1. INTRODUCTION:

Famotidine is a histamine H_2-receptor antagonist that inhibits stomach acid production, and it is commonly used in the treatment of peptic ulser disease and gastroesophageal reflux disease. Famotidine is chemically 3-([2-(diaminomethyleneamino)thiazol- 4-yl]methylthio)- N'-sulfamoylpropanimidamide. Its molecular formula is $C_8H_{15}N_7O_2S_3$ and molecular weight is 337.45. On compare with cimetidine, the first H_2 antagonist, famotidine has no effect on the cytochrome P450 enzyme system, and does not appear to interact with other drugs. It is a relatively new H_2RA that is 7 and 20 times more potent than ranitidine and cimitidine, respectively, in acid secretion in adults. 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. Many HPLC methods were reported for determination of famotidine in human plasma[1-3], HPTLC[4] and HPLC method for analysis of famotidine in pharmaceutical form[6-10].

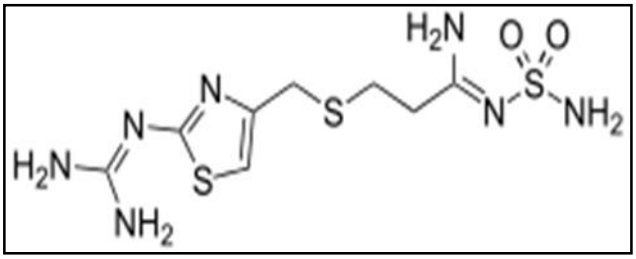


Figure-1: Structure of famotidine

Pharmakokinetic study:

Orally administered famotidine is incompletely absorbed from gastrointestinal tract, and it undergoes minimal first-pass metabolism. The bioavailability of famotidine following oral doses is 40–45%, this is slightly affected by food or antacid. After oral doses, peak plasma level is 75–100 ng/ml which occur in 1–3 h [11]. The peak plasma level is not altered by chronic administration, and elimination half-life is 2.5–3.5 h which is prolonged in renal failure. The drug is 15–20% bound to plasma proteins. The apparent volume of distribution of famotidine is reported to be 1.1–1.4 l/kg in normal renal function and does not appear to be altered substantially in patients with renal dysfunction [12, 13]. It is widely distributed into kidney, liver, pancreas, and submandibular gland. In rats, it is distributed into central nervous system [11, 12]. Famotidine is metabolized in the liver to famotidine S-oxide. The metabolite has no pharmacological activity on gastric

acid secretion. It is eliminated largely as unchanged drug and only 30–35% undergo metabolic routes. Orally administered drug undergoes minimal first-pass metabolism [12]. Famotidine is excreted markedly, 25–30% or 65–80%, unchanged by renal route, primarily by active tubular secretion and glomerular filtration within 24 h after oral or intravenous administration, respectively. The remaining drug is excreted via metabolic routes [12]. Total body clearance of the drug averages 381–483 ml/min, and renal clearance of the drug averages 250–450 ml/min [12]. As a result, famotidine should be used in a lower dosage and at longer dosing intervals when used in patients with sever renal insufficiency. In rats, the drug is found in breast milk, and does not cross the placenta [11, 12]. It is not known whether the drug crosses the placenta or distributes into milk in humans. The elimination of famotidine does not appear to be affect by age in adults.

Single crystal X-ray:

The most common experimental method of obtaining a detailed structure of a molecule, that allows resolution of individual atoms, single crystal X-ray diffraction (SXRD) is performed by analyzing the pattern of X-rays diffracted by an ordered array of many identical molecules (single crystal). Many pure compounds, from small molecules to organometallic complexes, proteins, and polymers, solidify into crystals under the proper conditions. When solidifying into the crystalline state, these individual molecules typically adapt one of only a few possible 3D orientations. When a monochromatic X-ray beam is passed through a single crystal, the radiation interacts with the electrons in the atoms, resulting in scattering of the radiation to produce a unique image pattern. Multiple images are recorded, with an area X-ray detector, as the crystal is rotated in the X-ray beam. Computationally intensive analysis of a set images results in a solution for the 3D structure of the molecule.

2. LITERATURE REVIEW

The literature review shows that various analytical methods were reported for its determination as API, pharmaceutical formulation. Brief details for the same are as under.

D. Zendelovska, T. Staflov, described high-performance liquid chromatographic determination of famotidine in human plasma using solid phase column extraction. Famotidine and the internal standard were chromatographically separated from plasma components using a Lichrocart Lichrospher 60 RP select B cartridge for solid-phase

separation with a mobile phase composed of 0.1 % (v/v) triethylamine in water (pH 3) and acetonitrile (92:8, v/v). UV detection was set at 270 nm. The calibration curve was linear in the concentration range of 10.0 – 350.0 ng mL^{-1}.

A. Zarghi, H. Komeilizadeh, M. Amini, L. Kimiagar described method for determination of famotidine in plasma and urine by high performance liquid chromatography. Because therapeutic concentrations of famotidine are low in plasma, a sensitive method is required to determine plasma famotidine concentrations in clinical studies. Ranitidine and cimetidine were used as internal standards for urine and plasma measurements. For plasma, the mobile phase was acetonitrile-water (15 : 85 v/v), 45 mM sodium dodecyl sulphate (SDS) and 20 mM disodium hydrogen phosphate adjusted to pH 3. A mixture of methanol-phosphate buffer (20 : 80 v/v) adjusted to pH 6·3 was used as the mobile phase for the determination of the compound in urine. The separation was performed on an analytical 150 x 3·9 mm i.d. reversed-phase Novapack C_{18} (4 μm particle size) column using UV detector (267 nm for plasma and 282 nm for urine). The detection limits for famotidine in plasma and urine were 7·5 ng ml^{-1} and 160 ng ml^{-1}, respectively. The inter- and intra-assay coefficients of variation were found to be less than 10%.

N. Helali, Darghouth F. Darghouth, L. Monser, RP-HPLC determination of famotidine and its potential impurities in pharmaceuticals. Separations were performed on a Supelcosil LC18 column with an isocratic mobile phase—13:87 (*v*/*v*) acetonitrile–0.1 M dihydrogen phosphate buffer containing 0.2% triethylamine (pH 3.0). The mobile phase flow rate was 1 mL min^{-1} and the detection wavelength was 265 nm. Response was linearly dependent on concentration between 1 and 80 μg mL^{-1} (regression coefficient, R^2, from 0.9981 to 0.9999). *RSD* from determination of method repeatability (intraday) and reproducibility (interday) were <2% (*n*=6). Lowest detectable concentrations ranged from 0.08 to 0.14 μg mL^{-1}. The proposed liquid chromatographic method can be satisfactorily used for routine quality control of famotidine in pharmaceutical formulations.

S. M. Pawar, B. S. Patil, Patil RY explain HPTLC method for simultaneous quantitation of famotidine and domperidone in bulk drug and formulation. Chromatographic separation of the drugs was performed on aluminum plates precoated with silica gel 60 F254 as the stationary phase and the solvent system consisted of n-butanol: water 6: 1(v/v). Densitometric evaluation of the separated zones was performed

at 280 nm. The two drugs were satisfactorily resolved with RF values 0.27±0.01 and 0.58±0.01 for FAM and DOM, respectively. The accuracy and reliability of the method was assessed by evaluation of linearity (200-1200 ng.spot-1 for FAM and 100-600 ng.spot-1 for DOM), precision (intra-day RSD 0.05–0.40 % and inter-day RSD 0.13–0.39 % for FAM, and intra-day RSD 0.10–0.20 % and inter-day RSD 0.13–0.60 % for DOM), accuracy (99.10±0.116 % for FAM and 98.57±1.078 % for DOM), and specificity, in accordance with ICH guidelines.

P. Deshpande, S. Gandhi, V. Bhavani, R. Bandewa, A. Dhiware, V. Diwale described high performance thin layer chromatographic method for determination of famotidine and domperidone in combined tablet dosage form. The mobile phase selected was Toluene: Methanol: Triethylamine (6: 3: 0.5 v/v/v) with UV detection at 290 nm. The retention factor for famotidine and domperidone were found to be 0.23±0.102 and 0.67±0.123. The method was validated with respect to linearity, accuracy, precision and robustness. Results found to be linear in the concentration range of 100-500 ng/band for both famotidine and domperidone. The method has been successfully applied for the analysis of drugs in pharmaceutical formulation. The % assay (Mean±S.D.) was found to be 98.63±0.257 for famotidine and 98.87±0.654 for domperidone.

M. S. Arayne, N. Sultana, M. H. Zuberi, F. A. Sissiqui, discover Simultaneous determination of metformin, cimetidine, famotidine, and ranitidine in human serum and dosage formulations using HPLC with UV detection. These drugs were separated on a Purospher Star RP18 endcapped (250 mm × 4.6 mm i.d.) column packed with 5-µm particles. The mobile phase, optimized through an experimental design, consisted of methanol-water-triethylamine (20:80:0.05), whose pH was adjusted to 3.0 with phosphoric acid (85%) pumped at a flow rate of 1.0 mL/min. UV detection was performed at 229 nm. The method was validated in the sample concentration range of 5.25 µg/mL for all the drugs, where it demonstrated good linearity with r = 0.9998, 0.9979, 0.9997, and 0.9987 (n = 6), respectively. For independent 100% level samples, the intra-day and inter-day precision was in the range i.e. < 2.0 for all the drugs. The method demonstrated robustness, resisting to small deliberate changes in pH, flow rate, and composition (organic:aqueous ratio) of the mobile phase. The limit of detection values were 0.071, 0.116, 0.134, and 0.110 µg/mL, while the limit of quantitation were 0.217, 0.352, 0.405, and 0.368 µg/mL for Metf, Cimt, Famt, and Rant, respectively. The

applicability of the method was demonstrated by determining the drug content in pharmaceutical formulations, where it exhibited good performance.

Y. R. Reddy, K. K. Kumar, M. R. P. Reddy, K. Mukkanti explain RP-UPLC method development and validation for the simultaneous estimation of ibuprofen and famotidine in pharmaceutical dosage form.The chromatographic separation was achieved on Acquity UPLC BEH C-18,50 mm x 2.1 mm and 1.7 µm column with gradient elution. The mobile phase A contains a mixture of 50 mM sodium acetate buffer (pH 5.5): methanol (85:15, v/v), and the mobile phase B contains a mixture of 50 mM sodium acetate buffer (pH 5.5): methanol (25:75, v/v). The flow rate was 0.3 mL min^{-1} , and the detection wavelength was 260 nm. Results: The limit of detection for Ibuprofen and Famotidine was 1.6 and 1.2 µg mL^{-1} , respectively. The limit of quantification (LOQ) for Ibuprofen and Famotidine was 5.1 and 4.3 µg mL^{-1} , respectively. Conclusion: This method was validated for accuracy, precision, and linearity. The method was also found to be stability indicating.

N. Helali, N. T. Tran, L. Monser, M. Taverna Capillary zone electrophoresis method for the determination of famotidine and related impurities in pharmaceuticals. A simple and rapid capillary zone electrophoresis (CZE) method with UV detection has been developed for the determination of famotidine and its potential impurities in pharmaceutical formulations. The electrophoretic separation of these compounds was performed using a fused silica capillary and 37.5 mmol L^{-1} phosphate buffer pH 3.5 as the electrolyte. Under the optimised conditions, six impurities could be resolved from the famotidine peak in less than 7 min. The calibration curves obtained for the seven compounds were linear over the concentration range investigated (from 1.5 to 78.5 µg mL^{-1}). The intra- and inter-day relative standard deviations for the migration times and corrected peak areas were less than 2% and 5%, respectively. The detection limits were found to be 0.09 µg mL^{-1} for famotidine, and from 0.1 to 0.62 µg mL^{-1} depending on the impurities. The method has been successfully applied to the determination of famotidine in commercial dosage forms.

N. R. Reddy, K. Prabhavathi, YV. B. Reddy, I, E. Chakravarthy developed A new spectrophotometric determination of famotidine from tablets. The method was based on bromination of the drug with excess brominating mixture in acidic medium. The yellow

colour developed was measured at 350 nm against distilled water blank. Beer's law was obeyed in the range of 40-200 µg/ml.

N. Helalo, L. Monser developed Stability indicating method for famotidine in pharmaceuticals using porous graphitic carbon column. Chromatographic separation was accomplished within 10 min on a porous graphitic carbon (PGC) column using 50:50 v/v ACN–water containing 0.5% pentane sulphonic acid (PSA) as the mobile phase. Separation was achieved with a flow rate of 1 mL/min and a detection wavelength of 265 nm. The calibration curves were linear over a concentration range of 1.5–100 µg/mL. The intra- and interday RSDs (n = 5) for the retention times and peak area were all less than 2%. The method was sensitive with an LOD (S/N = 3) of 0.1 µg/mL for FMT, imp. C and 0.05 µg/mL for imp. 2, A and D. All recoveries were greater than 98%. The method was demonstrated to be precise, accurate and specific with no interference from the tablet ingredients and separation of the drug peak from the peaks of the degradation products (oxidative degradation and acid and base degradation). The results indicated that the proposed method could be used for the determination of FMT in commercial dosage forms and as a stability-indicating assay.

3. AIM OF PRESENT WORK:

The above literature review reveals that there were many methods for the quantitative analysis of famotidine as a drug saubstance as well as pharmaceutical dosage form, few methods are there which deals with bionalytical study and stability study. The aim of present work is to developed a shortest and optimized method for quantitative analysis of famotidine development and stability testing as well as for routine analysis. Performed a degradation study and identified possible impurities observed during the study.

4. METHOD DEVELOPMENT:

4.1 Mobile phase and column selection:

Based on literature review and drug information many experimental trials were performed using selected buffer to obtained best chromatographic condition for assay analysis of famotidine. During experimental trails different column and organic solvent (i.e. methanol, acetonitrile etc.) used and at last accepted result obtained using following chromatographic condition.

5. METHOD VALIDATION:

5.1 Chromatographic Conditions:

Instrument	**:** Waters UPLC with empower software
Column	: ACQUITY UPLC HSS T_3 C18 (100*2.1) mm ,1.8 µ
Flow rate	: 0.3 mL/min
Mobile phase	: Buffer:Acetonitrilel (80:20)
Buffer	: 0.1% Orthophosphoric acid in water
Oven temperature	: 30°C
Wave length	: 266 nm
Injection volume	: 1µL
Run time	: 4 min

Mobile Phase Preparation:

1 mL of Ortho phosphoric acid (OPA) added to 1Ltr of HPLC grade water. Mix well and sonicate to degass.

Diluent Preparation:

Use mobile phase as a diluents.

Blank Preparation:

Diluent is used as a blank

Standard Preparation:

To prepare a stock solution (500 µg/mL) for assay analysis, weigh accurately about 50 mg famotidine reference standard and transfer into 100 mL volumetric flask. Add 70 mL of diluents to dissolve the substance by sonication for one minute and dilute it up to the mark with diluents.

Pipette out 5 mL of above standard stock solution, transfer into 50 mL volumetric flask and dilute it up to the mark with diluent. The concentration obtained is 50 µg/mL of famotidine.

Test Preparation:

To prepare a stock solution (500 µg/mL) for assay analysis, weigh accurately about 50 mg famotidine sample and transfer into 100 mL volumetric flask. Add 70 mL of diluents to dissolve the substance by sonication for one minute and dilute it up to the mark with diluents.

Pipette out 5 mL of above standard stock solution, transfer into 50 mL volumetric flask and dilute it up to the mark with diluent. The concentration obtained is 50 µg/mL of famotidine.

Procedure:

Inject blank followed by five replicated injection of standard preparation. Asymmetry of first injection of standard should not more than 2.0. Related standard deviation of replicate standard preparation should not more than 2.0 %. If system suitability pass than make duplicate injection of sample preparation.

5.2 Specificity Study:

The evaluation of the specificity of the method was determined against diluent and stress (forced) degradation. The specificity of the method toward the drug was established by means of the interference of the blank preparation and degradation products against drug during the forced degradation study.

Blank Preparation:

Diluent is used as a blank.

Standard Preparation:

To prepare a stock solution (500 µg/mL) for assay, weigh accurately about 50.12 mg famotidine reference standard and transfer into 100 mL volumetric flask. Add 70 mL of diluents to dissolve the substance by sonication for one minute and dilute it up to the mark with diluents.

Pipette out 5 mL of above standard stock solution, transfer into 50 mL volumetric flask and dilute it up to the mark with diluent. The concentration obtained is 50.12 µg/mL of famotidine.

Test Preparation:

To prepare a stock solution (500 µg/mL) for assay, weigh accurately about 50.14 mg famotidine sample and transfer into 100 mL volumetric flask. Add 70 mL of diluents to dissolve the substance by sonication for one minute and dilute it up to the mark with diluents.

Pipette out 5 mL of above standard stock solution, transfer into 50 mL volumetric flask and dilute it up to the mark with diluent. The concentration obtained is 50 .14µg/mL of famotidine.

5.2.1 Forced Degradation Study:

Stress study was carried out by application of chemical and physical forced degradation. To perform forced degradation study, the drug content 50 mg in 100mL volumetric flask was employed for acidic, alkaline and oxidant media and also for thermal and photolytic conditions. After the degradation treatments were completed, the stress content were allowed to equilibrate to room temperature and diluted with diluent to attain 500 µg/ml concentrations, further 5 mL sample solution diluted to 50mL to attained 50 µg/ml concentration of famotidine. Pattern of stress (degradation) conditions and preparation for same was described as under:

Acid Degradation:

Acidic degradation study was performed by heating the drug content in 0.1N HCl at about 80° C for 1 hour and after cooling to room temperature it was neutralized with 0.1N NaOH solution. Further solution was diluted to achieve concentrations 50 µg/ml with diluent.

Alkali Degradation:

Alkaline degradation was performed by heating the drug content in 0.1N NaOH at room temperature for 1 hour and then the mixture was neutralized with 0.1N HCl. It was further diluted with diluent to achieve 50 µg/ml concentrations.

Oxidative Degradation Study:

Oxidative degradation study was performed at room temperature by adding 3% H_2O_2 in the drug content for 30 min then diluted to 50µg/ml with diluent.

Blank preparation:

Blank preparation was also performed for all degradation to identify the peaks which arise due to blank. Blank was subjected under same all stress condition as that of sample.

Chromatogram of standard preparation of specificity study:

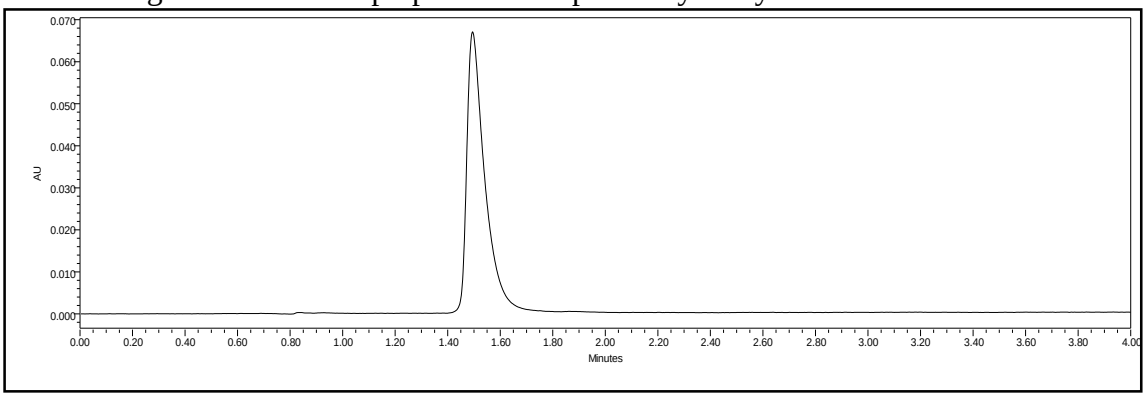
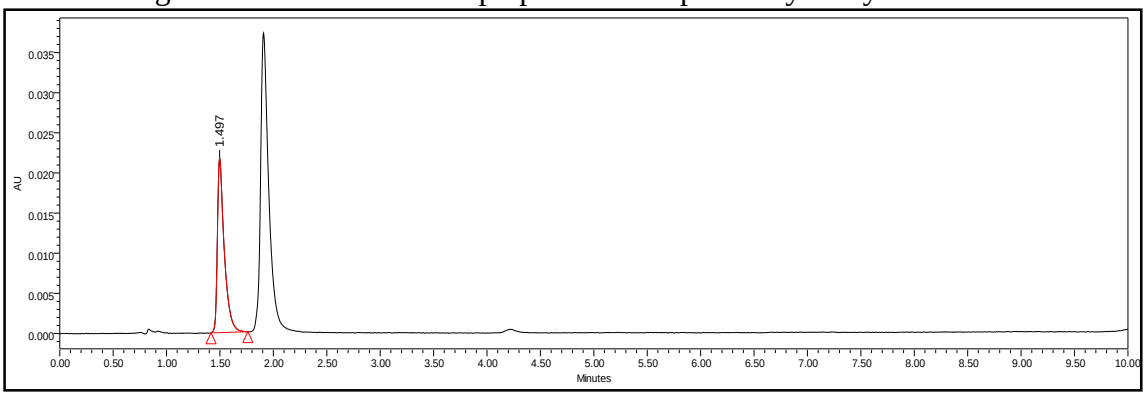


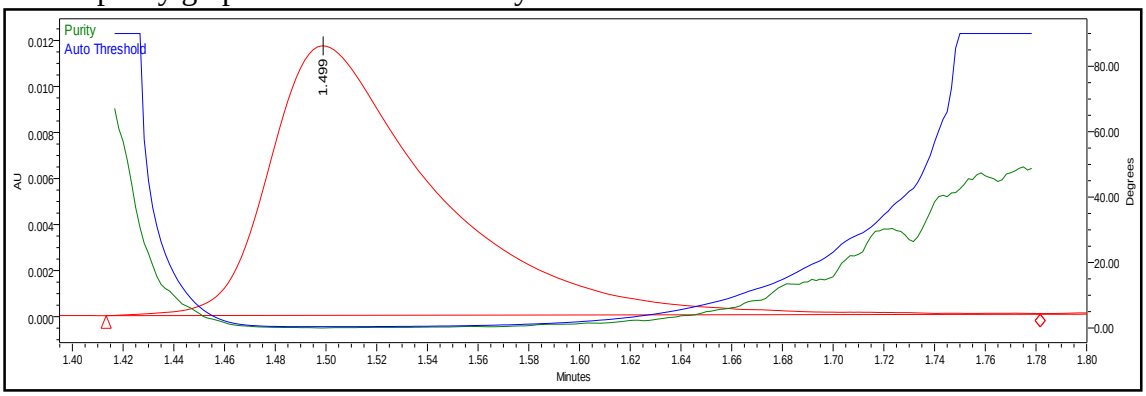
Table-1: Chromatographic sequence for Specificity study is represented through

No.	Description	Injection Replicate	Remarks
1	Blank	1	As such
2	Standard Preparation	5	
3	Test Preparation	2	
4	Bracketing Standard	1	
5	Blank preparation of acidic stress	1	Acidic Forced Degradation
6	Test preparation of acidic stress	2	
7	Bracketing Standard	1	
8	Blank preparation of alkali stress	1	
9	Test preparation of alkali stress	2	Alkali Forced Degradation
10	Bracketing Standard	1	
11	Blank preparation of oxidative stress	1	
12	Test preparation of oxidative stress	2	
13	Bracketing Standard	1	Oxidative Forced Degradation
14	Blank preparation of thermal stress	1	
15	Test preparation of thermal stress	2	
16	Bracketing Standard	1	

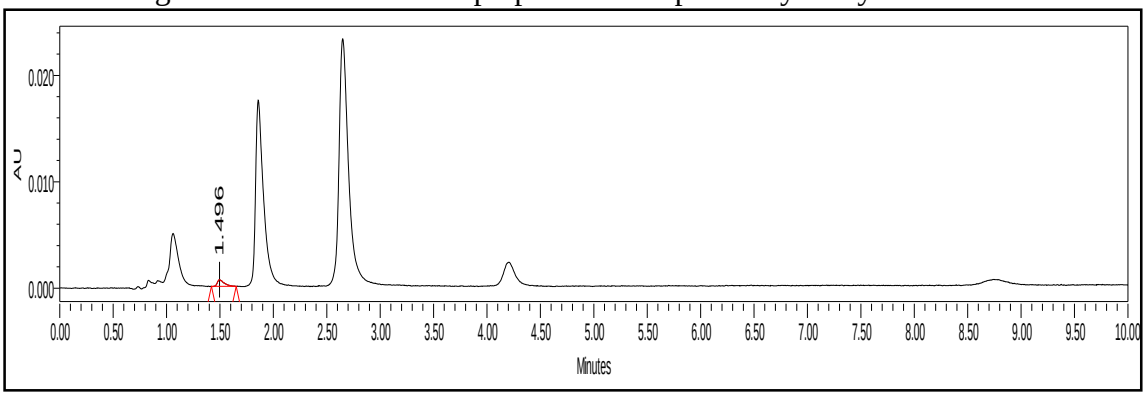
Chromatogram of acidic stress test preparation of specificity study:



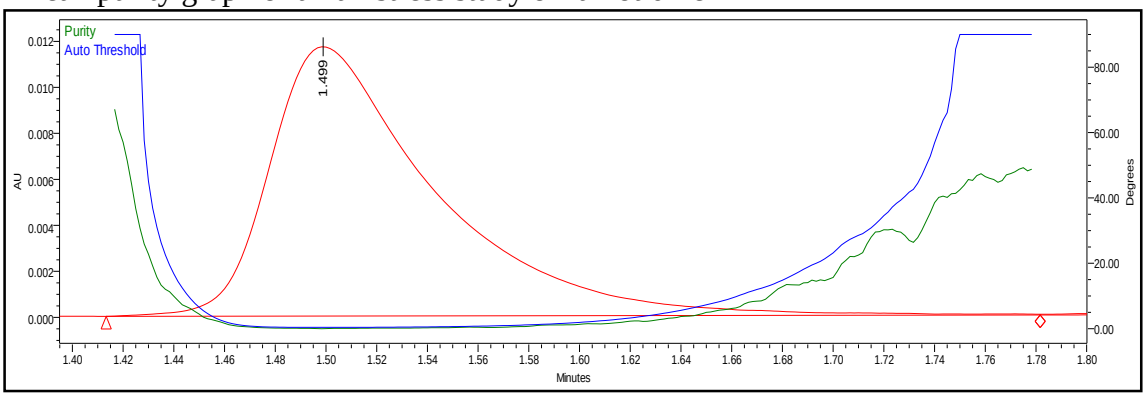
Peak purity graph of acidic stress study of famotidine:



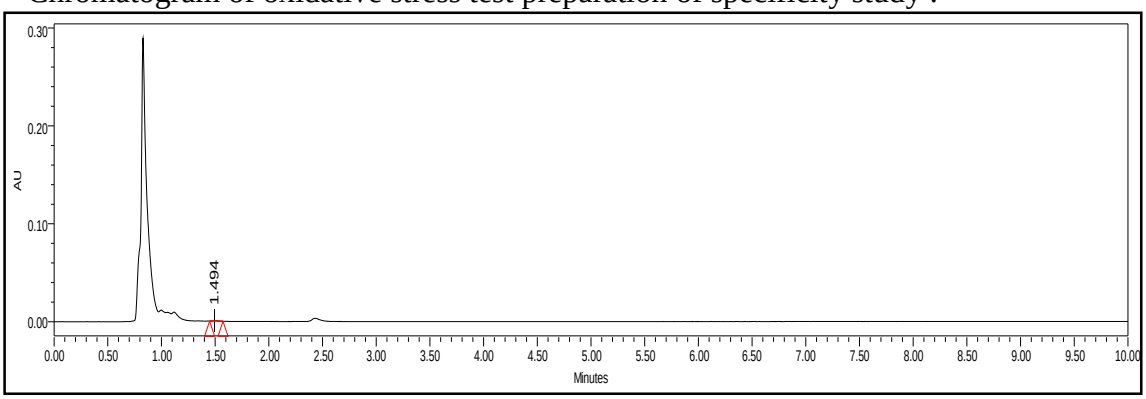
Chromatogram of alkali stress test preparation of specificity study :



Peak purity graph of alkali stress study of famotidine



Chromatogram of oxidative stress test preparation of specificity study :



Observations:

(1) Any interference was not observed from blank to the peak of interest, in addition to this peak purity was also within the acceptance criteria proved by the photo diode detector.

(2) From the above chromatogram it can be conclude that there is no interference of any degradation product to the peak of interest and impurity has been generated by each stress condition.

Table-2: Degradation result of stress condition

Degradation Condition	Time	Peak Purity		% Degradation
		Purity Threshold	Purity Angle	
Acidic	1 Hrs at 80°C	7.4	2.6	30.91%
Basic	0.5 Hrs at RT	9.8	3.7	28.36%
Oxidative	0.5 Hrs at RT	15.9	11.3	88.34%

5.3 Linearity and Range:

The linearity plot was prepared with 8 concentration levels (10, 20, 30, 40, 50, 60, 70,80 and 90 µg/ml of Famotidine). These concentration levels were respectively corresponding to 20, 40, 60, 80, 100, 120, 140, 160 and 180% of standard solution concentration. The peak areas vs. concentration data were evaluated by linear regression analysis.

Standard solution preparation:

Weigh accurately 50.07mg of famotidine standard and transferred into 100 ml volumetric flask. Added 70ml of diluent into the volumetric flask, dissolved by sonication and dilute up to the mark with diluent. The concentration obtained was 500.7 µg/ml of Famotidine.

5 ml of above standard stock solution was pipette out and transferred into 50 ml volumetric flask followed by diluted to volume with diluent. The concentration obtained is 50.07 µg/ml of Famotidine.

Linearity Standard Solution Preparation:

Stock Solution:

Weigh accurately 100.32 mg of famotidine standard and transferred into 200 ml volumetric flask. Added 140 ml of diluent into the volumetric flask, dissolved by

sonication and dilute up to the mark with diluent. The concentration obtained was 501.6 µg/ml of Famotidine.

Linearity Level 1 (20%):-

1 ml of stock solution was pipette out and transferred into 50 ml volumetric flak. The solution was diluted to volume with diluent. The concentration obtained is 10.03 µg/ml of Famotidine.

Linearity Level 2 (40%):-

2 ml of stock solution was pipette out and transferred into 50 ml volumetric flak. The solution was diluted to volume with diluent. The concentration obtained is 20.06 µg/ml of Famotidine.

Linearity Level 3 (60%):-

3 ml of stock solution was pipette out and transferred into 50 ml volumetric flak. The solution was diluted to volume with diluent. The concentration obtained is 30.09 µg/ml of Famotidine.

Linearity Level 4 (80%):-

4 ml of stock solution was pipette out and transferred into 50 ml volumetric flak. The solution was diluted to volume with diluent. The concentration obtained is 40.12 µg/ml of Famotidine.

Linearity Level 5 (100%):-

5 ml of stock solution was pipette out and transferred into 50 ml volumetric flak. The solution was diluted to volume with diluent. The concentration obtained is 50.16 µg/ml of Famotidine.

Linearity Level 6 (120%):-

6 ml of stock solution was pipette out and transferred into 50 ml volumetric flak. The solution was diluted to volume with diluent. The concentration obtained is 60.19 µg/ml of Famotidine.

Linearity Level 7 (140%):-

7 ml of stock solution was pipette out and transferred into 50 ml volumetric flak. The solution was diluted to volume with diluent. The concentration obtained is 70.22 µg/ml of Famotidine.

Linearity Level 8 (160%):-

8 ml of stock solution was pipette out and transferred into 50 ml volumetric flak. The solution was diluted to volume with diluent. The concentration obtained is 80.26 µg/ml of Famotidine.

Linearity Level 9 (180%):-

9 ml of stock solution was pipette out and transferred into 50 ml volumetric flak. The solution was diluted to volume with diluent. The concentration obtained is 90.29 µg/ml of Famotidine.

For each linearity level, the solution was injected in duplicate. Linearity was evaluated by linear regression analysis.

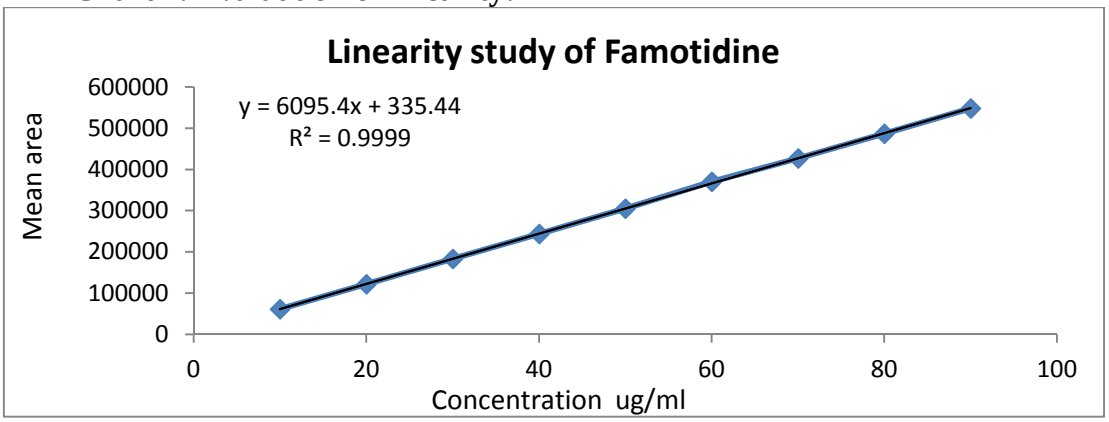
Table-3: Sequence of Linearity and range study

No.	Description	Injection Replicate
1	Blank	1
2	Standard Preparation	5
3	Linearity level-1 (20%)	2
4	Linearity level-2 (40%)	2
5	Linearity level-3 (60%)	2
6	Linearity level-4 (80%)	2
7	Linearity level-5 (100%)	2
8	Linearity level-6 (120%)	2
9	Linearity level-7 (140%)	2
10	Linearity level-8 (160%)	2
11	Linearity level-9 (180%)	2
12	Bracketing Standard	1

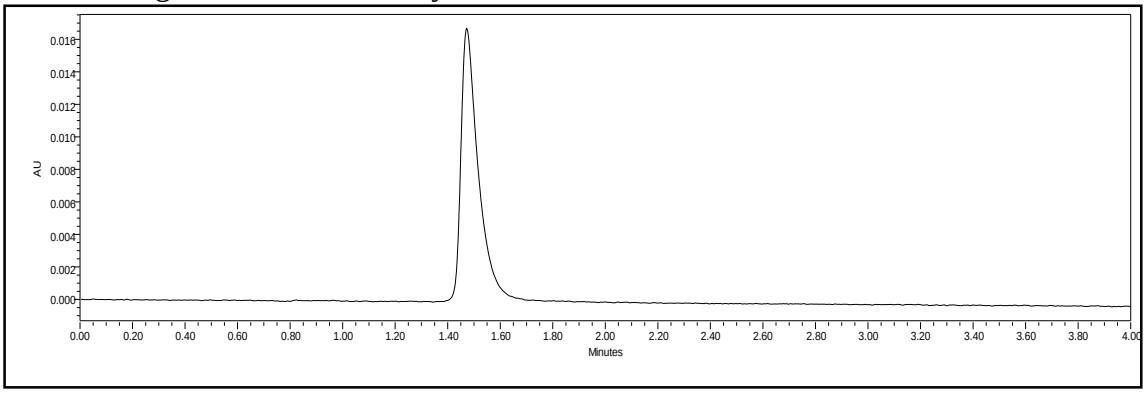
Table-4: Summary of linearity study

Linearity Level	% of Level	Concentration (µg/ml)	Mean Area
1	20	10	60864
2	40	20	121785
3	60	30	182861
4	80	40	243866
5	100	50	304970
6	120	60	370074
7	140	70	426847
8	160	80	486841
9	180	90	547835
Correlation Co-efficient			0.999
Slope			6095
Intercept			335.4

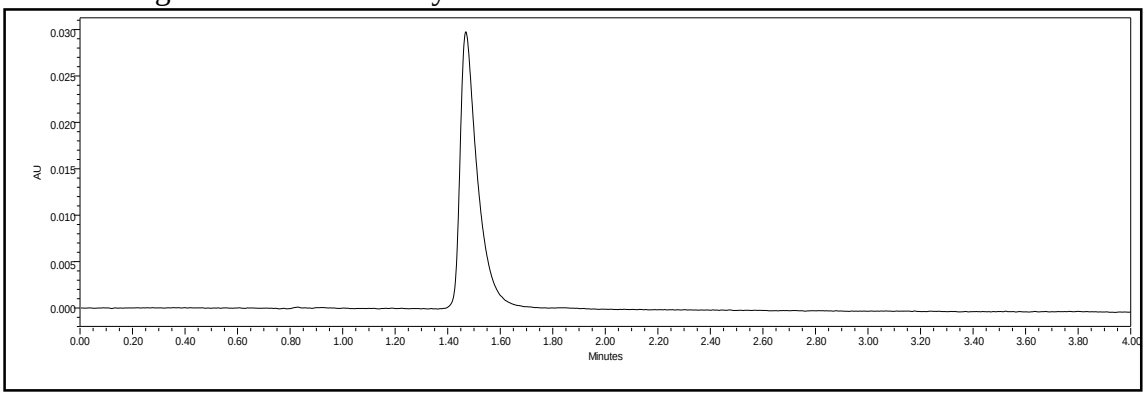
Chart 1: Evaluation of linearity:



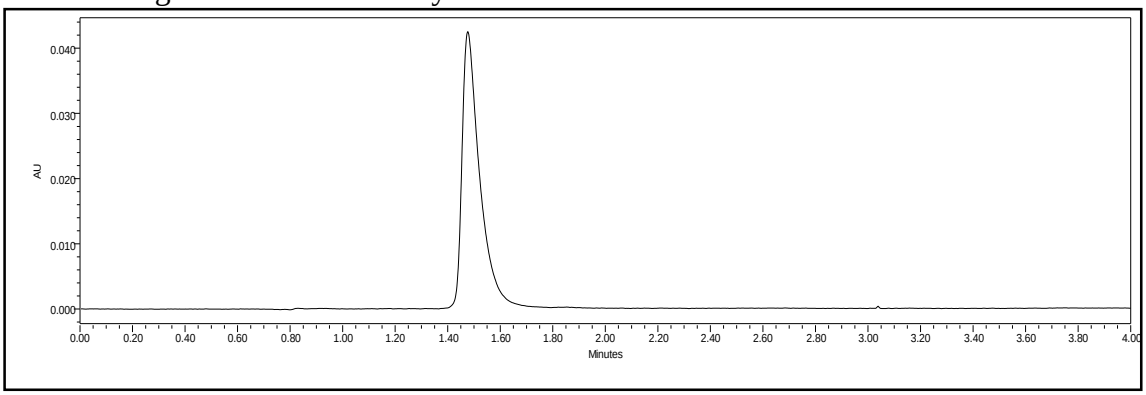
Chromatogram of 20% Linearity level:



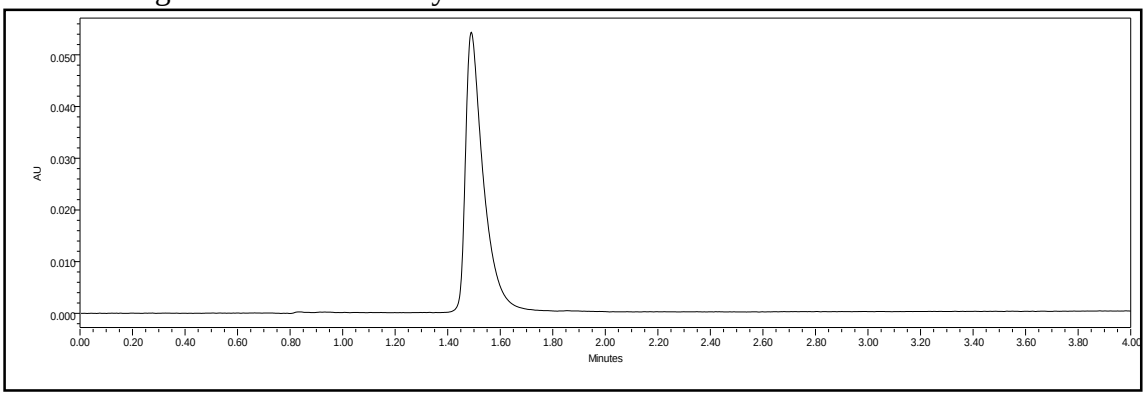
Chromatogram of 40% Linearity level:



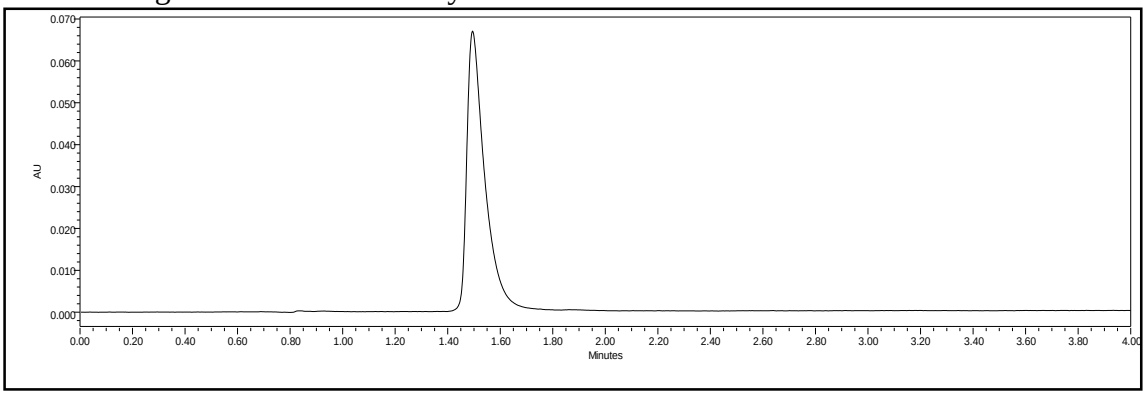
Chromatogram of 60% Linearity level:



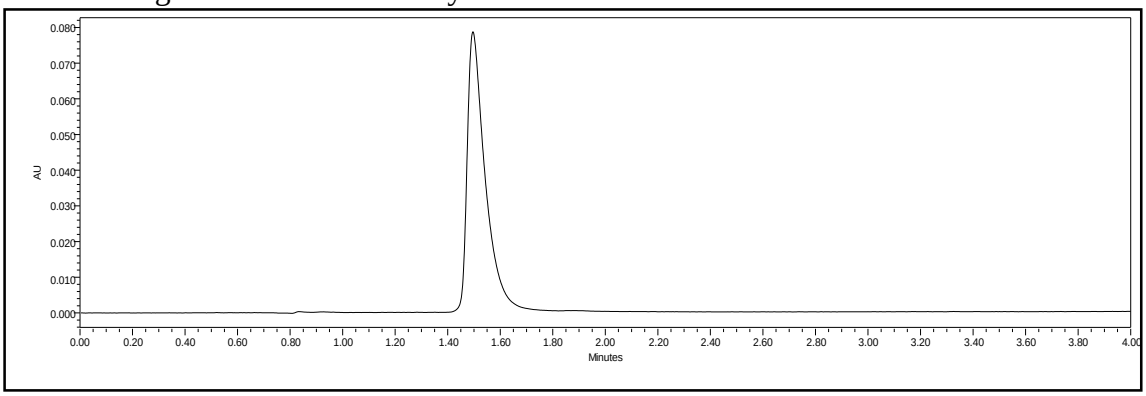
Chromatogram of 80% Linearity level:



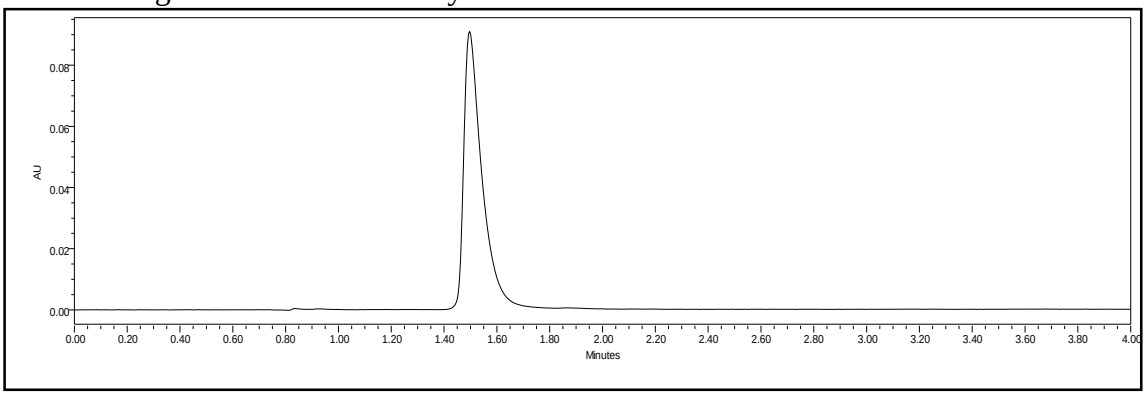
Chromatogram of 100% Linearity level:



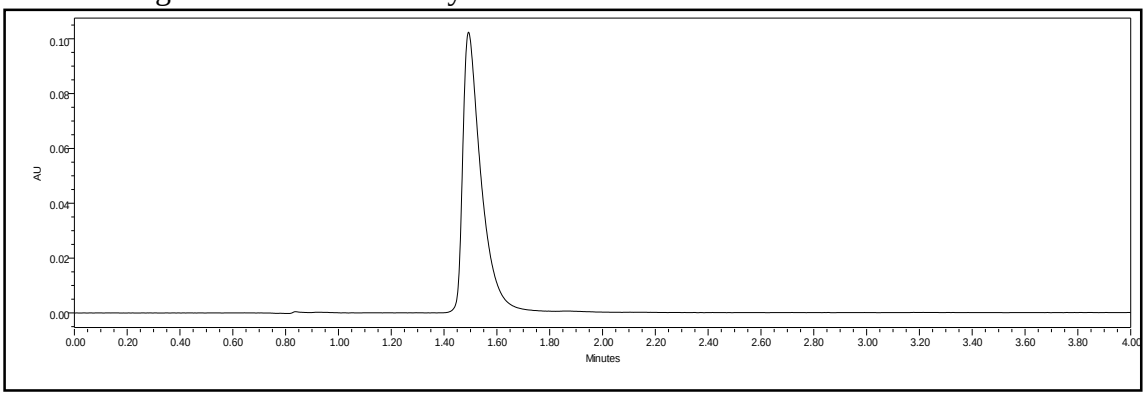
Chromatogram of 120% Linearity level:



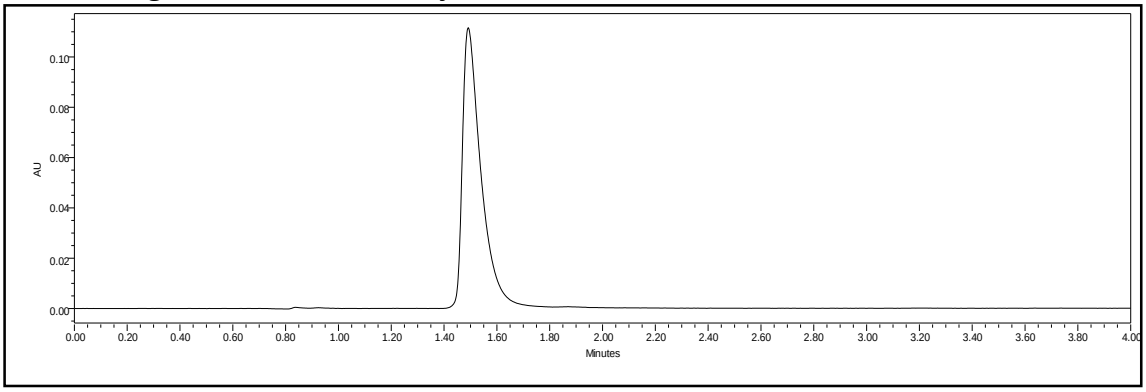
Chromatogram of 140% Linearity level:



Chromatogram of 160% Linearity level:



Chromatogram of 180% Linearity level:



Correlation coefficient of the linearity study was found to R^2 = 0.999 with linear regression equation y = 6095x + 335.5, which proves the method is highly linear over the working range 10 – 90 µg/ml.

5.4 Limit of detection and Limit of quantitation:

LOD is the lowest amount of the drug content which can be detected by the proposed method while LOQ is the lowest amount which can be quantified by the method. The guideline suggest minimum signal to noise ratio (S/N) more than 3.3 for LOD and more than 10 for LOQ. On the basis of linearity data theoretically it can be also calculated by the given formula,

$$LOD = 3.3\ \sigma/S \qquad LOQ = 10\ \sigma/S$$

Where σ = Residual Standard Deviation of regression line and S = Slope of regression line.

LOQ value is precised by six replicate injections and checked for linear response with respect to other linearity levels by extended linearity curve.

For LOD and LOQ study, blank, standard preparation, LOD preparation and LOQ preparation was prepared as under:

Blank preparation:

Diluent was used as blank.

Standard preparation:

Stock solution: Weigh accurately 50.14mg of famotidine standard and transferred into 100 ml volumetric flask. Added 70ml of diluent into the volumetric flask, dissolved by sonication and dilute up to the mark with diluent. The concentration obtained was 501.4 µg/ml of Famotidine.

5 ml of above standard stock solution was pipette out and transferred into 50 ml volumetric flask followed by diluted to volume with diluent. The concentration obtained is 50.14 µg/ml of Famotidine.

LOD and LOQ solution:

Solution-A:

Weigh accurately 50.06mg of famotidine standard and transferred into 100 ml volumetric flask. Added 70ml of diluent into the volumetric flask, dissolved by sonication and dilute up to the mark with diluent. The concentration obtained was 500.6 µg/ml of Famotidine.

5 ml of above standard stock solution was pipette out and transferred into 50 ml volumetric flask followed by diluted to volume with diluent. The concentration obtained is 50.06 µg/ml of Famotidine.

Pipette out 5 mL of this solution and transfer into 50 mL volumetric flask and dilute it up to the mark with diluents. The concentration obtained is 5.0 µg/ml of Famotidine. This solution is designated as Solution-A.

LOD preparation:

2 ml of above Solution-A was pipette out and transferred into 50 ml volumetric flask followed by diluted to volume with diluent. The concentration obtained is 0.2 µg/ml of Famotidine.

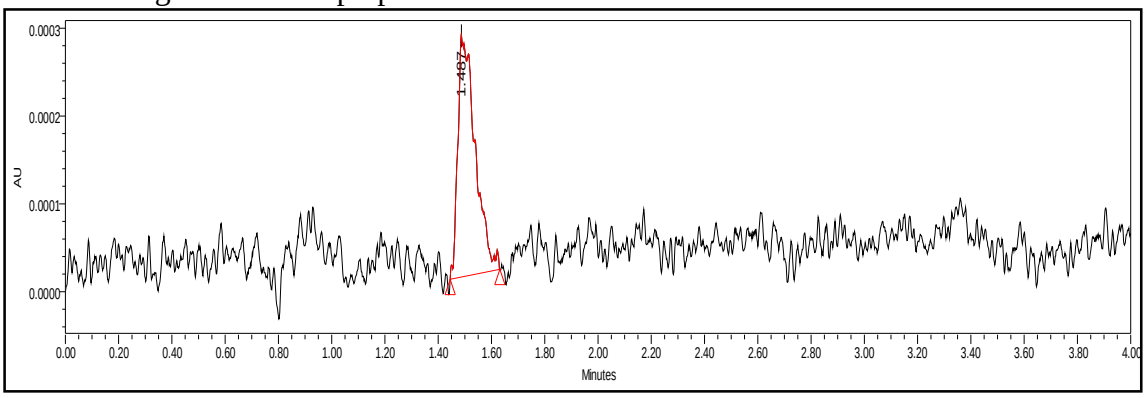
LOQ preparation:

5 ml of above Solution-A was pipette out and transferred into 50 ml volumetricflask followed by diluted to volume with diluent. The concentration obtained is 0.5 µg/ml of Famotidine.

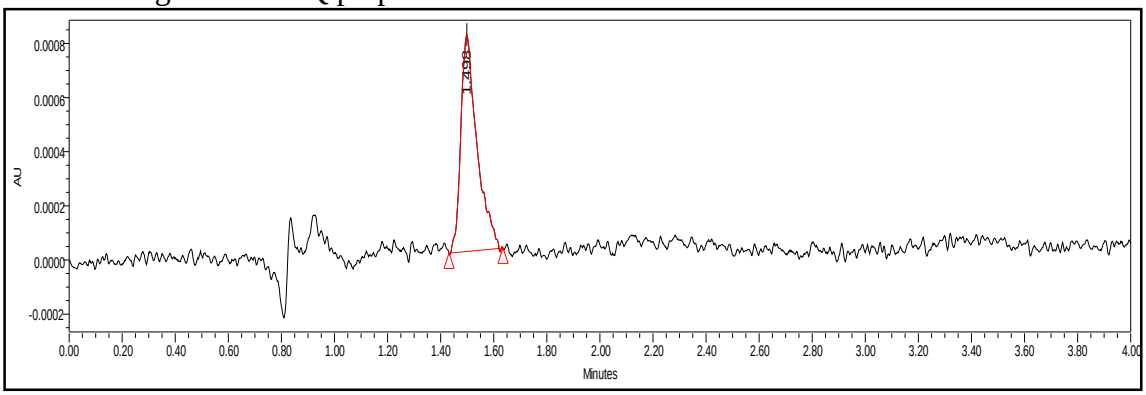
Table-5: Sequence of LOD and LOQ study

No.	Description	Injection Replicate
1	Blank	1
2	Standard Preparation	5
3	Blank	1
4	LOD	2
5	LOQ	6
6	Bracketing Standard	1

Chromatogram of LOD preparation:



Chromatogram of LOQ preparation:



LOQ of the analytical method can evaluated by establish linearity up to LOQ value. Hence linearity study is extended to LOQ value.

Chart 2: Confirmation of LOQ value by extended linearity study up to LOQ level

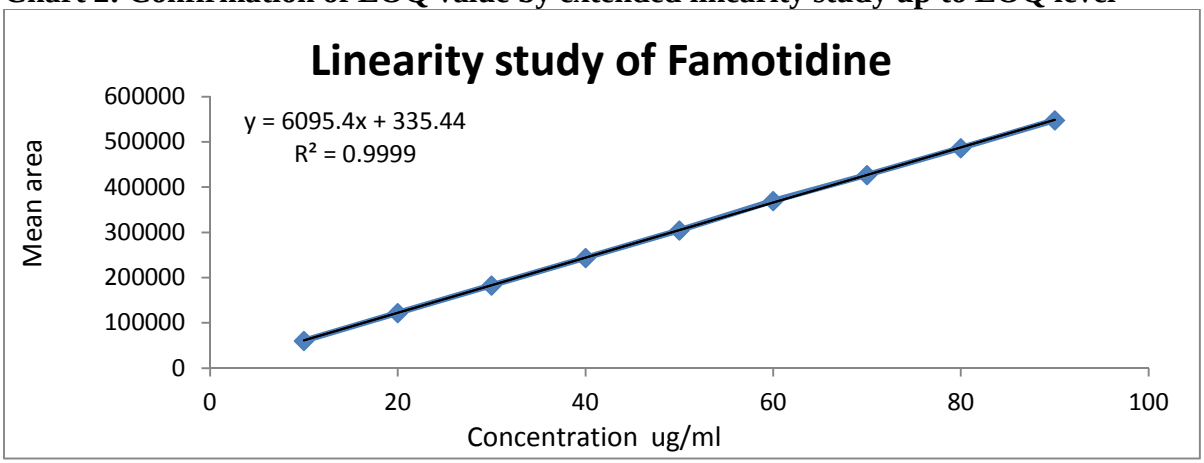


Table-6: Summary of LOD and LOQ study

Linearity Level	% of Level	Concentration (µg/ml)	Mean Area
1	LOQ	0.5	3388
2	20	10	60864
2	40	20	121785
3	60	30	182861
4	80	40	243866
5	100	50	304970
6	120	60	370074
7	140	70	426847
8	160	80	486841
9	180	90	547835
Correlation Co-efficient			0.999
Slope			6095
Intercept			337.1

All the results of LOD and LOQ data were within the acceptance criteria, hence it can be conclude that the LOD and LOQ of the method was 0.2 µg/ml and 0.5 µg/ml respectively which correspond to 0.4% and 1.0% of working concentration.

5.5 Precision Study:

Precision study was established by evaluating method precision and intermediate precision study.

Method precision of the analytical method was determined by analyzing six sets of sample preparation. Assay of all six replicate sample preparations was determined and mean % assay value, standard deviation, % relative standard deviation and 95% confidence interval for the same was calculated.

Intermediate precision of the analytical method was determined by performing method precision on another day by another analyst using different make of raw materials under same experimental condition. Assay of all six replicate sample preparations was determined and mean % assay value, standard deviation, % relative standard deviation and 95% confidence interval for the same was calculated. Overall assay value of method precision and intermediate precision was compared and % difference and overall % relative standard deviation was calculated.

For method precision, blank, standard preparation and six sets of test preparations was prepared as per method as under:

Blank preparation:

Diluent was used as blank.

Standard preparation:

Stock solution: Weigh accurately 50.06mg of famotidine standard and transferred into 100 ml volumetric flask. Added 70ml of diluent into the volumetric flask, dissolved by sonication and dilute up to the mark with diluent. The concentration obtained was 500.6 µg/ml of Famotidine.

5 ml of above standard stock solution was pipette out and transferred into 50 ml volumetric flask followed by diluted to volume with diluent. The concentration obtained is 50.06 µg/ml of Famotidine.

Test preparation:

Weigh accurately 50.01 mg of famotidine standard and transferred into 100 ml volumetric flask. Added 70ml of diluent into the volumetric flask, dissolved by sonication and dilute up to the mark with diluent. The concentration obtained was 500.1 µg/ml of Famotidine.

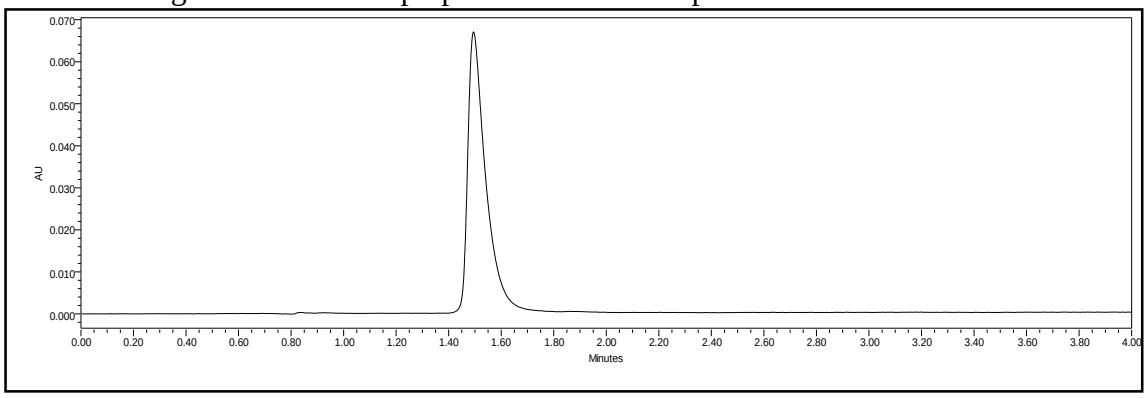
5 ml of above standard stock solution was pipette out and transferred into 50 ml volumetric flask followed by diluted to volume with diluent. The concentration obtained is 50.01 µg/ml of Famotidine.

The same procedure was used for preparing the six Test preparation Sets. Same approach was applied for the intermediate precision study on the second day with different analyst.

Table-7: Sequence of Precision study

No.	Description	Injection Replicate
1	Blank	1
2	Standard Preparation	5
3	Test preparation (Set-1)	2
4	Test preparation (Set-2)	2
5	Test preparation (Set-3)	2
6	Test preparation (Set-4)	2
7	Test preparation (Set-5)	2
8	Test preparation (Set-6)	2
9	Bracketing Standard	1

Chromatogram of Standard preparation in method precision



Chromatogram of sample solution in method precision

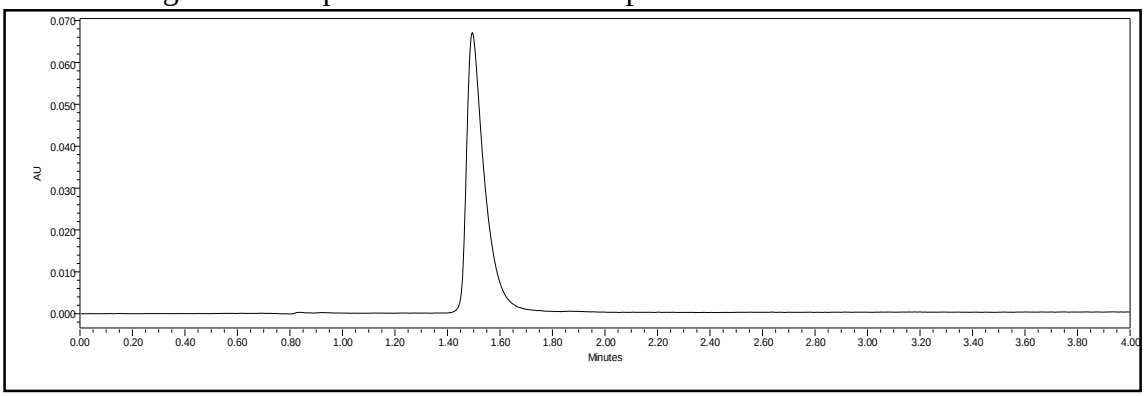


Table-8: Data for method precision

Observation					
Data for Standard preparation					
Replicate	Area		Standard Weight	50.06	
1	304852		Standard Potency	99.47	
2	304257				
3	304756				
4	305093				
5	305174				
Average	304826				
Stdev	361.15				
%RSD	0.12				
Data for Test preparation					
Set No.	Replicate	Area	Mean Area	Wt. of Sample	% Assay
1	1	304932	304831	50.01	99.57
	2	304729			
2	1	305127	305223	50.12	99.48
	2	305318			
3	1	305594	305303	50.29	99.17
	2	305011			
4	1	305623	305771	50.31	99.28
	2	305919			
5	1	304982	305014	50.06	99.53
	2	305046			
6	1	305113	305538	50.09	99.64
	2	305962			

% Assay calculation is as under:

$$\% \text{ Assay} = \frac{A_T}{A_S} x \frac{W_1}{100} x \frac{5}{50} x \frac{100}{W_2} x \frac{50}{5} x P$$

Where,

A_T = Average Area of Test Preparation.

A_S = Average Area of Standard Preparation.

W_1 = Weight of Working Standard (mg).

W_2 = Weight of Test Sample (mg).

P = Potency of Working Standard (%).

Table-9: Summary of Intermediate precision study

Observation

Data for Standard preparation

Replicate	Area		Standard Weight	50.07
1	304764		Standard Potency	99.47
2	305019			
3	304823			
4	305012			
5	305863			
Average	305096			
Stdev	443.29			
%RSD	0.15			

Data for Test preparation

Set No.	Replicate	Area	Mean Area	Wt. of Sample	% Assay
1	1	305046	304935	50.07	99.51
	2	304823			
2	1	305018	304969	50.14	99.38
	2	304919			
3	1	305263	305123	50.31	99.09
	2	304982			
4	1	305237	305580	50.27	99.32
	2	305922			
5	1	304726	304972	50.11	99.44
	2	305218			
6	1	305822	305579	50.01	99.84
	2	305335			

% Assay calculation is as under:

$$\% \text{ Assay} = \frac{A_T}{A_S} x \frac{W_1}{100} x \frac{5}{50} x \frac{100}{W_2} x \frac{50}{5} xP$$

Where,

A_T = Average Area of Test Preparation.

A_S = Average Area of Standard Preparation.

W_1 = Weight of Working Standard (mg).

W_2 = Weight of Test Sample (mg).

P = Potency of Working Standard (%).

Table-10: Summary of Precision study

Summary of precision study:				
Study	**%Assay**	**Mean Assay %**	**Std. Dev.**	**%RSD**
Method Precision	99.57	99.445	0.18	0.18%
	99.48			
	99.17			
	99.28			
	99.53			
	99.64			
Intermediate Precision	99.51	99.43	0.25	0.25%
	99.38			
	99.09			
	99.32			
	99.44			
	99.84			
Overall	99.4375			
	0.21			
	0.21%			

Overall the data for the precision study suggest % Assay value for each Test Preparation is between 98 – 102% which is under the acceptance criteria while % RSD of all results are less than 2%. Hence from all the observation it can conclude that the proposed method is highly precise.

5.6 Accuracy Study:

This Experiment can be performed by the recovery test. Recovery of the method was evaluated at 3 different concentration levels (Generally corresponding to 50, 100 and 150% of test solution concentration) by addition of known amounts of standard to placebo preparation. For each concentration level, 3 sets were prepared and injected in duplicate.

Blank preparation:

Diluent was used as blank.

Standard preparation:

Stock solution: Weigh accurately 50.02 mg of famotidine standard and transferred into 100 ml volumetric flask. Added 70ml of diluent into the volumetric flask, dissolved by sonication and dilute up to the mark with diluent. The concentration obtained was 500.2 µg/ml of Famotidine.

5 ml of above standard stock solution was pipette out and transferred into 50 ml volumetric flask followed by diluted to volume with diluent. The concentration obtained is 50.02 µg/ml of Famotidine.

Sample preparations for accuracy levels are as under:

Accuracy level 1 (50%):

Test stock solution:

Weigh accurately 25.05 mg of famotidine standard and transferred into 100 ml volumetric flask. Added 70ml of diluent into the volumetric flask, dissolved by sonication and dilute up to the mark with diluent. The concentration obtained was 250.5 µg/ml of Famotidine.

5 ml of above standard stock solution was pipette out and transferred into 50 ml volumetric flask followed by diluted to volume with diluent. The concentration obtained is 25.05 µg/ml of Famotidine.

The same procedure was applied for preparing the three sets.

Accuracy level 2 (100 %):

Test stock solution:

Weigh accurately 50.09 mg of famotidine standard and transferred into 100 ml volumetric flask. Added 70ml of diluent into the volumetric flask, dissolved by sonication and dilute up to the mark with diluent. The concentration obtained was 500.9 µg/ml of Famotidine.

5 ml of above standard stock solution was pipette out and transferred into 50 ml volumetric flask followed by diluted to volume with diluent. The concentration obtained is 50.09 µg/ml of Famotidine.

Famotidine. The same procedure was applied for preparing the three sets.

Accuracy level 3 (150 %):

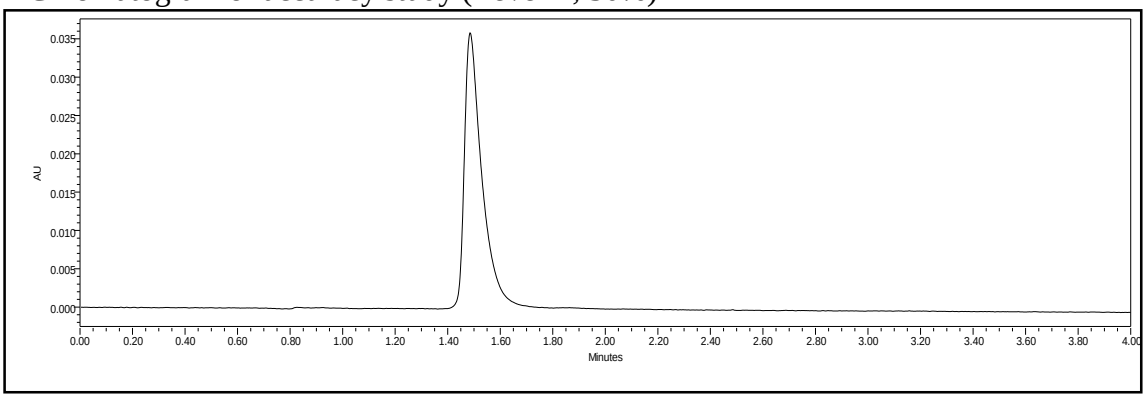
Test stock solution:

Weigh accurately 75.02 mg of famotidine standard and transferred into 100 ml volumetric flask. Added 70ml of diluent into the volumetric flask, dissolved by sonication and dilute up to the mark with diluent. The concentration obtained was 750.1 µg/ml of Famotidine.

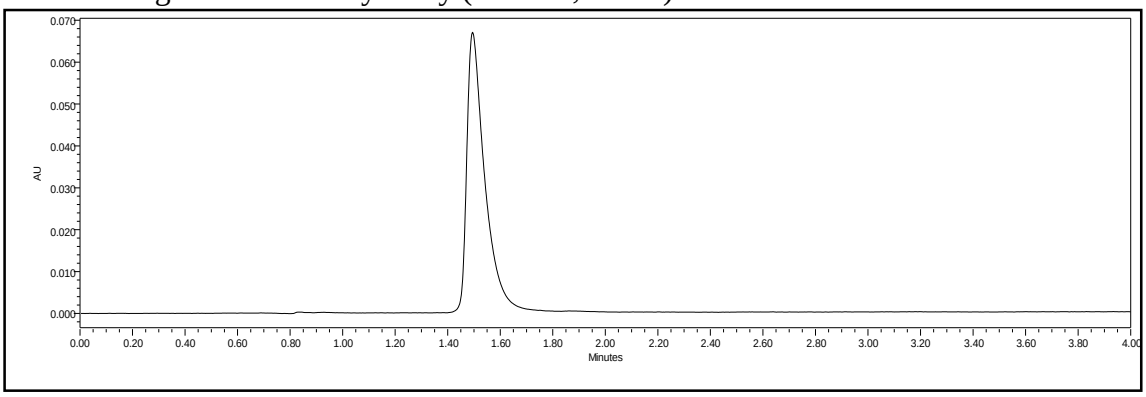
5 ml of above standard stock solution was pipette out and transferred into 50 ml volumetric flask followed by diluted to volume with diluent. The concentration obtained is 75.01 µg/ml of Famotidine.

The same procedure was applied for preparing the three sets

Chromatogram of accuracy study (Level-1, 50%)



Chromatogram of accuracy study (Level-2, 100%)



Chromatogram of accuracy study (Level-3, 150%)

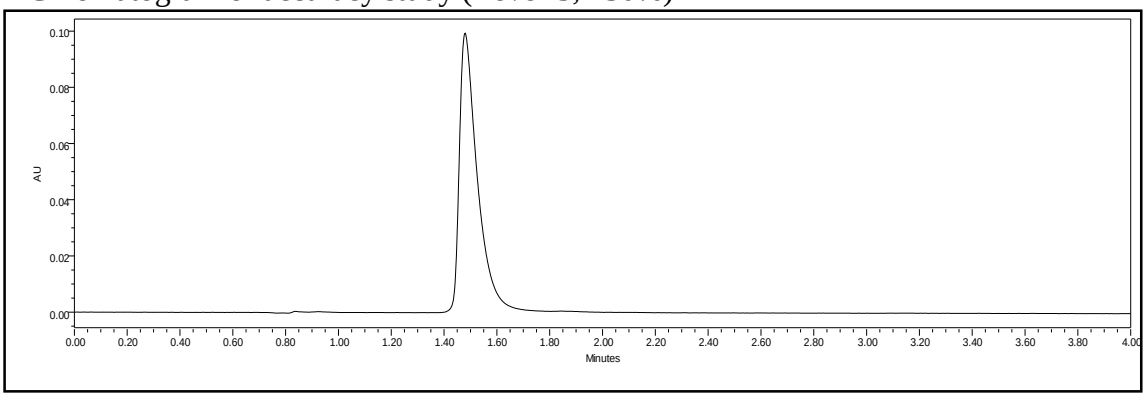


Table-11: Sequence of Accuracy Study

No.	Description	Injection Replicate
1	Blank	1
2	Standard Preparation	5
3	Accuracy level-1 preparation: Set-1	2
4	Accuracy level-1 preparation: Set-2	2
5	Accuracy level-1 preparation: Set-3	2
6	Accuracy level-2 preparation: Set-1	2
7	Accuracy level-2 preparation: Set-2	2
8	Accuracy level-2 preparation: Set-3	2
9	Accuracy level-3 preparation: Set-1	2
10	Accuracy level-3 preparation: Set-2	2
11	Accuracy level-3 preparation: Set-3	2
12	Bracketing Standare	1

Table-12: Summary of accuracy study

Accuracy (Recovery) Study							
Accuracy Level	**Set No**	**Amount added (µg/ml)**	**Amount Found (µg/ml)**	**Recovery (%)**	**Average recovery**	**Std Dev.**	**% RSD**
I (50%)	1	25.05	25.06	100.02	100.09	0.26	0.26
	2	25.12	25.09	99.87			
	3	25.17	25.26	100.37			
II (100%)	1	50.09	50.23	100.28	99.91	0.34	0.34
	2	50.01	49.92	99.83			
	3	50.16	49.97	99.61			
II (150%)	1	75.02	74.78	99.68	99.55	0.22	0.22
	2	75.01	74.76	99.67			
	3	75.11	74.58	99.29			

Calculation formulas for recovery study are as under:

$$\text{Amount added (µg/ml)} = \frac{Wt.taken}{Volume\,1} x \frac{Volume\,2}{Volume\,3} x\,1000$$

$$\text{Amount found (µg/ml)} = \frac{Mean\ area\ of\ test\ preparation}{Average\ area\ of\ standard\ preparation} x\ standard\ \text{conc.}$$

$$\%\ \text{Recovery} = \frac{Amount\ found}{Amount\ added} x\,100$$

Table-13: Data of accuracy study

Observation				
Data for Standard preparation				
Replicate	Area		Standard Weight	50.02
1	304863		Standard Potency	99.47
2	304515		Standard Conc.	50.02
3	304094			
4	305017			
5	304954			
Average	324873			
Stdev	384.83			
%RSD	0.12			
Data for Test preparation				
Accuracy Level	Set No	Replicate	Area	Mean Area
1(50%)	1	1	163118	162730
		2	162342	
	2	1	162853	162937
		2	163021	
	3	1	164203	164083
		2	163963	
2(100%)	1	1	304531	326244
		2	304956	
	2	1	324131	324247
		2	324363	
	3	1	324303	324527
		2	324751	
3(150%)	1	1	485953	485709
		2	485465	
	2	1	485349	485551
		2	485753	
	3	1	485209	484389
		2	483569	

From the all above data it has been proven that the % recovery is within the limit of 98 to 102 % this is in the limit of acceptance criteria and % RSD value of % recovery of replicate set is below 2 % .Hence this suggest that proposed method is highly accurate.

5.6 Robustness Study:

Robustness of the method was evaluated by assaying test solutions under slight but deliberate changes in analytical conditions, such as change in flow rate, change in

proportions of Buffer-Acetonitrile (82:18 and 78:22,v/v), Change in flow rate and change in column-lot.

5.6.1 Change in flow rate:

Blank preparation:

Diluent was used as blank.

Standard preparation:

Stock solution: Weigh accurately 50.17 mg of famotidine standard and transferred into 100 ml volumetric flask. Added 70ml of diluent into the volumetric flask, dissolved by sonication and dilute up to the mark with diluent. The concentration obtained was 501.7 µg/ml of Famotidine.

5 ml of above standard stock solution was pipette out and transferred into 50 ml volumetric flask followed by diluted to volume with diluent. The concentration obtained is 50.17 µg/ml of Famotidine.

Test preparation:

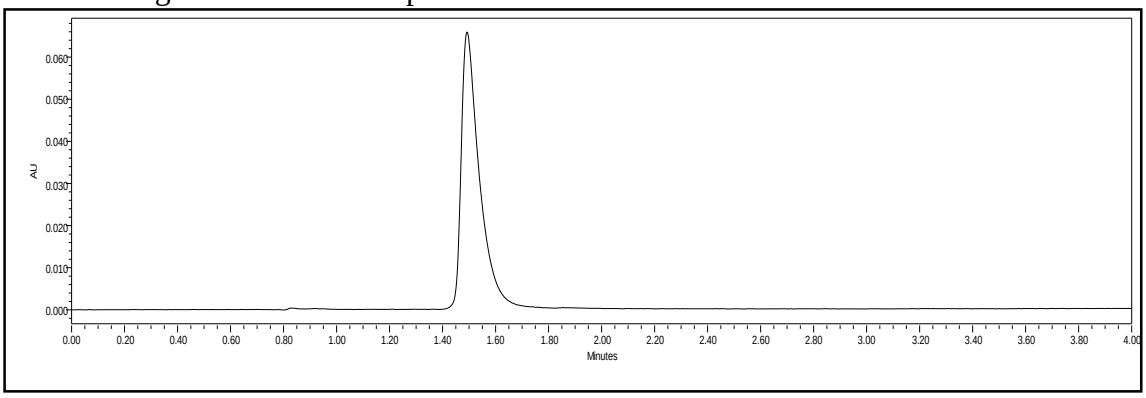
Weigh accurately 50.10 mg of famotidine standard and transferred into 100 ml volumetric flask. Added 70ml of diluent into the volumetric flask, dissolved by sonication and dilute up to the mark with diluent. The concentration obtained was 501.0 µg/ml of Famotidine.

5 ml of above standard stock solution was pipette out and transferred into 50 ml volumetric flask followed by diluted to volume with diluent. The concentration obtained is 50.10 µg/ml of Famotidine.

Table-14: Sequence for flow rate robustness study

No.	Description	Injection Replicate	Chromatographic Parameter
1	Blank	1	Flow rate: 0.27 mL/min
2	Standard Preparation	5	
3	Test Preparation	2	
4	Bracketing Standard	1	
5	Blank	1	Flow rate: 0.33 mL/min
6	Standard Preparation	5	
7	Test Preparation	2	
8	Bracketing Standard	1	

Chromatogram of robustness parameter flow rate 0.27 ml/min



Chromatogram of robustness parameter flow rate 0.33 ml/min

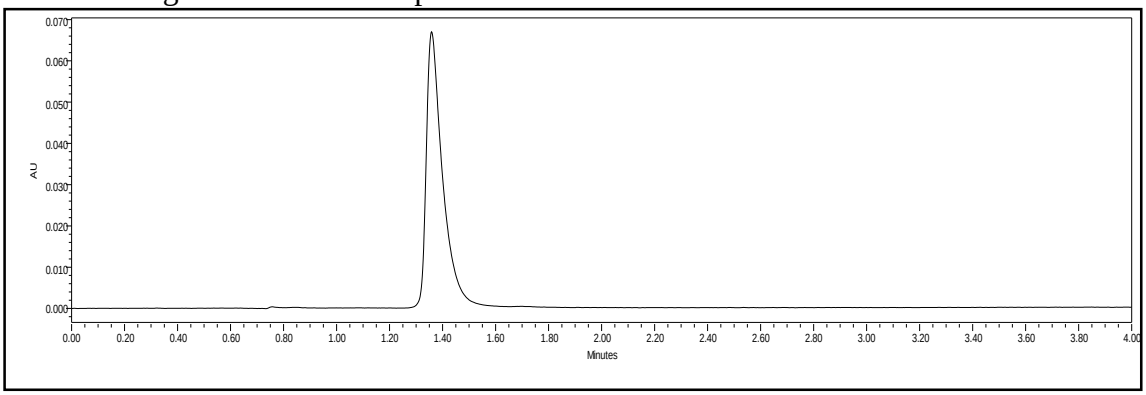


Table-15: Summary for flow change parameter of robustness study

At 0.27 mL/min flow rate		**At 0.33 mL/min flow rate**	
Data for standard preparation		*Data for standard preparation*	
Replicate	**Area**	**Replicate**	**Area**
1	321547	1	262415
2	321851	2	262853
3	322093	3	262171
4	321432	4	262689
5	321559	5	262017
Mean	321696	**Mean**	262429
Std.dev.	270.32	**Std.dev.**	347.81
%RSD	0.08	**%RSD**	0.13
Data for Test preparation		*Data for Test preparation*	
Replicate	**Area**	**Replicate**	**Area**
1	321920	1	262218
2	321496	2	261769
Mean	321708	**Mean**	261994
Standard wt. (mg)	50.17	Standard wt. (mg)	50.17
Test wt. (mg)	50.1	Test wt. (mg)	50.1
%Assay	99.61	%Assay	99.44

The calculation formula for the determination of assay is,

$$\% \text{ Assay} = \frac{A_T}{A_S} x \frac{W_1}{100} x \frac{5}{50} x \frac{100}{W_2} x \frac{50}{5} xP$$

Where,

A_T = Average Area of Test Preparation.

A_S = Average Area of Standard Preparation.

W_1 = Weight of Working Standard (mg).

W_2 = Weight of Test Sample (mg).

P = Potency of Working Standard (%).

5.6.2 Change in mobile phase composition:

In this experiment the test samples were analyzed at the mobile phase proportion of (Buffer:Acetonitrile) 80:20 and 78:22 v/v each and the results were observed in terms of assay value.

Blank preparation:

Diluent was used as blank.

Standard preparation:

Refer standard preparation under flow rate parameter of robustness study

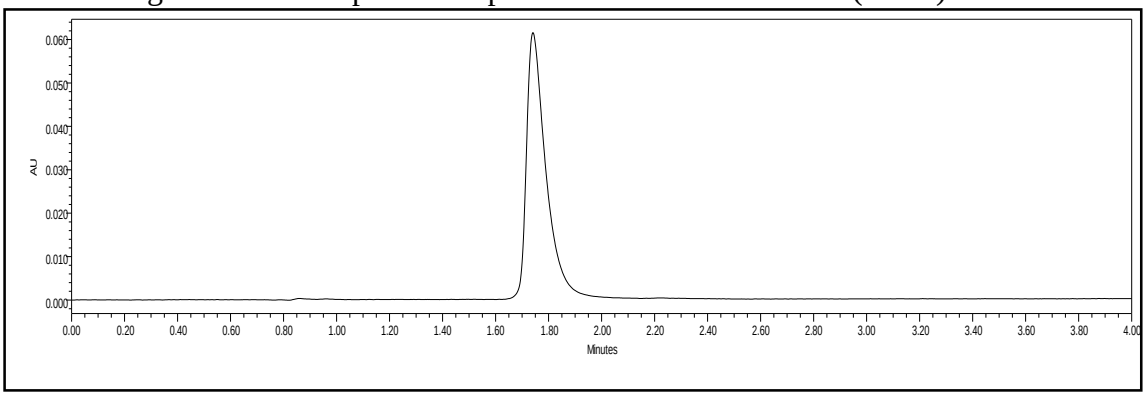
Test preparation:

Refer test preparation under flow rate parameter of robustness study.

Table-16: Sequence for change in composition robustness study

No.	Description	Injection Replicate	Chromatographic Parameter
1	Blank	1	Buffer:Acetonitrile (82:18)
2	Standard Preparation	5	
3	Test Preparation	2	
4	Bracketing Standard	1	
5	Blank	1	Buffer:Acetonitrile (78:22)
6	Standard Preparation	5	
7	Test Preparation	2	
8	Bracketing Standard	1	

Chromatogram of mobile phase composition Buffer:Acetonitrile (82:18)



Chromatogram mobile phase composition Buffer:Acetonitrile (78:22)

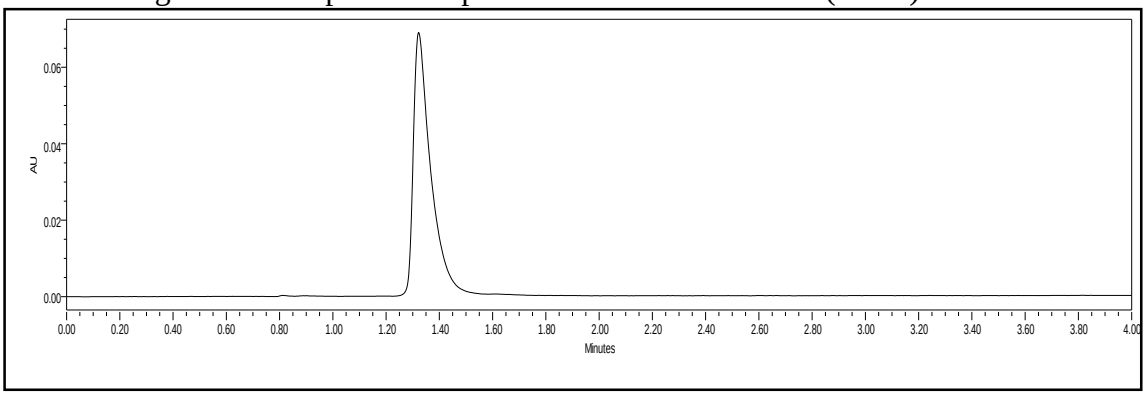


Table-17: Summary for change mobile phase composition

Buffer:Acetonitrile (82:18)		**Buffer:Acetonitrile (78:22)**	
Data for standard preparation		*Data for standard preparation*	
Replicate	**Area**	**Replicate**	**Area**
1	315589	1	287814
2	315017	2	287156
3	315851	3	287449
4	314935	4	287930
5	315449	5	286993
Mean	315368	**Mean**	287468
Std.dev.	387.10	**Std.dev.**	405.12
%RSD	0.12	**%RSD**	0.14
Data for Test preparation		*Data for Test preparation*	
Replicate	**Area**	**Replicate**	**Area**
1	315214	1	286635
2	315589	2	287195
Mean	315402	**Mean**	286915
Standard wt. (mg)	50.14	Standard wt. (mg)	50.14
Test wt. (mg)	50.11	Test wt. (mg)	50.11
%Assay	99.36	%Assay	99.19

The calculation formula for the determination of assay is,

$$\% \text{ Assay} = \frac{A_T}{A_S} x \frac{W_1}{100} x \frac{5}{50} x \frac{100}{W_2} x \frac{50}{5} xP$$

Where,

A_T = Average Area of Test Preparation.

A_S = Average Area of Standard Preparation.

W_1 = Weight of Working Standard (mg).

W_2 = Weight of Test Sample (mg).

P = Potency of Working Standard (%).

5.6.3 Robust Parameter: Change column lot:

In this parameter, column used in analytical method was changed to different lot. Sample was assayed by changing the lot of column.

Blank preparation:

Diluent was used as blank.

Standard preparation:

Refer standard preparation under flow rate parameter of robustness study

Test preparation:

Refer standard preparation under flow rate parameter of robustness study

Table-18: Sequence for change in composition robustness study

No.	Description	Injection Replicate	Chromatographic Parameter
1	Blank	1	Change column lot
2	Standard Preparation	5	
3	Test Preparation	2	
4	Bracketing Standard	1	

Chromatogram of robustness parameter column change

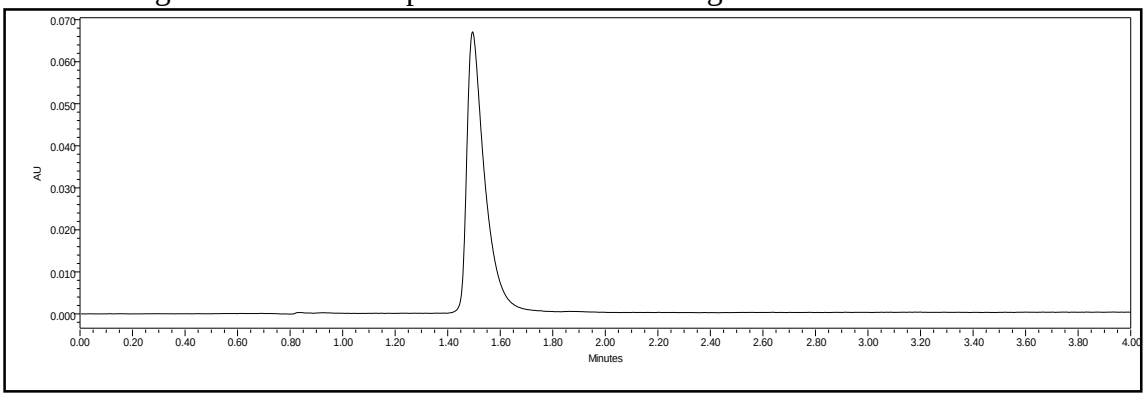


Table-19: Summary for column change parameter of robustness study

Column lot change	
Data for standard preparation	
Replicate	**Area**
1	304963
2	304284
3	303941
4	303147
5	304651
Mean	304197.2
Std.dev.	701.51
%RSD	0.23
Data for Test preparation	
Replicate	**Area**
1	304289
2	304807
Mean	304548
Standard wt. (mg)	50.14
Test wt. (mg)	50.11
%Assay	99.64

The calculation formula for the determination of assay is,

$$\% \text{ Assay} = \frac{A_T}{A_S} x \frac{W_1}{100} x \frac{5}{50} x \frac{100}{W_2} x \frac{50}{5} xP$$

Where,

A_T = Average Area of Test Preparation.

A_S = Average Area of Standard Preparation.

W_1 = Weight of Working Standard (mg).

W_2 = Weight of Test Sample (mg).

P = Potency of Working Standard (%).

Table-20: Summary of robustness study

Summary of Robustness Study				
Robust Condition	**% Assay**	**Retention time (min.)**	**System Suitability**	
			Theoretical Plates	**Asymmetry**
Flow Change 0.27 ml/min	9.61	1.58	5374	1.19
Flow Change 0.33ml/min	99.44	1.36	5239	1.14
MP Proportion Change A:B= 82:18	99.36	1.74	5253	1.21
MP Proportion Change A:B= 78:22	99.19	1.32	5182	1.18
Column Lot Change	99.67	1.49	5832	1.15

The data and the chromatogram given above suggest that there is no considerable influence of the change in flow rate, mobile phase composition and column lot change on the result of the analysis by this method or on chromatographic suitability of this method. Hence, it can be conclude from this experiment that the method is highly robust.

5.7 Solution stability study:

Solution stability period for the solutions of standard preparation and test preparation was evaluated. The solutions were stored at 5° C and ambient temperature without protection against light and tested at interval of 6, 12 and 24 hrs. The responses for the aged solution were evaluated using a freshly prepared standard solution..

Blank preparation:

Diluent was used as blank.

Standard preparation:

Stock solution: Weigh accurately 50.14 mg of famotidine standard and transferred into 100 ml volumetric flask. Added 70ml of diluent into the volumetric flask, dissolved by sonication and dilute up to the mark with diluent. The concentration obtained was 501.4 µg/ml of Famotidine.

5 ml of above standard stock solution was pipette out and transferred into 50 ml volumetric flask followed by diluted to volume with diluent. The concentration obtained is 50.14 µg/ml of Famotidine.

Test preparation:

Stock solution: Weigh accurately 50.21 mg of famotidine standard and transferred into 100 ml volumetric flask. Added 70ml of diluent into the volumetric flask, dissolved by sonication and dilute up to the mark with diluent. The concentration obtained was 502.1 µg/ml of Famotidine.

5 ml of above standard stock solution was pipette out and transferred into 50 ml volumetric flask followed by diluted to volume with diluent. The concentration obtained is 50.21 µg/ml of Famotidine.

Table-21: Summary of solution stability

Time intervals	Absolute difference in assay for standard solution %		Absolute difference in assay for sample solution %	
	At 5°C	At room temperature	At 5°C	At room temperature
After 6 hours	0.16	0.19	0.13	0.17
After 12 hours	0.29	0.31	0.22	028
After 24 hours	0.59	0.92	0.55	0.89

Conclusion:

Solution stability time period for standard solution is 24 hours at 5°C and room temperature. Solution stability time period for test solution is 24 hours at 5°C and at room temperature .

5.8 System suitability study:

A system suitability test for the chromatographic system was performed before each validation experiment. Five replicate injections of standard preparation were injected and asymmetry, theoretical plate and % RSD of peak area were determined for same. Only after the system suitability results were in acceptance criteria the experiments were precede further.

The Theoretical plates should be more than 5000, Asymmetry should be less than 2.0 and % RSD should be less than 2.0. As the data suggest the system suitability was within the criteria in each validation experiment. Hence the system was found suitable to perform

the validation experiment which confirms the reliability of the data generated during the method validation.

Table-22: Summary of system suitability test

Summary of System Suitability Test			
Experiment Name	Theoretical Plates	Asymmetry	% RSD
Specificity	5234	1.17	0.14
Linearity and Range	5148	1.14	0.17
LOD and LOQ	5324	1.16	1.9
Method Precision	5289	1.17	0.12
Int. Precision	5228	1.16	0.15
Accuracy	5109	1.16	0.12
Robustness	5223	1.17	1.21
Solution Stability	5218	1.12	0.10

6. IMPPURITY CHARACTERIZATION BY SINGLE CRYSTAL XRD:

Degradation procedure:

Famotidine is dissolved in diluent and basic condition generated using sodium hydroxide solution. Famotidine degraded completely into an impurity within 1hours at room temperature. Impurity recovered by vaccume distillation. Impurity sample washed with small quantity of water and crystallization performed in methanol. Recrystallization of impurity carry out in methanol till desired purity of impurity (purity > 98%) obtained. Crystal obtained were examine under microscope, Two to three good crystals were selected and cut it into proper shape. Structure of the impurity were determined by single crystal XRD analysis.

Data collection:

A colorless prism crystal of $C_8H_{14}N_6S_2$ having approximate dimensions of 0.640 x 0.550 x 0.460 mm was mounted on a glass fiber. All measurements were made on a Rigaku SCX mini diffractometer using graphite monochromated Mo-Ka radiation.

The crystal-to-detector distance was 52.00 mm.

Cell constants and an orientation matrix for data collection corresponded to a primitive monoclinic cell with dimensions:

a = 5.507(1) Å

b = 15.989(3) Å β = 98.509(4)^o

c = 16.896(4) Å

$V = 1471.3(5)\ Å^3$

For Z = 4 and F.W. = 258.36, the calculated density is 1.166 g/cm^3. The reflection conditions of:

h0l: l = 2n

0k0: k = 2n

uniquely determine the space group to be:

$P2_1/c$ (#14)

The data were collected at a temperature of 20 ± 1°C to a maximum 2θ value of 54.5°. A total of 540 oscillation images were collected. A sweep of data was done using w oscillations from -120.0 to 60.0° in 1.0° steps. The exposure rate was 10.0 [sec./°]. The detector swing angle was -30.80°. A second sweep was performed using w oscillations from -120.0 to 60.0° in 1.0° steps. The exposure rate was 10.0 [sec./°]. The detector swing angle was -30.80°. Another sweep was performed using w oscillations from -120.0 to 60.0° in 1.0° steps. The exposure rate was 10.0 [sec./°]. The detector swing angle was -30.80°. The crystal-to-detector distance was 52.00 mm. Readout was performed in the 0.146 mm pixel mode.

Data Reduction:

Of the 14152 reflections that were collected, 3279 were unique (R_{int} = 0.0199); equivalent reflections were merged. Data were collected and processed using CrystalClear (Rigaku).

The linear absorption coefficient, m, for Mo-Ka radiation is 3.486 cm^{-1}. An empirical absorption correction was applied which resulted in transmission factors ranging from 0.731 to 0.852. The data were corrected for Lorentz and polarization effects.

Structure Solution and Refinement:

The structure was solved by direct methods[15] and expanded using Fourier techniques. The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were refined using the riding model. The final cycle of full-matrix least-squares refinement[16] on F^2 was based on 3279 observed reflections and 145 variable parameters and converged (largest parameter shift was 0.03 times its esd) with unweighted and weighted agreement factors of:

$$R1 = \Sigma \, ||Fo| - |Fc|| \, / \, \Sigma \, |Fo| = 0.3675$$

$$wR2 = [\Sigma \, (w \, (Fo^2 - Fc^2)^2) / \Sigma \, w(Fo^2)^2]^{1/2} = 0.7656$$

The standard deviation of an observation of unit weight[17] was 4.81. Unit weights were used. The maximum and minimum peaks on the final difference Fourier map corresponded to 15.24 and -1.64 $e^-/Å$[16], respectively.

Neutral atom scattering factors were taken from Cromer and Waber[18]. Anomalous dispersion effects were included in Fcalc[19]; the values for Df' and Df'' were those of Creagh and McAuley[20]. The values for the mass attenuation coefficients are those of Creagh and Hubbell[21]. All calculations were performed using the CrystalStructure[22] crystallographic software package except for refinement, which was performed using SHELXL-97[23].

Crystal Data :

Empirical Formula	: $C_8H_{14}N_6S_2$
Formula Weight	: 258.36
Crystal Color, Habit	: Colorless, prism
Crystal Dimensions	: 0.640 X 0.550 X 0.460 mm
Crystal System	: Monoclinic
Lattice Type	: Primitive
Lattice Parameters	a = 5.507(1) Å
	b = 15.989(3) Å
	c = 16.896(4) Å
	β = 98.509(4) ^{o}
	V = 1471.3(5) $Å^3$
Space Group	: $P2_1/c$ (#14)
Z value	: 4
D_{calc}	: 1.166 g/cm^3
F_{000}	: 544.00
μ(MoKα)	: 3.486 cm^{-1}

Intensity Measurements :

Diffractometer	: SCX mini
Radiation	: MoKα (λ = 0.71075 Å)
	graphite monochromated

Voltage, Current	: 50kV, 30mA
Temperature	: 20.0°C
Detector Aperture	: 75 mm (diameter)
Data Images	: 540 exposures
ω oscillation Range	: -120.0 - 60.0°
Exposure Rate	: 10.0 sec./°
Detector Swing Angle	: -30.80°
ω oscillation Range	: -120.0 - 60.0°
Exposure Rate	: 10.0 sec./°
Detector Swing Angle	: -30.80°
ω oscillation Range	: -120.0 - 60.0°
Exposure Rate	: 10.0 sec./°
Detector Swing Angle	: -30.80°
Detector Position	: 52.00 mm
Pixel Size	: 0.146 mm
$2\theta_{max}$	: 54.5°
No. of Reflections Measured	: Total: 14152
	: Unique: 3279 (R_{int} = 0.0199)
Corrections	: Lorentz-polarization
	Absorption
	(trans. factors: 0.731 - 0.852)

Structure Solution and Refinement:

Structure Solution	: Direct Methods (SIR92)
Refinement	: Full-matrix least-squares on F^2
Function Minimized	: Σ w $(Fo^2 - Fc^2)^2$
Least Squares Weights	: $w = 1/ [\sigma^2(Fo^2) + (0.2000 \cdot P)^2$
	$+ 0.0000 \cdot P]$
	where $P = (Max(Fo^2,0) + 2Fc^2)/3$
$2\theta_{max}$ cutoff	: 54.5°
Anomalous Dispersion	: All non-hydrogen atoms

No. Observations (All reflections)	: 3279
No. Variables	: 145
Reflection/Parameter Ratio	: 22.61
Residuals: R1 (I>2.00σ(I))	: 0.3675
Residuals: R (All reflections)	: 0.3806
Residuals: wR2 (All reflections)	: 0.7656
Goodness of Fit Indicator	: 4.813
Max Shift/Error in Final Cycle	: 0.029
Maximum peak in Final Diff. Map	: 15.24 $e^-/Å^3$
Minimum peak in Final Diff. Map	: -1.64 $e^-/Å^3$

Table-23: Atomic coordinates and B_{iso}/B_{eq}

Atom	x	Y	z	Beq
S1	1.0686(7)	0.2347(3)	0.6910(3)	4.7(2)
S2	0.9208(6)	0.5392(3)	0.6042(2)	4.0(2)
N1	0.750(2)	0.3447(8)	0.6326(7)	3.8(3)
N2	0.654(3)	0.2036(9)	0.5904(8)	4.5(3)
N3	0.358(2)	0.3030(7)	0.5259(7)	3.7(2)
N4	0.307(3)	0.1596(7)	0.501(1)	4.9(3)
N5	1.109(3)	0.7241(8)	0.5702(8)	4.5(3)
N6	1.359(2)	0.7736(7)	0.6786(8)	4.0(3)
C1	1.126(3)	0.341(2)	0.723(1)	5.1(4)
C2	0.939(2)	0.3864(8)	0.6851(6)	3.0(2)
C3	0.796(3)	0.2679(8)	0.6323(7)	3.1(2)
C4	0.443(3)	0.2275(9)	0.5389(9)	3.9(3)
C5	0.921(3)	0.481(2)	0.6991(8)	8.2(9)
C6	1.254(3)	0.556(1)	0.606(1)	4.6(3)
C7	1.347(2)	0.6307(9)	0.6589(8)	3.5(3)
C8	1.253(3)	0.715(2)	0.6302(9)	4.5(4)

$B_{eq} = 8/3\ \pi^2(U_{11}(aa^*)^2 + U_{22}(bb^*)^2 + U_{33}(cc^*)^2 + 2U_{12}(aa^*bb^*)\cos\gamma + 2U_{13}(aa^*cc^*)\cos\beta + 2U_{23}(bb^*cc^*)\cos\alpha)$

Table-24: Atomic coordinates and B_{iso} involving hydrogen atoms

Atom	x	Y	z	Biso
H1	1.2593	0.3606	0.7581	6.17
H2	0.6953	0.1519	0.5965	5.37
H3A	0.4342	0.3447	0.5504	4.44
H3B	0.2263	0.3112	0.4927	4.44
H4A	0.1712	0.1686	0.4703	5.90
H4B	0.3611	0.1094	0.5091	5.90
H5A	1.0587	0.4987	0.7379	9.90
H5B	0.7711	0.4929	0.7208	9.90
H5	1.0799	0.6825	0.5506	5.44
H6A	1.3269	0.8255	0.6682	4.83
H6B	1.4608	0.7601	0.7203	4.83
H6C	1.3424	0.5060	0.6256	5.52
H6D	1.2865	0.5660	0.5516	5.52
H7A	1.3036	0.6215	0.7118	4.15
H7B	1.5252	0.6317	0.6644	4.15

Table-25: Anisotropic displacement parameters

Atom	U11	U22	U33	U12	U13	U23
S1	0.052(3)	0.063(3)	0.063(3)	0.021(2)	0.007(2)	0.019(2)
S2	0.041(2)	0.049(3)	0.058(3)	-0.007(2)	-0.002(2)	0.009(2)
N1	0.032(5)	0.051(7)	0.059(7)	0.014(4)	0.003(4)	0.026(5)
N2	0.068(8)	0.045(6)	0.057(7)	-0.020(6)	0.009(6)	-0.013(5)
N3	0.044(6)	0.037(6)	0.052(6)	0.000(5)	-0.018(5)	0.001(5)
N4	0.068(8)	0.029(6)	0.085(9)	-0.005(5)	-0.004(7)	-0.009(6)
N5	0.067(8)	0.050(7)	0.049(6)	-0.007(6)	-0.013(6)	0.006(5)
N6	0.042(6)	0.040(6)	0.066(7)	-0.007(5)	-0.012(5)	-0.011(5)
C1	0.036(7)	0.09(2)	0.070(9)	0.002(7)	-0.003(6)	0.034(9)
C2	0.038(6)	0.043(6)	0.029(5)	-0.003(5)	-0.007(4)	0.006(5)
C3	0.039(6)	0.040(6)	0.035(6)	0.003(5)	-0.001(5)	0.003(5)
C4	0.047(7)	0.052(8)	0.047(7)	-0.017(6)	-0.001(5)	-0.012(6)
C5	0.028(6)	0.26(4)	0.019(5)	-0.00(1)	-0.016(5)	0.03(1)
C6	0.064(9)	0.043(8)	0.07(1)	-0.005(6)	0.023(8)	-0.018(7)
C7	0.029(5)	0.049(7)	0.049(7)	-0.003(5)	-0.005(5)	0.006(5)
C8	0.036(7)	0.08(1)	0.058(8)	-0.030(7)	0.014(6)	-0.025(7)

The general temperature factor expression: $\exp(-2\pi^2(a^{*2}U_{11}h^2 + b^{*2}U_{22}k^2 + c^{*2}U_{33}l^2 + 2a^*b^*U_{12}hk + 2a^*c^*U_{13}hl + 2b^*c^*U_{23}kl))$

Table-26:Bond lengths (Å)

Atom	Atom	distance		Atom	Atom	Distance
S1	C1	1.79(2)		S1	C3	1.757(12)
S2	C5	1.86(2)		S2	C6	1.851(17)
N1	C2	1.428(15)		N1	C3	1.254(17)
N2	C3	1.416(18)		N2	C4	1.396(19)
N3	C4	1.300(18)		N4	C4	1.415(19)
N5	C8	1.200(19)		N6	C8	1.33(2)
C1	C2	1.34(2)		C2	C5	1.53(4)
C6	C7	1.54(2)		C7	C8	1.49(3)

Table 27. Bond lengths involving hydrogens (Å)

Atom	Atom	distance		Atom	Atom	distance
N2	H2	0.86		N3	H3A	0.86
N3	H3B	0.86		N4	H4A	0.86
N4	H4B	0.86		N5	H5	0.75
N6	H6A	0.86		N6	H6B	0.86
C1	H1	0.93		C5	H5A	0.97
C5	H5B	0.97		C6	H6C	0.97
C6	H6D	0.97		C7	H7A	0.97
C7	H7B	0.97				

Table 28. Bond angles (°)

Atom	Atom	Atom	angle		Atom	Atom	Atom	angle
C1	S1	C3	88.9(7)		C5	S2	C6	100.8(7)
C2	N1	C3	109.4(10)		C3	N2	C4	117.3(12)
S1	C1	C2	106.4(11)		N1	C2	C1	118.6(13)
N1	C2	C5	119.8(10)		C1	C2	C5	121.5(12)
S1	C3	N1	116.7(9)		S1	C3	N2	115.2(10)
N1	C3	N2	128.1(11)		N2	C4	N3	127.2(13)
N2	C4	N4	113.9(13)		N3	C4	N4	118.9(12)
S2	C5	C2	110.7(11)		S2	C6	C7	111.5(11)
C6	C7	C8	115.9(11)		N5	C8	N6	127.3(17)
N5	C8	C7	122.9(15)		N6	C8	C7	109.7(12)

Table-29: Bond angles involving hydrogens (º)

Atom	Atom	Atom	angle		Atom	Atom	Atom	angle
C3	N2	H2	121.4		C4	N2	H2	121.4
C4	N3	H3A	120		C4	N3	H3B	120
H3A	N3	H3B	120		C4	N4	H4A	120
C4	N4	H4B	120		H4A	N4	H4B	120
C8	N5	H5	109.5		C8	N6	H6A	120
C8	N6	H6B	120		H6A	N6	H6B	120
S1	C1	H1	126.8		C2	C1	H1	126.8
S2	C5	H5A	109.5		S2	C5	H5B	109.5
C2	C5	H5A	109.5		C2	C5	H5B	109.5
H5A	C5	H5B	108.1		S2	C6	H6C	109.3
S2	C6	H6D	109.3		C7	C6	H6C	109.3
C7	C6	H6D	109.3		H6C	C6	H6D	108
C6	C7	H7A	108.3		C6	C7	H7B	108.3
C8	C7	H7A	108.3		C8	C7	H7B	108.3
H7A	C7	H7B	107.4					

Table-30: Torsion Angles(º)

Atom 1	Atom 2	Atom 3	Atom 4	Angle		Atom 1	Atom 2	Atom 3	Atom 4	angle
C1	S1	C3	N1	1.5(10)		C1	S1	C3	N2	-178.3(10)
C3	S1	C1	C2	-0.7(11)		C5	S2	C6	C7	-81.1(13)
C6	S2	C5	C2	-89.5(10)		C2	N1	C3	S1	-1.7(15)
C2	N1	C3	N2	178.1(11)		C3	N1	C2	C1	1.1(15)
C3	N1	C2	C5	-177.3(10)		C3	N2	C4	N3	-2(3)
C3	N2	C4	N4	-178.2(11)		C4	N2	C3	S1	-75.9(11)
C4	N2	C3	N1	4(2)		S1	C1	C2	N1	-0.0(16)
S1	C1	C2	C5	178.4(8)		N1	C2	C5	S2	-61.7(14)
C1	C2	C5	S2	119.9(13)		S2	C6	C7	C8	-66.7(13)
C6	C7	C8	N5	2(2)		C6	C7	C8	N6	-76.1(11)
Remarks: (Those having bond angles > 160 or < 20 degrees are excluded.)										

Table-31: Possible hydrogen bonds

Donor	H	Acceptor	D...A	D-H	H...A	D-H...A	
N3	H3A	N1	2.683(15)	0.86	2.06	128.90	intramol.
N3	H3B	N5[1]	2.866(17)	0.86	2.07	153.86	
N4	H4A	N5[1]	3.058(19)	0.86	2.34	141.09	
N5	H5	S2	3.213(14)	0.75	2.66	132.27	intramol.
N5	H5	N3[1]	2.866(17)	0.75	2.57	105.44	

Symmetry Operators:

(1) -X+1,-Y+1,-Z+1

Table 32: Intramolecular contacts less than 3.60 Å

Atom	Atom	distance		Atom	Atom	Distance
S2	N1	3.303(13)		S2	N5	3.213(14)
S2	C8	3.342(17)		N1	N3	2.683(15)
N1	C4	2.843(18)		N2	C2	3.581(18)
N3	C3	2.839(15)		N5	C6	2.84(2)
C2	C6	3.59(2)		C5	C7	3.49(3)

Table-33: Intramolecular contacts less than 3.60 Å involving hydrogens

Atom	Atom	distance		Atom	Atom	Distance
S1	H2	2.749		S2	H5	2.66
S2	H7A	2.89		N1	H1	3.266
N1	H2	3.149		N1	H3A	2.058
N1	H3B	3.489		N1	H5A	3.35
N1	H5B	2.792		N2	H3A	2.6
N2	H3B	3.174		N2	H4A	3.145
N2	H4B	2.469		N3	H2	3.17
N3	H4A	2.504		N3	H4B	3.109
N4	H2	2.481		N4	H3A	3.124
N4	H3B	2.465		N5	H6A	2.494
N5	H6B	3.01		N5	H6D	2.745
N5	H7A	2.967		N5	H7B	2.981
N6	H5	2.861		N6	H7A	2.525
N6	H7B	2.471		C1	H5A	2.568
C1	H5B	3.116		C1	H6C	3.413
C2	H3A	3.388		C2	H6C	3.205
C3	H1	3.41		C3	H3A	2.562
C5	H1	2.758		C5	H6C	2.82
C5	H7A	3.07		C6	H5A	2.773
C6	H5	2.37		C7	H5A	3.063
C7	H5	2.326		C7	H6A	3.121
C7	H6B	2.357		C8	H6C	3.374
C8	H6D	2.741		H1	H5A	2.47
H1	H5B	3.406		H1	H6C	3.306
H2	H3A	3.441		H2	H4A	3.336
H2	H4B	2.286		H3A	H4A	3.358
H3B	H4A	2.323		H3B	H4B	3.314
H5A	H6C	2.633		H5A	H7A	2.458
H5	H6A	3.198		H5	H6B	3.519
H5	H6C	3.335		H5	H6D	2.182
H5	H7A	2.983		H5	H7B	2.997
H6A	H7A	3.349		H6A	H7B	3.289
H6B	H7A	2.375		H6B	H7B	2.309
H6C	H7A	2.382		H6C	H7B	2.299
H6D	H7A	2.836		H6D	H7B	2.392

Table-34: Intermolecular contacts less than 3.60 Å

Atom	Atom	distance		Atom	Atom	distance
S1	$N3^{1}$	3.583(13)		S1	$N6^{2}$	3.512(14)
S1	$C4^{1}$	3.528(16)		S2	$N3^{3}$	3.544(11)
N1	$N6^{2}$	3.522(18)		N2	$N5^{4}$	3.39(2)
N3	$S1^{5}$	3.583(13)		N3	$S2^{3}$	3.544(11)
N3	$N5^{3}$	2.866(17)		N3	$N5^{4}$	3.583(19)
N4	$N5^{3}$	3.058(19)		N5	$N2^{4}$	3.39(2)
N5	$N3^{3}$	2.866(17)		N5	$N3^{4}$	3.583(19)
N5	$N4^{3}$	3.058(19)		N5	$C3^{4}$	3.540(18)
N5	$C4^{3}$	3.404(19)		N5	$C4^{4}$	3.38(3)
N6	$S1^{6}$	3.512(14)		N6	$N1^{6}$	3.522(18)
N6	$C1^{6}$	3.52(2)		N6	$C1^{7}$	3.251(19)
N6	$C2^{6}$	3.517(18)		N6	$C3^{6}$	3.429(18)
C1	$N6^{2}$	3.52(2)		C1	$N6^{8}$	3.251(19)
C2	$N6^{2}$	3.517(18)		C3	$N5^{4}$	3.540(18)
C3	$N6^{2}$	3.429(18)		C4	$S1^{5}$	3.528(16)
C4	$N5^{3}$	3.404(19)		C4	$N5^{4}$	3.38(3)

Symmetry Operators:

(1) X+1,Y,Z

(2) -X+2,Y+1/2-1,-Z+1/2+1

(3) -X+1,-Y+1,-Z+1

(4) -X+2,-Y+1,-Z+1

(5) X-1,Y,Z

(6) -X+2,Y+1/2,-Z+1/2+1

(7) -X+3,Y+1/2,-Z+1/2+1

(8) -X+3,Y+1/2-1,-Z+1/2+1

Table-35[A]: Intermolecular contacts less than 3.60 Å involving hydrogens

Atom	Atom	distance		Atom	Atom	distance
S1	H6B[1]	3.493		S1	H6B[2]	2.824
S1	H7A[1]	3.343		S1	H7B[2]	3.474
S2	H3A[3]	3.548		S2	H3B[3]	2.944
S2	H6C[4]	3.301		S2	H6D[4]	3.501
S2	H6D[5]	3.189		S2	H7B[4]	2.937
N1	H5[5]	3.391		N1	H6A[1]	3.467
N1	H6B[1]	3.195		N1	H6C[4]	3.409
N1	H6D[5]	3.403		N2	H5[5]	3.489
N2	H6B[1]	3.469		N2	H7A[1]	3.566
N3	H5[3]	2.574		N3	H5[5]	3.532
N3	H6D[5]	3.269		N3	H7B[5]	3.529
N4	H5[3]	3.336		N5	H3A[3]	3.538
N5	H3B[3]	2.069		N5	H4A[3]	2.341
N6	H1[6]	2.612		C1	H3A[7]	3.579
C1	H6A[1]	3.324		C1	H6A[2]	3.3
C1	H6B[2]	2.67		C2	H6A[1]	3.21
C2	H6B[1]	3.538		C3	H5[5]	3.357
C3	H6B[1]	3.041		C4	H5[3]	3.371
C4	H5[5]	3.524		C5	H6C[4]	3.268
C5	H7B[4]	3.246		C6	H5B[7]	3.357
C7	H5B[7]	3.266		C8	H3B[3]	3.135
C8	H4A[3]	3.264		H1	N6[2]	2.612
H1	H6A[2]	2.494		H1	H6B[2]	2.219
H2	H7A[1]	3.275		H3A	S2[3]	3.548
H3A	N5[3]	3.538		H3A	C1[4]	3.579
H3A	H5[3]	3.113		H3A	H5[5]	3.408
H3A	H6C[4]	2.951		H3A	H6D[5]	2.858
H3B	S2[3]	2.944		H3B	N5[3]	2.069
H3B	C8[3]	3.135		H3B	H5[3]	1.738
H3B	H6D[3]	3.43		H3B	H6D[5]	3.494
H3B	H7B[5]	3.291		H4A	N5[3]	2.341
H4A	C8[3]	3.264		H4A	H5[3]	2.749
H4A	H6A[3]	3.333		H5B	C6[4]	3.357
H5B	C7[4]	3.266		H5B	H6A[1]	3.358
H5B	H6C[4]	2.661		H5B	H7A[4]	3.281
H5B	H7B[4]	2.698		H5	N1[5]	3.391
H5	N2[5]	3.489		H5	N3[3]	2.574

Table-35[B]: Intermolecular contacts less than 3.60 Å involving hydrogens (continued)

Atom	Atom	distance		Atom	Atom	Distance
H5	$N3^{5}$	3.532		H5	$N4^{3}$	3.336
H5	$C3^{5}$	3.357		H5	$C4^{3}$	3.371
H5	$C4^{5}$	3.524		H5	$H3A^{3}$	3.113
H5	$H3A^{5}$	3.408		H5	$H3B^{3}$	1.738
H5	$H4A^{3}$	2.749		H6A	$N1^{8}$	3.467
H6A	$C1^{8}$	3.324		H6A	$C1^{6}$	3.3
H6A	$C2^{8}$	3.21		H6A	$H1^{6}$	2.494
H6A	$H4A^{3}$	3.333		H6A	$H5B^{8}$	3.358
H6B	$S1^{8}$	3.493		H6B	$S1^{6}$	2.824
H6B	$N1^{8}$	3.195		H6B	$N2^{8}$	3.469
H6B	$C1^{6}$	2.67		H6B	$C2^{8}$	3.538
H6B	$C3^{8}$	3.041		H6B	$H1^{6}$	2.219
H6C	$S2^{7}$	3.301		H6C	$N1^{7}$	3.409
H6C	$C5^{7}$	3.268		H6C	$H3A^{7}$	2.951
H6C	$H5B^{7}$	2.661		H6D	$S2^{7}$	3.501
H6D	$S2^{5}$	3.189		H6D	$N1^{5}$	3.403
H6D	$N3^{5}$	3.269		H6D	$H3A^{5}$	2.858
H6D	$H3B^{3}$	3.43		H6D	$H3B^{5}$	3.494
H7A	$S1^{8}$	3.343		H7A	$N2^{8}$	3.566
H7A	$H2^{8}$	3.275		H7A	$H5B^{7}$	3.281
H7B	$S1^{6}$	3.474		H7B	$S2^{7}$	2.937
H7B	$N3^{5}$	3.529		H7B	$C5^{7}$	3.246
H7B	$H3B^{5}$	3.291		H7B	$H5B^{7}$	2.698

Symmetry Operators:

(1) -X+2,Y+1/2-1,-Z+1/2+1

(2) -X+3,Y+1/2-1,-Z+1/2+1

(3) -X+1,-Y+1,-Z+1

(4) X-1,Y,Z

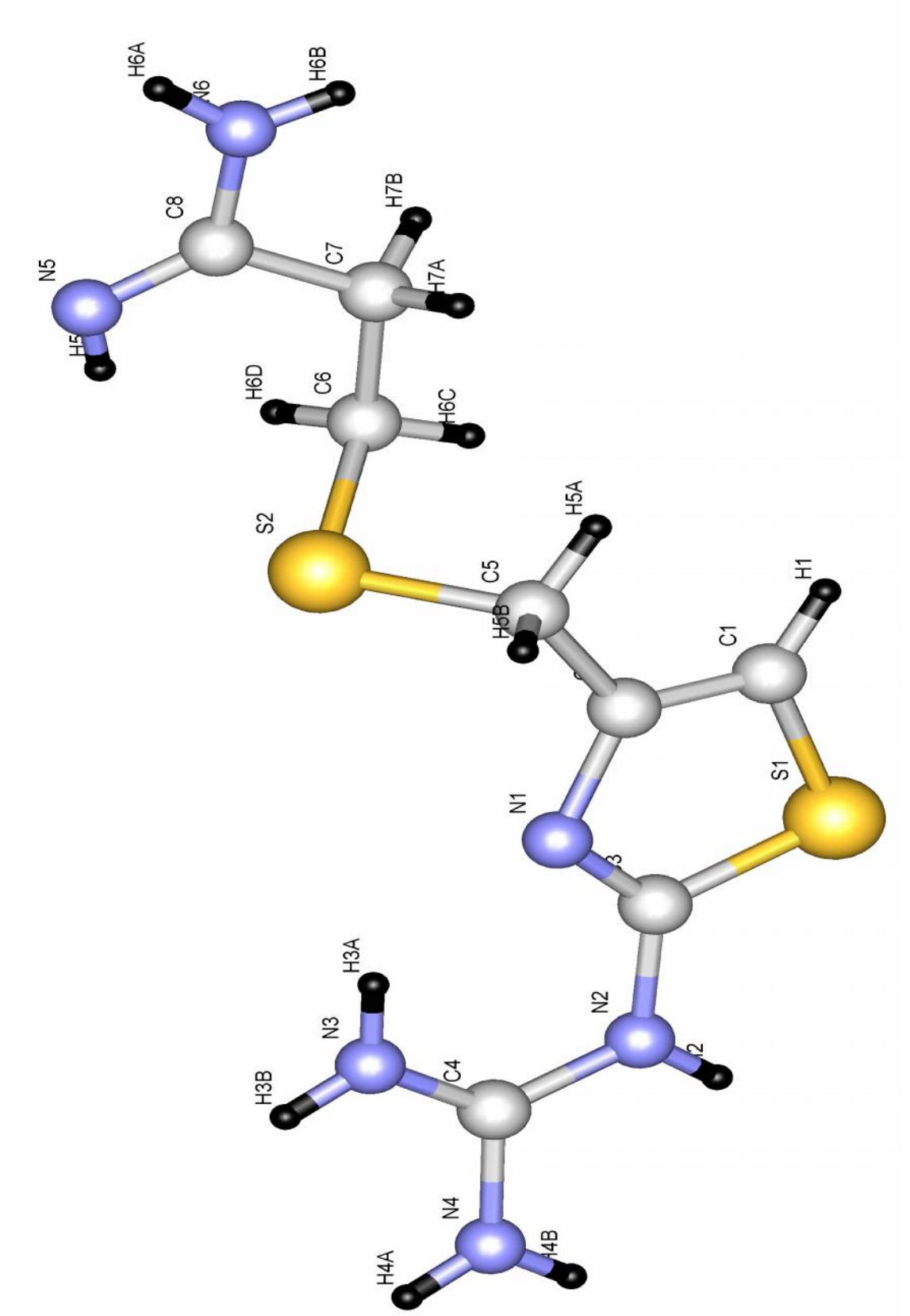
(5) -X+2,-Y+1,-Z+1

(6) -X+3,Y+1/2,-Z+1/2+1

(7) X+1,Y,Z

(8) -X+2,Y+1/2,-Z+1/2+1

Structure of Famotidine Impurity:



3-[[2-(diaminomethylideneamino)-1,3-thiazol-4-yl]methylsulfanyl]propanimidamide

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$\Sigma w(F_o^2-F_c^2)^2$ where w = Least Squares weights.

[17] Standard deviation of an observation of unit weight:

$[\Sigma w(F_o^2-F_c^2)^2/(N_o-N_v)]^{1/2}$

where: N_o = number of observations

N_v = number of variables

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