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Development and validation of spectrophotometric and HPLC methods For the determination of Famotidine in bulk drug formulations.

M.V. Kumudhavalli*, S.Alexandar, and B. Jaykar,
Department of Pharmaceutical Analysis,Vinayaka Mission's College of Pharmacy,
Yercaud main road, Kondappanaikenpatty, Salem-636008, Tamilnadu ,India

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ABSTRACT

Two simple, rapid and selective methods were developed for the determination of Famotidine in drug substance and its pharmaceutical preparations. The first method (I) is based on UV Spectroscopy.UV spectra of Famotidine are recorded. The absorption maxima (λ_{max}) were observed at 286nm. Beer's law was obeyed over the concentration range from 2 to 20 µg/ml and it shows linearity The validation of the proposed method was further confirmed by Recovery studies at 50%, 100% and 150%. The percentage recovery values from 96.83%w/w, 102.7%w/w, 97.70%w/w, 99.07%w/w. This serves as a good index of accuracy and reproducibility of the study. The second method is based on the Reverse phase HPLC method was developed by using Methanol: Acetonitrile in the ratio of 50:50 as a mobile phase and Hypersil C18 (5 micron, 250 4.6 mm) column as a stationary phase. Flow rate was 1.5 ml/min. The uv detector was operated at 286nm. The method was validated for specificity, linearity, precision, accuracy and limit of quantification. The recovery studies showed that the observed percentage recovery of Famotidine was found to be 98.4 to 98.9 %. The retention time of Famotidine was found to be 5.907min. The developed method was accurate and precise which was evident from the analytical data and recovery studies.

KEY WORDS: Famotidine; UV- Spectroscopy, Reverse-phase HPLC.

INTRODUCTION

Famotidine, 3-[({2-[(aminoimino-methyl) amino] 4-thiazolyl} methyl) thio]-N-(aminosulfonyl) propanimidoamide is a new H2-receptor antagonist used in the treatment of gastric and duodenal ulcers [1, 2]. The empirical formula of Famotidine is $C_8H_{15}N_7O_2S_3$ and its molecular weight is 337.43. Famotidine is available in 20mg and 40mg for oral administration. It suppresses [3] the secretion of gastric acid induced by histamine and food effectively. It is an alternative to cimetidine and ranitidine for healing duodenal ulcers[4,5]. A literature survey revealed that different high-performance liquid chromatographic (HPLC) methods for determination of famotidine in combination and in biological fluids have been reported [6-12]. There is no method reported for the estimation of famotidine alone .Hence , The aim of the present work was to develop selective and sensitive UV spectrophotometric and RPHPLC method developement and determination of famotidine with shortest possible retention time. The proposed method has also been validated according to the ICH guidelines [13].

MATERIALS AND METHODS

Experimental

Chemicals and reagents:

All reagents used were of an analytical grade. HPLC grade Acetonitrile and Methanol were obtained from E.Merck (India) LTD, Mumbai. Pure Famotidie usp was obtained as a gift sample from Dr.Reddy's Lab Ltd.hydrabad. Ultra purified water, purified using an ELGA water purification unit (Bucks, UK), was used to prepare the sample and standard solutions. Pure famotidin USP was obtained as a gift sample from Dr.Reddy's Lab ltd, Hydrabad. The used pharmaceutical preparation Famocid 40mg tablets were purchased from local market and it was manufactured by Sun pharma.

***Corresponding author.**
Dr.M.V.Kumudha valli,M.Pharm,Ph.D
Department of Pharmaceutical Analysis,
Vinayaka Mission's College of Pharmacy,
Yercaud main road, Kondappanaikenpatty,
Salem-636008, Tamilnadu ,India

Methods

Instrumentation

Shimadzu (UV 1601) UV- visible spectrophotometer with quartz cells of 1 cm optical length incorporated with a PC computer was used, HPLC instrument equipped with UV-detection chromatographic separation was achieved at ambient temperature on column symmetry C18 (4.6x150mm, 3.5µm).The flow rate and runtime was set 1ml/min and 10 minutes. The wavelength selected was 286/nm. The injection volume was 20µl.

Preparation of Mobile Phase and Diluent.

The mobile phase was prepared by mixing methanol and distilled water in the ratio of 50:50 and degassed in an ultrasonic water bath for 10minutes. Then the resultant solution was filtered through 0.45µ filter under vacuum filtration. The mobile phase was used as diluent.

PROCEDURE

1. Calibration for Spectrophotometric method.

(A) Preparation of Famotidine Standard and Sample Solution.

(i) Preparation of Stock Solution

The stock solution was prepared by weighing accurately 10mg of Famotidine and transferred into a clean and dry 100ml volumetric flask. About 70ml of diluents was added and sonicated. The volume was made upto the mark with the same diluent, to get c concentration of 10mg/ml. The standard drug solution was diluted so as to get the final concentration of the standard solution, in the range of 2 to 20 mg/ml concentration. The absorbance was taken ar 286nm. As the result obtained were satisfactory, the method was applied for pharmaceutical formulations.

(ii) Preparation of Sample Solution

For estimation of Famotidine USP in tablet formulation, 20 tablets of the brand were weighed and triturated to fine powder. Tablet powder equivalent to 10mg of Femotidin USP was weighed and dissolved and further diluted with quantity sufficient with diluents. It was kept for sonitication for 10 mins, this was then filtered through whatman filter paper no. 41 to get a concentration of 100mg /ml. Various dilutions of the tablet solutions were prepared and analysed, under optimum condition, the calibration graph was plotted as a reletion of absorbance against concentration of the drug.

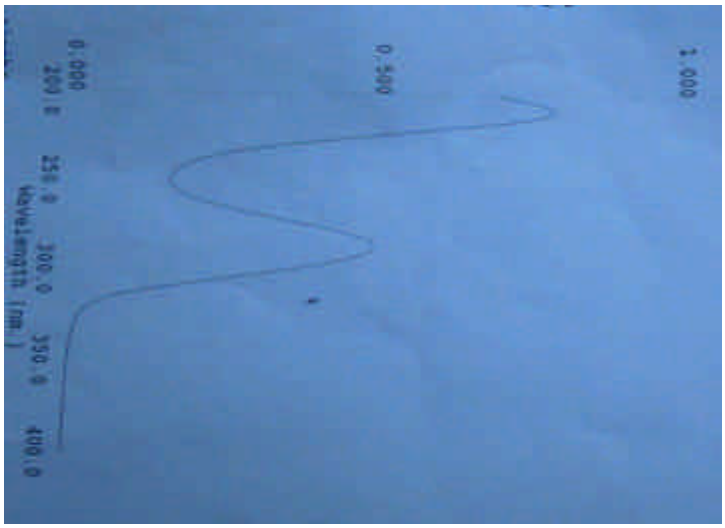


Fig. No: 1 UV-Spectrum of Famotidine in Methanol& D.H_2O(50:50)

METHOD-II CALIBRATION FOR HPLC METHOD.

(i) Preparation of Stock Solution

Aliquots of Famotidine drug solution (1ml/ml in the concentration range from 10 to 100ng/ml were transferred in to 10 ml calibrated volumetric Flask and completed to the mark with the mobile phase solvent. Triplicate 20 µl of each solution were injected using the above chromatographic conditions. The calibration curve was constructed by plotting the peak area versus the corresponding concentrations of famotidine and the regression equation was computed

(ii) Preparation of sample solution:

Accurately weighed quantity 10 mg equivalent of famotidine was transferred to 100 ml calibrated volumetric flask, dissolved in diluents and the volume was adjusted to the mark. The solution was further diluted to get concentration of 10 µg/ml, subjected to analyses and amount of famotidine was determined.

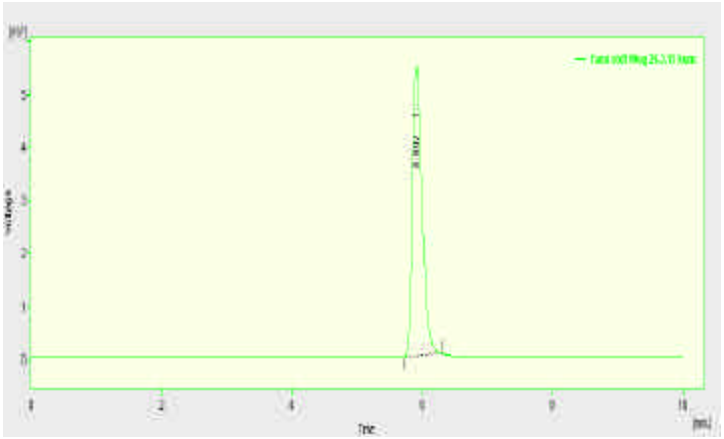
Initialization of the Instrument

First, the column was placed on the instrument and switch on the instrument and washed with Double Distilled water for 30 min. Then run the mobile phase for 30 min for column saturation.

OPTIMIZED METHOD

Chromatographic Condition for Optimized Method

Column name	C_{18}(2);250x4.6mm, 5µ, Phenomenox Luna Column
Mobile phase	Methanol :acetonitrile in the ratio of (50:50)
Flow rate	1.0ml/min
Detection	UV at 286nm
Temperature	Ambient
Injection volume	20ul



RESULT AND DISCUSSION

METHOD I SPECTROPHOTOMETRIC METHOD.

The developed UV-Spectrophotometric method utilizes Methanol as a solvent which is cheap as compared to Acetonitrile and the quality of the developed method is very good as evident from the analytical and statistical parameters calculated.

The UV Spectra of Famotidine are recorded. The absorption maxima (λ_{max}) were observed at 286nm.Famotidine shows linearity in the concentration range of 2 to 20 µg/ml. . The validation of the proposed method was further confirmed by Recovery studies at 50%, 100% and 150%. The percentage recovery values from 96.83%w/w, 102.7%w/w, 97.70%w/w, 99.07%w/w. This serves as a good index of accuracy and reproducibility of the study.

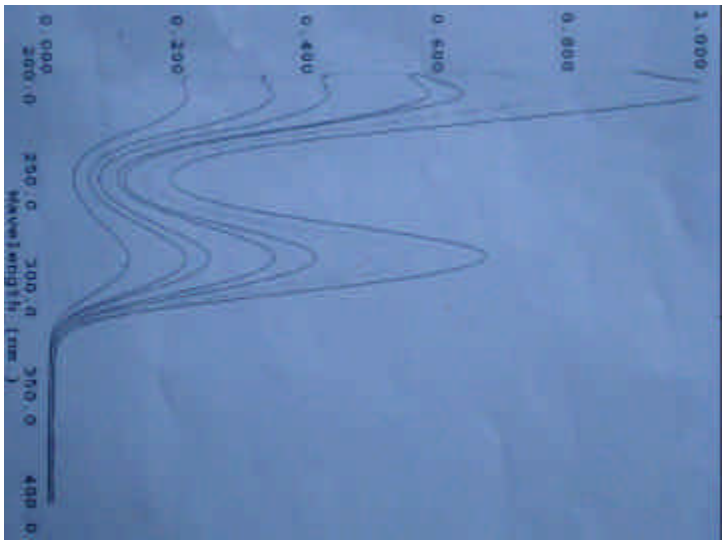


Figure no: 5: Figure showing the overlay spectrum of Famotidine at 2 to 20 µg/ml

Table:4 Calibration data of Famotidine

S. No	Concent-ration (µg/ml)	Absorbance at 286 nm Trail -I	Trail –II	Trail –III	Mean Absorbance
1	2	0.065	0.066	0.067	0.066
2	4	0.1554	0.1553	0.1555	0.1554
3	6	0.1930	0.1925	0.1927	0.1927
4	8	0.2364	0.2224	0.2453	0.2347
5	10	0.3284	0.3364	0.3574	0.3407
6	12	0.4169	0.4079	0.4196	0.4148
7	14	0.5124	0.5315	0.5216	0.5218
8	16	0.5365	0.5473	0.5572	0.547
9	18	0.6130	0.6240	0.6372	0.6247
10	20	0.6791	0.6872	0.6762	0.6808

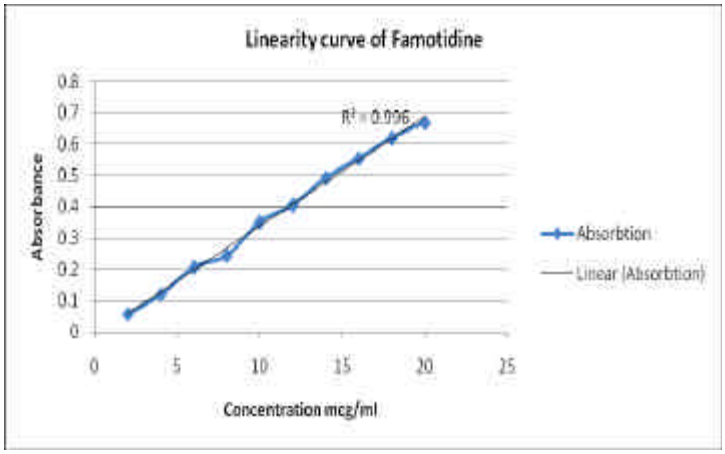


Figure no:6 Figure showing standard absorbance curve of Famotidine by UV-methano

VALIDATION OF PROPOSED METHOD

1. ACCURACY FOR FAMOTIDINE at 50%, 100%, 150%:

Table no:7

Drug	Initial Amount [µg/ml]	Amount added [µg/ml]	Amount recovered [µg/ml,n=3]	%Recovered	%R.S.D
50%	20	10	38.76	96.83	0.489
100%	20	20	41.11	102.7	0.500
150%	20	30	38.95	97.70	0.411
AVERAGE					99.07
S.D					0.463
R.S.D					0.466

Figure no:7 Spectrum shows overlay of accuracy of 50%, 100% and 150%.

METHOD-II CALIBRATION FOR HPLC METHOD

Validation of Developed Method

The developed method was validated according to ICH guidelines.The mobile phase was prepared and arranged all parameter as per the above optimized method.

Validation Parameters

1. System suitability
2. Precision
3. Accuracy (recovery)
4. Linearity of test method
5. Robustness of test method

1. Data for system suitability

Table no:8 System suitability parameters for Famotidine

System suitability parameters (Conc 4µg/ml)	Retention time (min)	AUC (µv)	No. of Theoretical plates	Tailing factor
Trial-1	5.123	94310	7078	1.323
Trial-2	5.102	94400	7168	1.310
Trial-3	5.117	94350	7104	1.291
Mean	5.114	94353	7116	1.391
S.D	0.0087			
R.S.D	0.1572			

RESULT:S

Standard solution Famotidine was determined under proposed condition

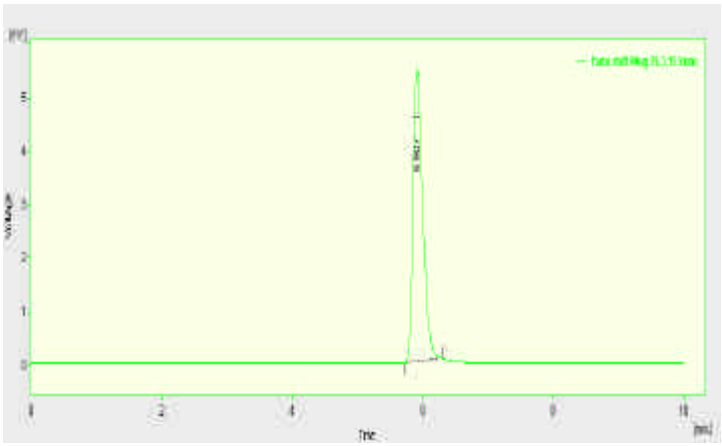


Figure no:8 Chromatogram for system suitability

Table no:9 Precision data of Famotidine

S. No	Dilution (µl/ml)	Tria-1	Trial-2	Trial-3	Average	Conc From calibration curve	Amount present in each tablet	Percentage (%)
1	20	94310	94170	94008	94162	20	40.42	101
2	40	192602	197216	198520	196112	40	40.20	100
3	60	313908	317968	310162	314012	60	40.12	100
4	80	422761	427316	428316	426131	80	40.09	100
5	100	561345	561471	568676	563830	100	40.07	100
Average							40.15	100
S.D								1.852
R.S.D								0.857

Observation:

The S.D and R.S.D of % Famotidine was found to be 1.852 and 0.857 respectively.

Report:

The above propose method was lies with in the standard limit. Hence the proposed method was found to be precise and accurate.

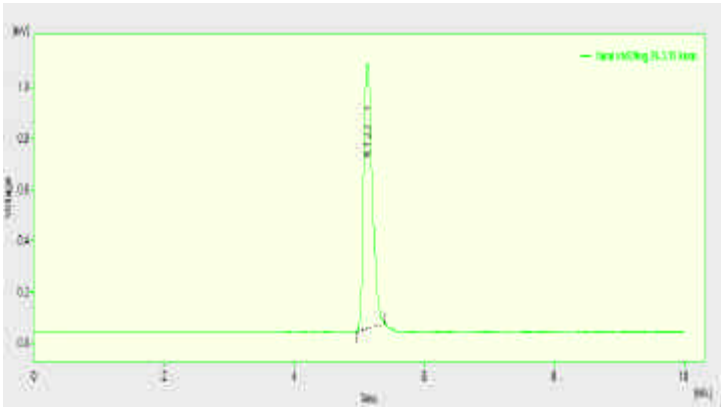


Fig no:9 chromatogram shows precision

Table no:10 Intermediate precision (Day to Day)

S.no	Dilution detail (µl-ml)	Trial-1	Trial-2	Trial-3	Average	Amount	Percentage in each tablet
Day-1	40	192602	197216	198520	196112	40.20	100
Day-2	80	422761	427316	428316	426131	40.09	100
Average						40.14	100
S.D							1.739
R.S.D							1.0705

Observation:

The S.D and R.S.D of % Famotidine was found to be 1.739 and 1.0705 respectively.

Report:

The above propose method was lies with in the standard limit. Hence the propose method was found to be precise and accurate.

3.ACCURACY:

Table no:11. Recovery for 50%,100%,150% level in Famotidine

Drug	Initial Amount [µg/ml]	Amount added [µg/ml]	Amount recovered [µg/ml,n=3]	% Recovered	% R.S.D
50%	20	10	40.34	99.17	0.4129
100%	20	20	40.42	100	1
150%	20	30	40.38	101	0.9900
AVERAGE					100
S.D					0.8031
R.S.D					0.8009

Observation:

The S.D and R.S.D of % Famotidine was found to be 0.8031 and 0.8009 respectively.

Report:

The above propose method was lies with in the standard limit. Hence the proposed method was found to be precise and accurate.

Recovery for Famotidine at 50%

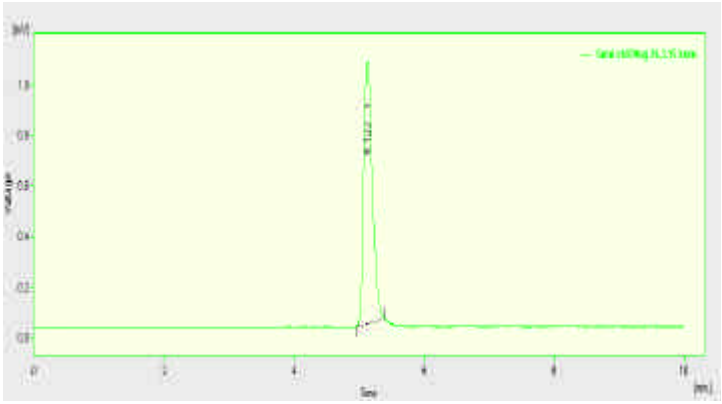


Figure no:10 Recovery for Famotidine at 100%

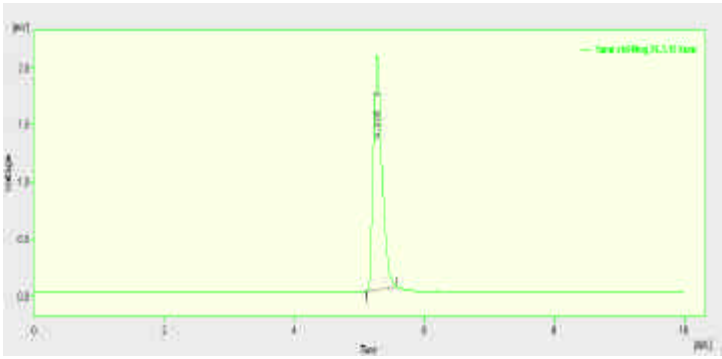


Figure no:11 Recovery for Famotidine at 150%

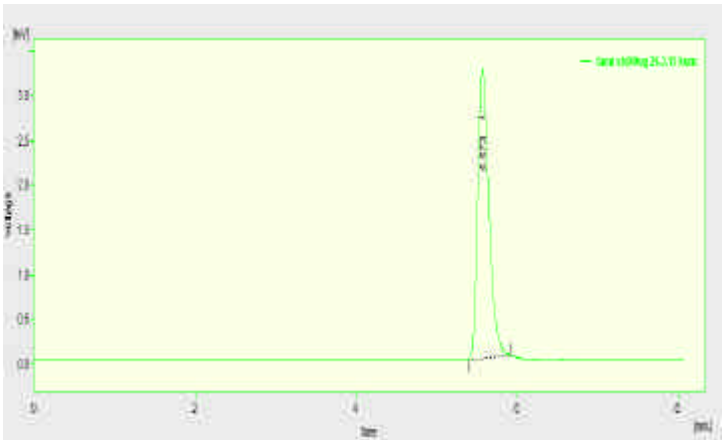


Figure no:12

Table no:12 Linearity data of Famotidine

Concentration µg/ml	Peak area
20	94310
40	192602
60	313908
80	422761
100	561345

Each values is the mean of 3 Readings

LINEARITY

STANDARD CURVE OF FAMOTIDINE

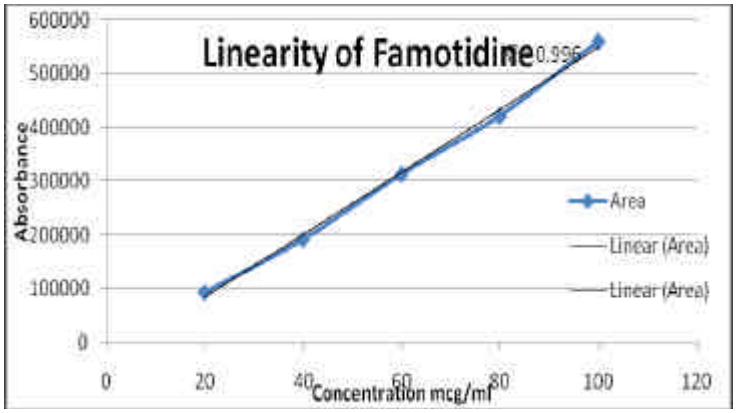


Figure no:13 Figure showing the linearity curve of Famotidine by RP-HPLC method.

Linearity-Chromatogram-1

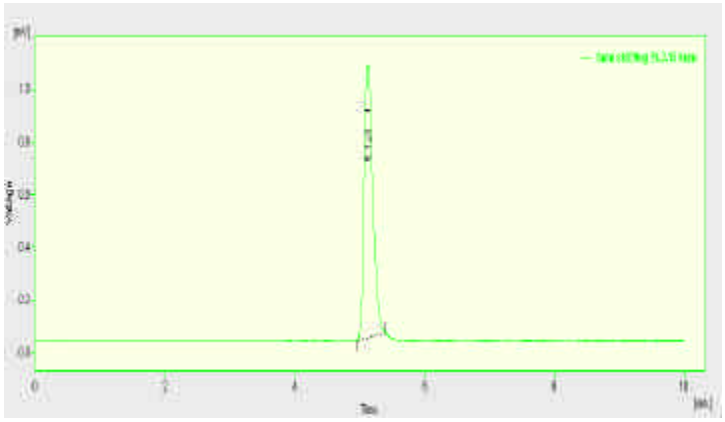


Figure no: 14- 20 µg/ml concentration

Linearity-Chromatogram-2

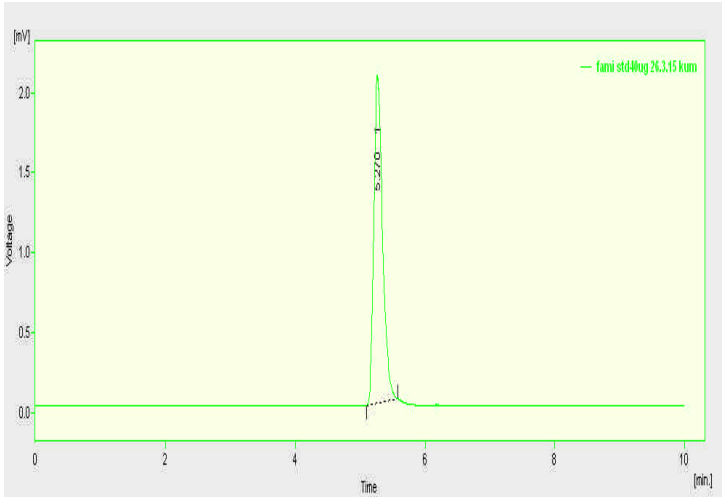


Figure no:15 - 40 µg/ml concentration

Linearity-Chromatogram

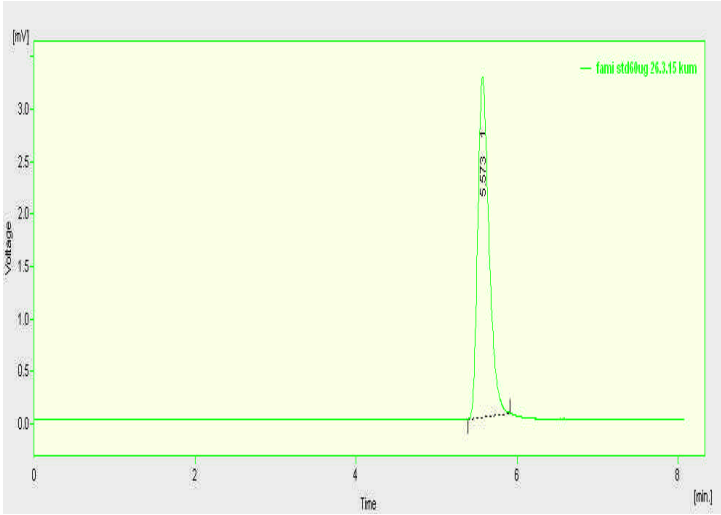


Figure no:16- 60 µg/ml concentration

Linearity-Chromatogram-4

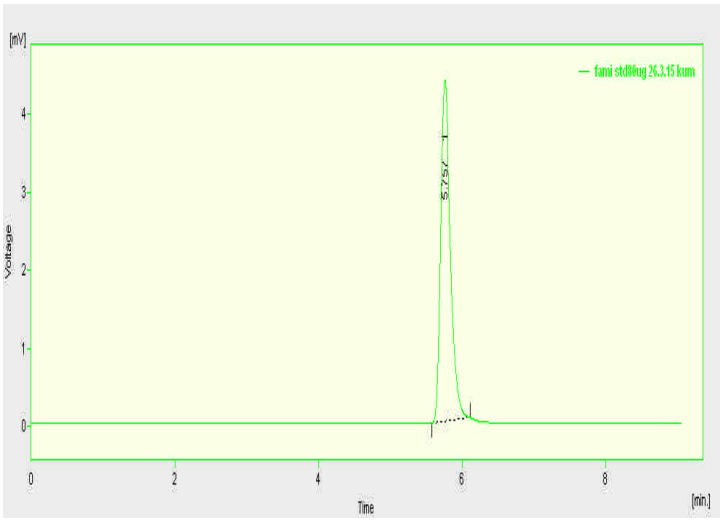


Figure no:17- 80 µg/ml concentration

Linearity-Chromatogram-5

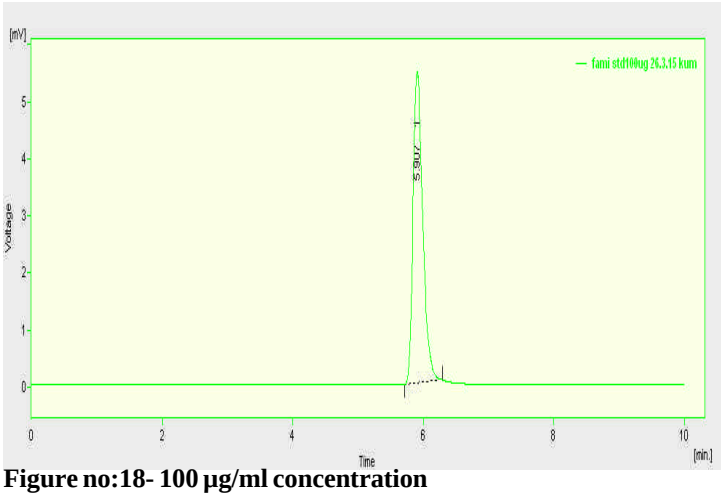


Figure no:18- 100 µg/ml concentration

SUMMARY AND CONCLUSION

The UV-Spectrophotometery and RP-HPLC method for the simple, precise and accurate was developed and validation for estimation of Famotidine in tablet dosage form.The developed UV-Spectrophotometric method utilizes Methanol as a solvent which is cheap as compared to Acetonitrile and the quality of the developed method is very good as evident from the analytical and statistical parameters calculated.The UV Spectra of Famotidine are recorded. The absorption maxima (λ_{max}) were observed at 286nmFamotidine shows linearity in the concentration range of 2 to 20 µg/ml. . The validation of the proposed method was further confirmed by Recovery studies at 50%, 100% and 150%. The percentage recovery values from 96.83%w/w, 102.7%w/w, 97.70%w/w, 99.07%w/w. This serves as a good index of accuracy and reproducibility of the study.

The reverse phase HPLC method was developed using Methanol:Acetonitrile in the ratio of 50:50 as a mobile phase and Hypersil C18 (5 micron, 250x4.6 mm) column as a stationary phase. The recovery studies showed that the observed percentage recovery of Famotidine was found to be 98.4 to 98.9 %. The retention time of Famotidine was found to be 5.907min. The developed method was accurate and precise which was evident from the analytical data and recovery studies.The repeatability of the method was confirmed by repeating the assay procedure with five different concentrations of three replicates in each. The assay percentage values in “Famotidine” ranging from 40.42% to 40.07%W/W for respectively. The quantitative results obtained were subjected to statistical validation. The values of R.S.D are less than 2%, indicating the accuracy and precision of the method‘The recovery studies at 50%, 100% ,150% were showed that the observed percentage recovery of Famotidine was found to be from 99.17%, 100% and 101% w/w respectively.

From the present study, it is clear that both the methods of analysis are simple, accurate, specific and precise in operation and can be employed in the routine analysis.

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