Analytical Method Development for Drugs and Impurities Degradation Using Chromatography Technique

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ABSTRACT

High Performance Liquid Chromatography (HPLC) is a powerful technique which helps in development and validation of analytical methods for drug analysis, including degradation studies. The paper focuses on the application of HPLC for analysis of drug impurities and degradation products. The experimental result demonstrates the robustness, accuracy, sensitivity of HPLC method for detection of degraded products and identifying impurities in pharmaceutical drugs. The method was validated according to ICH guidelines for precision, linearity, specificity, accuracy, and robustness. The study concludes on the method being suitable for regulatory compliance and quality assurance in the pharmaceutical industry.

Keywords: HPLC, Famotidine, Degradation, Impurity Detection, Method Development, Validation, Pharmaceutical Analysis.

I INTRODUCTION

Environmental conditions like light, humidity, temperature and other have cause the degradation of the pharmaceutical drugs. It is evident since it led to the formation of the impurities that may affect the safety and efficacy of the drug. Henceforth, the sensitive analytical method i.e., High Performance liquid Chromatography (HPLC) have offered excellent resolution, accuracy, and sensitivity to the impurity's detection. The study aims on the development of the HPLC method for degradation products detection and impurities in Famotidine, a histamine H2-receptor antagonist which usually treats gastric acid related disorders. The stability of Famotidine is crucial which certainly lead to impurity formation in line with the regulatory requirements and managing the safety and efficacy of drug by quantifying those impurities through its shelf life.

HPLC has been widely used for separation and quantification of drug substances and their impurities. The method has various advantages which includes high resolution, sensitivity, and reproducibility that makes it an ideal technique for pharmaceutical stability testing. In this study, the development and validation of the HPLC method for detection of degradation products and impurities in Famotidine is focused. It provides a reliable and sensitive approach to monitor the quality of Famotidine formulation during stability studies.

II PROPOSED METHOD

2.1 HPLC Methodology for Impurity Detection

To analyze the famotidine and its impurities, the chromatographic conditions have certainly focused on the active pharmaceutical ingredients (API) which aims on achieving optimal separation and resolution for degradation products. The chromatographic conditions involve column C18, 250 mm*4.6mm, 5µm. The mobile phase has gradient mixture of Solvent A (Water) and Solvent B (Methanol). The flow rate of 1.0 mL/min and wavelength at 254 nm. The instrument has equipped with UV detector.

The Stress Conditions involves acid

degradation (1.0 N HCl), Base Degradation (1.0 N NaOH), Oxidative degradation (10% of H_2O_2), Thermal Degradation (Heating at 60^0C for 24 hours), Photolytic degradation (UV exposure 254 nm and 365 nm), Humidity degradation (90% RH for 24 hours). The HPLC based analytical method have determined with organic impurities in Famotidine USP which ensure intended application requirements.

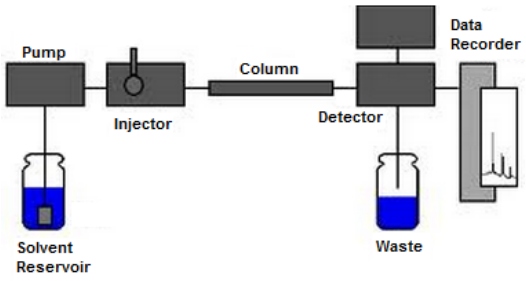


Figure 1: HPLC Instrument

It has tested following USP specification and ICH Q2 (R1) guidelines to cover the essential parameters including forced degradation studies. The run time for 57 minutes with analytical procedure involving the gradient programming with adequate resolution of Famotidine and its related impurities. The mobile phases were prepared using sodium 1-hexanesulfonate buffer, methanol, and acetonitrile.

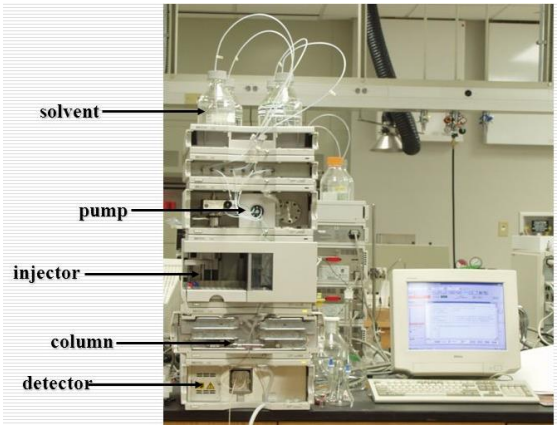


Figure 2: HPLC Instrumentation actual Diagram

The sample and standard solutions were prepared with precise concentrations and dilutions to ensure reproducibility. The study utilized calibrated HPLC systems, analytical balances and pH meters where the HPLC or analytical grade to source from reputed manufacturers like Shimazdu.

The pump in the chromatography is the key factor for driving liquid within the system at specific flow rate. It has been analyzed that the pump usually adjusts the composition of the mobile phase whenever the need of increasing the volume is required. However, this process turns out to be continuous and utilizing the pumps like constant-flow reciprocating pumps, syringe or displacement pumps, and pneumatic pumps for this purpose. The mixing unit on the other hand have been equipped with helium-based degassing system that operates under high pressure to ensure proper solvent preparation. The temperature is ambient with formulation of subjected forced degrade products under various stress conditions. Henceforth, the solvent delivery system has regulated polarity of the mobile phase by running the sample in smooth and pulse free flow with injector playing key role.

2.2. Preparation of Mobile Phase –

The mobile phase has buffer preparation of 1.882 g of sodium 1-hexanesulfonate in 1000 mL of water adjust the pH 3.5 with dilute acetic acid and filter it. The mobile phase solution A involve ratio of 900:94:6 and mix and degase solution. Mobile phase Solution B include sonicate acetonitrile 1000 mL in volumetric flask. The standard stock solution 0.5 mg/mL of USP famotidine RS/Famotidine working standard in Solution A. The dissolve of 12.5 mg of famotidine working standards in Solution A and dilute to 25 mL with Solution A (0.5 mg/mL).

The following equipment were used for forced degradation study include Waters and Alliance Model and Hypersil ODS-2 with Thermo Scientific and Analytical Balance involve Famotidine (FTTM (A)-0030520 sample for analysis.

III EXPERIMENT AND RESULT

The forced degradation study aims to evaluate the stability of Famotidine by subjecting to various stress conditions and identifying the potential impurities and degradation products. The study validated the stability-indicating and

capable of resolving Famotidine from its degradation products and related impurities.

The proposed scheme added on the Famotidine USP through Active Pharmaceutical Ingredient under study where acid degradation, basic degradation, oxidative degradation, and High-purity water and methanol for preparing mobile phases. The system suitability testing is carried out with validated way for its ability to analyze Famotidine and standard solutions of Famotidine to injected way with key parameters like retention time, resolution, purity angle, %RSD were assessed. The acceptance criteria include minimum of 3.5 between Famotidine and its impurities. The RSD have not more than 5% for peak areas of replicates.

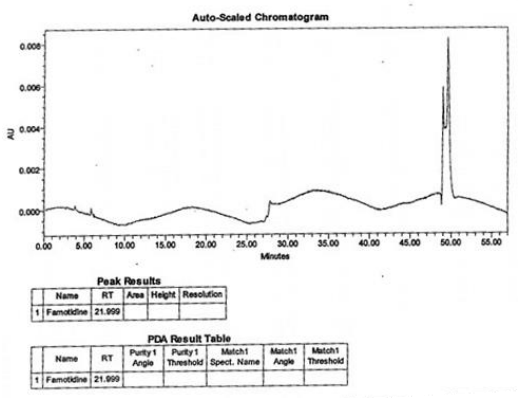


Figure 3: Blank Famotidine USP

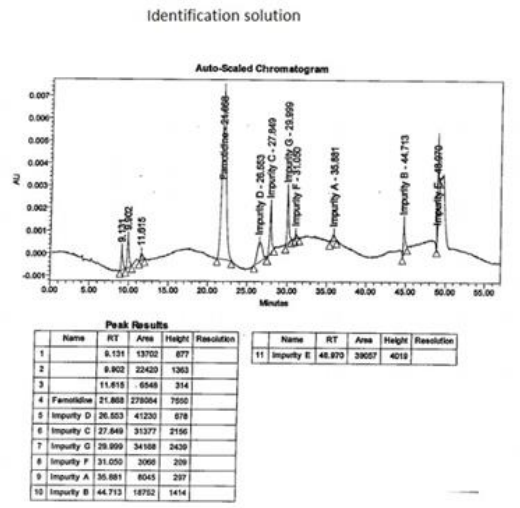


Figure 4: Identification Solution

The preparation of stress samples where Famotidine with exposed to 1.0N HCl at room temperature for 1 hour. The reaction was neutralized with 1.0 NaOH. The Famotidine was treated way to 1.0N NaOH for 1 hour with neutralization with 1.0N HCl.

The chromatogram Plot X-axis represent time in minutes (Retention time) and Y-axis represent absorbance (AU-Absorbance Units) to have a clear peak at around 21.999 minutes to represent the substance of interest (Famotidine) with no additional area, height, or resolution in visible portion. The sample indicates the minimal interference as there are no significant overlapping peaks.

The Famotidine is main compound identified at RT = 21.663 minutes. It has several impurities labelled D, G, C, F, A, B, and E were identified with varying peak areas where Impurity E (RT=48.970 minutes) to be most prominent areas to impurity based on its large peak area. The chromatograph has separately famotidine and its impurities to have a proportion of impurities to be quantified with the critical way to assess the sample's quality and compliance with pharmaceutical standards.

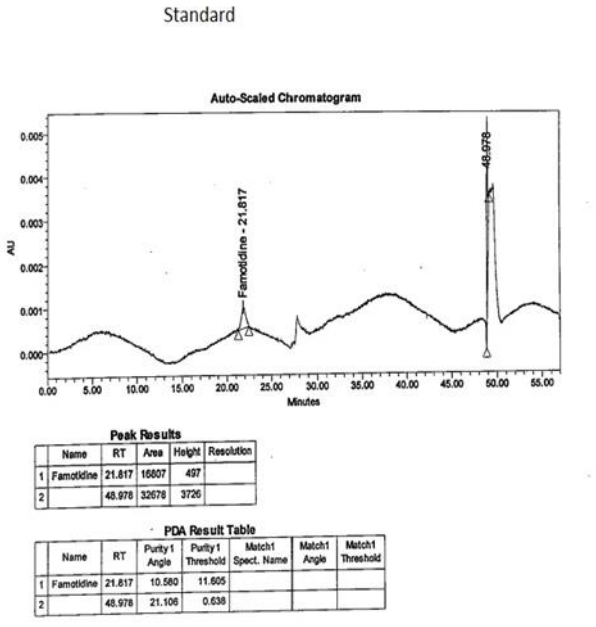
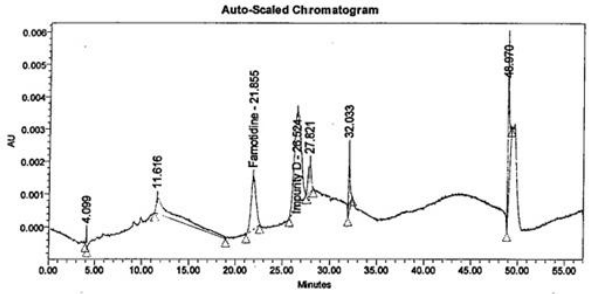


Figure 5: Standard Famotidine Sample

The chromatogram has set out the dominant peak at 22.016 minutes with high peak area and height validated with its abundance in the

sample. The minor impurities are detected at 11.836, 27.774 (Impurity C) and 32.103 minutes. Impurity C have quantified but remains at a significant lower area compared to Famotidine where the test solution primarily contains famotidine with small number of impurities. The Purity analysis confirms the Famotidine is adequate separately and free from co-elution to ensure high sample quality.

System Suitability



Peak Results

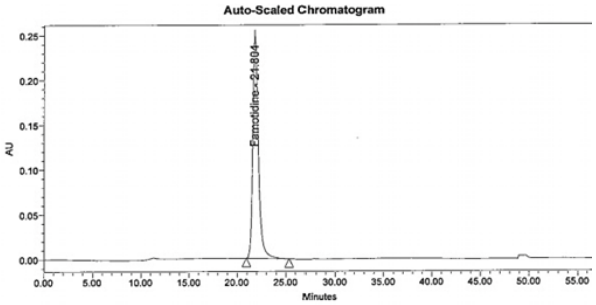
	Name	RT	Area	Height	Resolution
1		4.099	2271	459	
2		11.616	64972	478	2.971924e+000
3	Famotidine	21.855	58464	1634	3.484781e+000
4	Impurity D	26.524	149596	2969	3.984254e+000
5		27.821	16086	935	1.407040e+000
6		32.033	19856	1968	1.289514e+001
7		48.970	52750	5106	8.451767e+001

Figure 6: System Suitability of Famotidine and Impurity D

The method chromatogram has simultaneous detection of Famotidine with sharp peak and maximum theoretical plates with the best purity threshold percentage with simultaneous quantification. The mean contents of the formulation purity angle are between the acceptable range of 10.580 to 21. 106 of the label amounts. This indicate the new method is accurate and precise for quantification of fixed dosages of Famotidine by HPLC technique.

The system suitability test is essential in validating the analytical techniques and confirmation of the resolution amongst numerous peaks of interest. The parameters have met complete acceptance every time. % RSD for the areas of six replicate was found to be Impurity D of 26.524 to 27.82. The tailing factors was less than 2 and theoretical plates of greater than 2000 indicates that the technique was satisfied given the conditions were shown in the figure 6.

Test solution-1



Peak Results

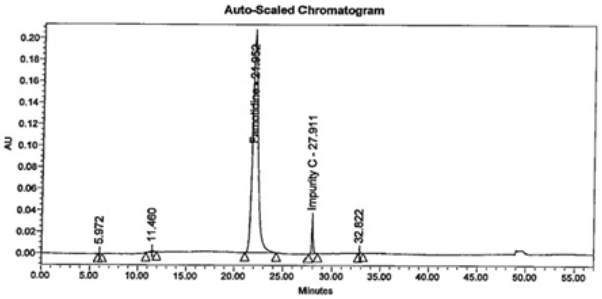
	Name	RT	Area	Height	Resolution
1	Famotidine	21.804	9866457	247956	

PDA Result Table

	Name	RT	Purity 1 Angle	Purity 1 Threshold	Match1 Spect. Name	Match1 Angle	Match1 Threshold
1	Famotidine	21.804	0.074	0.274			

Figure 7: (a) Test Solution 1 Famotidine

Test solution-2



Peak Results

	Name	RT	Area	Height	Resolution
1		5.972	4262	512	
2		11.460	38226	1073	
3	Famotidine	21.952	9396524	201838	
4	Impurity C	27.911	323270	32210	
5		32.822	15983	1327	

PDA Result Table

	Name	RT	Purity 1 Angle	Purity 1 Threshold	Match1 Spect. Name	Match1 Angle	Match1 Threshold
1		5.972	16.796	4.411			
2		11.460	4.860	5.289			

Figure 7: (b) Test Solution 2 (Impurity C and Famotidine)

The study has added on the development and validation of the method have alter on the various parameters such as buffer system with different pH values, wavelength, solvent composition, and flow rate to achieve simultaneous detection of Famotidine with sharp peak and minimal tailing factor within the shorter period. It has configured with best

results to achieve a mobile phase combined with buffer and organic solvent through PDA detector. The typical chromatogram represented the different aspects within the mixture.

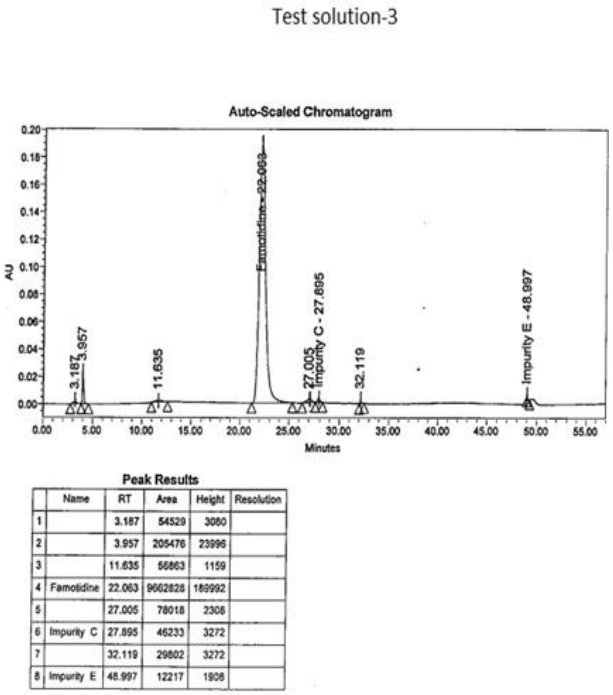


Figure 8: (a) Test Solution-3 (Famotidine, Impurity C, Impurity E)

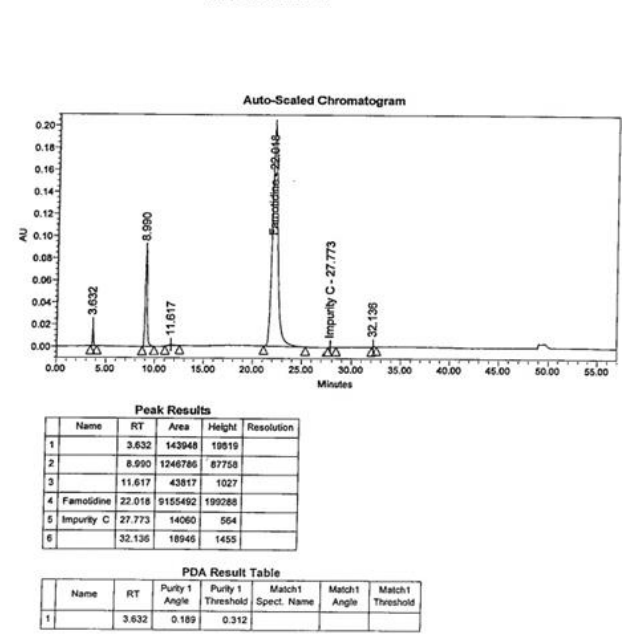


Figure 8: (b) Test Solution-4 (Famotidine, Impurity C)

The peak areas demonstrates that the six separate drug samples are injected for over three days at varying concentration and consistency and accuracy to confirm the intra-day and inter-day results that are developed with high accuracy and repeatability within the relative standard deviation (RSD) % within the acceptable limits. However, Famotidine within accepted limit RSD % < 2 with no significant difference between obtained results retaining other analysts.

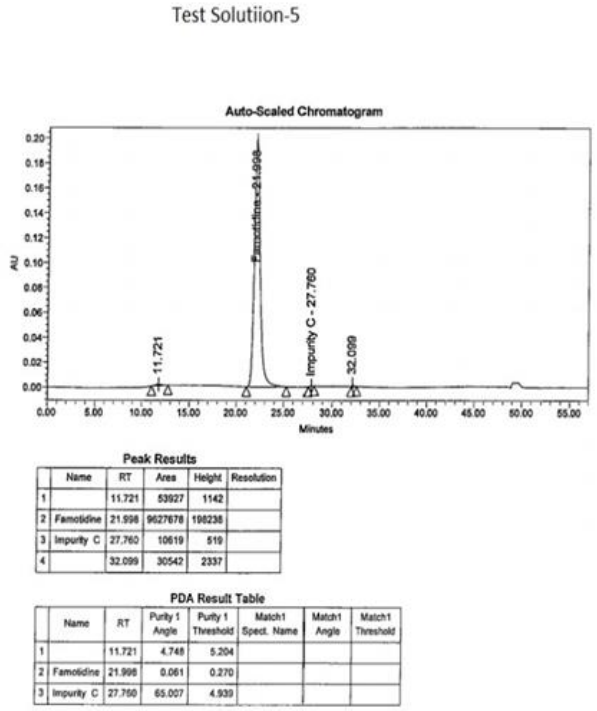


Figure 9: (a) Test Solution -5 (Famotidine, Impurity C)

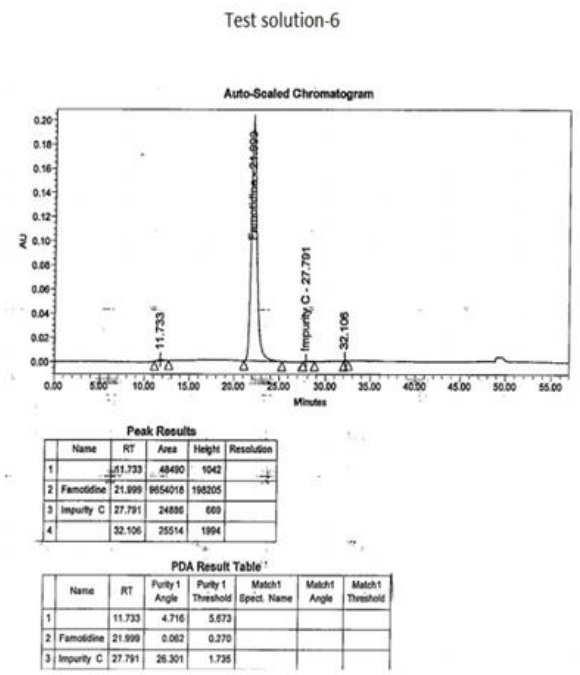


Figure 9: (b) Test Solution-6 (Impurity C and Famotidine)

Both Famotidine and Impurity C analytical calibration curves were within the required ranges as evidenced by the closeness of the correlation area and chromatograms showing linearity within the figure 9 (a).

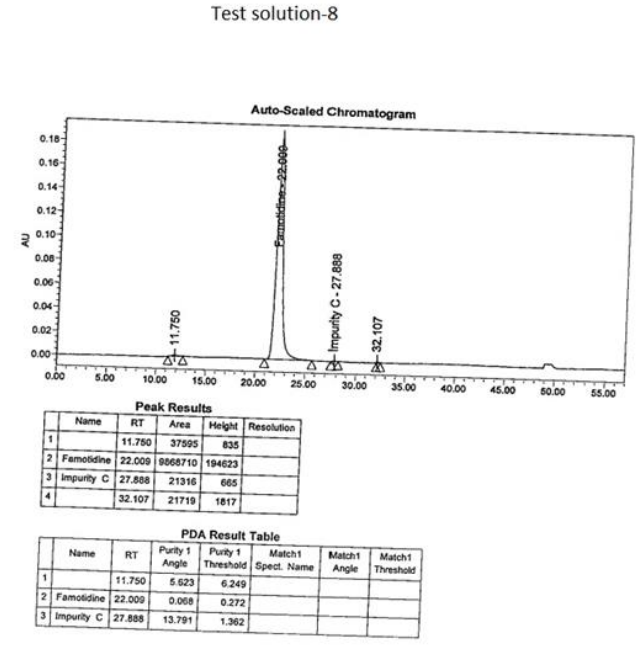


Figure 10: Test Solution-8 (Famotidine and Impurity C)

The technique is robust to modest purposeful modification in terms of the area, height, RT of the sample in the mobile phase. It has shown no significant changes when minor variations are added to the chromatographic conditions. The RSD percent for the Famotidine and Impurity C retention time were < 2, indicating the suggested analytical techniques to have modest alterations. This can also represent the data for both the drugs to be incorporated in the PDA result table in 10 (a) figure. The system suitability test is essential in validating analytical techniques and confirming the resolution amongst numerous peaks of interest. The perilous parameters in the study have met with the complete acceptance every time. The tailing factors are less than 2 which helps in indicating the techniques with the satisfied way to given the conditions and system suitability chromatograms.

The peak purity angle is consistently less than purity threshold for all injection indicating the peak is pure and no significant interferences with the other substance sample. The RSD of 1.10% for the area of Famotidine replicate with the acceptable limit of 5.0%. this suggest that the method is precise and provide consistent across multiple injections. The resolution of 3.98 exceeds the minimum required value of 3.5 demonstrates the chromatographic system can be effectively separate famotidine from its related compounds especially compound D. The system suitability test and method validation results indicate the chromatographic conditions that is suitable for analysis of Famotidine with good precision and peak purity and resolution. This can meet with the necessary requirements for robustness and accuracy in analysing the compounds, reliable results in further analysis.

The data provided the system suitability test (SST) conducted using Famotidine Standard solution. This ensures the chromatographic system to work with the proceeding with further analyses. The mass balance study has ensured the amount of analyte in the sample is accurate measures where the observed response corresponds to true amount of substance present in the sample. The purity angle is

consistently lower than the purity threshold with the peak to be pure and there is no significant interference from other compounds or impurities. This ensures the signal corresponds to analyte itself. The low RSD value 0.31% confirms with the precise method and marking on the peak areas that are consistent across replicate injections. The analytes of famotidine have indicated the system is operating correctly and ensuring the mass of Famotidine can be accurately quantified. The result has demonstrated the HPLC method to be validated and reliable way to manage the peak purity with purity angle < threshold and confirming the accuracy and consistency for further analysis.

Condition	Purity Angle	Purity Threshold	Total Degradation (%)	Assay (%)	Mass Balance (%)
Acid (1.0N HCl)	0.053	0.265	2.36	94.63	98.4
Base (1.0N NaOH)	0.099	0.268	2.59	94.99	99.0
Oxidation (10% H2O2)	0.063	0.275	7.94	90.72	100.1
Thermal Degradation	0.061	0.270	0.40	96.17	98.0
Photolytic (UV 254 nm)	0.062	0.270	0.45	97.22	99.1
Photolytic (UV 365 nm)	0.110	0.274	0.32	98.18	99.9
Humidity	0.068	0.272	0.36	97.02	98.8

Table 1: Shows peak of Famotidine with pure all conditions (purity angle < purity threshold)

The mass balance was achieved (≥ 95%) under all conditions. The famotidine exhibit on the significant degradation under oxidation (10% H2O2: 7.94%) and slight degradation in acidic and basic conditions. The analytical method successfully separated all degradation impurities with minimal degradation and minor degradation with the other stress conditions and acceptance criterion to confirm the reliability of the method.

IV CONCLUSION

The force degradation has validated that the study determines organic impurities in Famotidine by analytical method (HPLC). This has indicated towards specific, stability-indicating, and suitable for routine analysis. The method can be confidently used for quality control and stability testing of drug. All known impurities peaks were resolved from each other and the main peak of Famotidine. The peak due to Famotidine obtained spectrally pure i.e. the purity angle achieved less than the purity threshold for each condition of forced degradation. Acid Degradation = 2.36%, Basic Degradation = 2.59%, Oxidation Degradation = 7.94%, Thermal Degradation = 0.40%, Photolytic Degradation @ UV 254 nm = 0.45%, Photolytic Degradation @ UV 365 nm = 0.32%, Humidity Degradation = 0.36%. It has accounted on the mass balance too. Mass balance for Acid Degradation = 98.4%, Mass balance for Basic Degradation = 99.0%, Mass balance for Oxidation Degradation = 100.1%, Mass balance for Thermal Degradation = 98.0%, Mass balance for Degradation @ UV 254 nm = 99.1%, Mass balance for Degradation @ UV 365 nm = 99.9%, Mass balance for Humidity Degradation = 98.8%. The Technique Used in the study is High-Performance Liquid Chromatography (HPLC) which consist of Column of Hypersil ODS-2, 250 mm × 4.6 mm, 5 µm, Temperature: 50°C, Flow Rate: Gradient method, Injection Volume: 20 µL, Detection Wavelength: 265 nm and Run Time: 57 minutes

The Gradient Program System was used to optimize separation between Famotidine and its impurities, with a stabilization phase included at the end of the run. The Mobile Phase consist of A: Buffer (sodium 1-hexanesulfonate solution at pH 3.5) with acetonitrile and methanol (900:94:6 v/v). B: 100% acetonitrile. Famotidine working standard and its related compounds (B, C, D, E, F, cyanoamidine, and amidine) were prepared to ensure accurate

quantification. The study may extend to analyze a broader range of impurities in other drug components. It has evaluated the method performance under different environmental and storage conditions to assess its robustness. The use of other analytical techniques adds impurity identification and quantification for HPLC method. The use of efficient validation and optimization of the method aid on the comprehensive quality of control of Famotidine. It also ensures its safety and efficacy in pharmaceutical applications.

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