic parameters are shown in **Table 16**.

TABLE 15: CONCENTRATION OF STANDARD FAMOTIDINE AND PREPARED FAMOTIDINE SNEDDS IN PLASMA AT DIFFERENT TIME POINTS

S. no.	Time (h)	Plasma concentration of Standard famotidine (ng/mL)	Plasma concentration of prepared Famotidine SNEDDS (ng/mL)
1	0	0	0
2	0.25	198.99	310.35
3	0.5	390.77	646.54
4	1	509.78	1019.92
5	1.5	1009.67	1931.68
6	2	1278.56	3517.04
7	2.5	987.78	2739.65
8	3	467.99	1634.23
9	4	309.54	975.28
10	6	221.09	531.39
11	8	150.08	300.87
12	24	60.33	125.52

TABLE 16: PHARMACOKINETIC PARAMETERS OF STANDARD FAMOTIDINE AND PREPARED FAMOTIDINE SNEDDS

Parameters	Standard Famotidine	Famotidine SNEDDS
Biological Half-life (h)	2.5	2.5
C_{max} (ug/mL)	1.279	3.517
T_{max} (h)	2	2
AUC $_{0-t}$ (ug.hr/mL)	6.23	11.28
AUC $_{0-\infty}$ (ug.hr/mL)	6.41	11.73

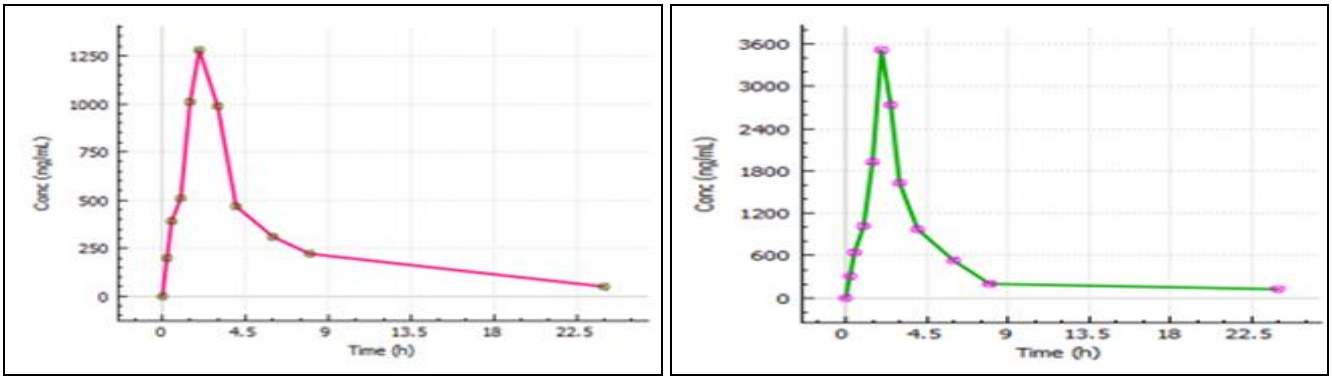

FIG. 6: MEAN PLASMA CONCENTRATION-TIME PROFILE FOR STANDARD FAMOTIDINE (PINK) AND FAMOTIDINE SNEDDS (GREEN)

The famotidine SNEDDS showed 3 fold increase in C_{max} and about 5.5 fold increase in AUC $_{0-\infty}$ than that of standard famotidine suspension, which revealed the possibility of enhancement of solubility and bioavailability. Thus, according to the statistical analysis of pharmacokinetic data, we

can conclude that famotidine SNEDDS improved the bioavailability of famotidine significantly compared to that of standard famotidine suspension. The chromatographic peak of Famotidine was eluted at a retention time of 5.03 minutes and that of internal standard Ranitidine hydrochloride at 11.6 minutes.

CONCLUSION: A new RP-HPLC method was developed for the estimation of famotidine in Solid-self nano emulsifying drug delivery system (SNEDDS) using LC-20AT SHIMADZU High-Performance Liquid Chromatography. The mobile phase consisted of phosphate buffer pH 6.8: methanol (50:50 %v/v) and Enable 18 H (5µ, 250 × 4.6 mm) C18 column was used.

The developed method was optimized prior to validation studies in terms of stationary phase, mobile phase composition and flow rate. The chromatographic peak of famotidine was eluted at a retention time of 5.03 min and that of internal standard ranitidine hydrochloride at 11.6 minutes.

The developed method was validated as per USFDA guidelines for different validation parameters. The method was selective, rugged and suitable for routine measurement of subject samples. The method has significant advantages in terms of reproducible liquid-liquid extraction procedure and short chromatographic run time.

The extraction procedure gave consistent and reproducible recoveries for analyte and internal standard from plasma. The established LLOQ was significantly low and was suitable for conducting pharmacokinetic study with the test formulation of Famotidine. The developed bioanalytical method was applied successfully for the pharmacokinetic study of Famotidine SNEDDS, and thus the objective of the study was met as there was an increase in C_{max} and AUC values Famotidine SNEDDS which showed the possibility of an increase in bioavailability of Famotidine.

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