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DEVELOPMENT OF FORCED DEGRADATION STABILITY INDICATING HPLC METHOD FOR THE ESTIMATION OF FEXINIDAZOLE IN MARKETED FORMULATION

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ABSTRACT

The development of a forced degradation stability-indicating method using High-Performance Liquid Chromatography (HPLC) for the estimation of Fexinidazole in marketed formulations is presented. This study aims to assess the stability of Fexinidazole under various stress conditions and establish a reliable HPLC method for its quantification. System suitability parameters were optimized, resulting in high theoretical plates (2547.840±8.397), a favorable tailing factor (1.177±0.025), and consistent retention time (4.576±0.012). Linearity was demonstrated over a concentration range of 5-25 µg/ml with a correlation coefficient (r^2) of 0.999. Recovery studies confirmed accuracy with mean recovery rates close to 100% at different concentration levels. Precision and robustness tests showed the method to be reliable and consistent, with low variability. The limits of detection (LOD) and quantification (LOQ) were 0.15 mg/ml and 0.45 mg/ml, respectively, indicating high sensitivity. Analysis of tablet samples yielded an assay of 99.80% with minimal variability. Forced degradation studies revealed that Fexinidazole is relatively stable under oxidative and thermal conditions but exhibits significant degradation under acidic and alkaline conditions. This HPLC method is effective for routine quality control and stability testing, ensuring the safety and efficacy of Fexinidazole in marketed formulations throughout its shelf life.

Key words: Stability-indicating method, High-Performance Liquid Chromatography (HPLC), Fexinidazole, Method development, Validation

INTRODUCTION

Forced degradation studies are important aspect of pharmaceutical development, particularly for ensuring the stability and efficacy of drug products over their shelf life. These studies involve subjecting a drug to extreme conditions to accelerate the rate of degradation, allowing for the identification of potential degradation products and the understanding of degradation pathways. High-Performance Liquid Chromatography (HPLC) is widely utilized in these studies due to its high resolution, precision, and sensitivity in separating and quantifying drug substances and their degradation products.

Forced degradation studies provide vital information on the chemical behavior of drug substances under various stress conditions, including acidic, basic, oxidative, thermal, and photolytic environments. This information helps in developing stability-indicating methods, which ensure that the

analytical method can accurately measure the active pharmaceutical ingredient (API) without interference from degradation products (Blessy *et al.,* 2014). Additionally, these studies help in understanding degradation pathways, identifying potential degradation products, and establishing the shelf life and appropriate storage conditions for the drug product (Bakshi & Singh, 2002). Furthermore, conducting forced degradation studies is essential for regulatory compliance, as agencies like the FDA and ICH mandate thorough stability testing to ensure drug safety and efficacy (ICH Q1A(R2), 2003).

Fexinidazole, a nitroimidazole derivative, has shown efficacy in treating parasitic infections, including human African trypanosomiasis (sleeping sickness). Given its therapeutic importance, ensuring the stability of fexinidazole in its marketed formulations is essential. The stability of a drug is a critical factor in its overall efficacy and safety, making forced degradation studies an indispensable part of its development process.

HPLC is the method of choice for forced degradation studies due to its versatility and accuracy. The technique involves several steps, starting with sample preparation, where the drug is subjected to various stress conditions. Following this, chromatographic conditions are optimized, including parameters like mobile phase composition, flow rate, column type, and detection wavelength (Ali *et al.,* 2010). Detection and quantification of the parent drug and its degradation products are then performed, followed by data analysis to interpret chromatograms and determine degradation pathways and product stability.

Forced degradation studies using HPLC are fundamental in ensuring the stability and safety of fexinidazole in its marketed formulation. These studies not only comply with regulatory requirements but also provide valuable insights into the chemical behavior and degradation mechanisms of the drug, ultimately contributing to the development of robust and reliable pharmaceutical products.

The aim of this study is to develop a robust and reliable forced degradation stability-indicating method using High-Performance Liquid Chromatography (HPLC) for the estimation of fexinidazole in its marketed formulation. This involves subjecting fexinidazole to various stress conditions, including acidic, basic, oxidative, thermal, and photolytic environments, to understand its degradation behavior and identify degradation products.

The primary objective is to establish an HPLC method that can effectively separate and quantify fexinidazole and its degradation products with high precision, accuracy, and sensitivity. This method will be validated according to ICH guidelines to ensure its suitability for stability testing. Additionally, the study aims to elucidate the degradation pathways of fexinidazole, providing critical insights into its chemical stability and identifying potential degradation products. This information will be essential for determining the appropriate storage conditions, predicting the shelf life of the drug product, and ensuring its safety and efficacy throughout its lifecycle.

MATERIALS AND METHODS

Mobile Phase Selection

Initially to estimate Fexinidazole number of mobile phase in different ratio were tried.

Taking into thought the system suitability parameter like RT, Tailing factor, no. of theoretical plates and HETP, the mobile phase found to be most suitable for analysis was 15mM KH_2PO_4 and Acetonitrile in the ratio of 20:80 adjust the pH 4 with OPA. The mobile phase was filtered through 0.45 µm filter paper to remove particulate matter and then degassed. Flow rate employed for analysis was 1.0 ml/min.

Selection of wavelength

100 mg of Fexinidazole was weighed accurately and transferred to a 100 ml volumetric flask, and the volume was adjusted to the mark with the mobile phase. From above solutions of 0.1 ml was transferred to 10 ml volumetric flasks, and make up the volume up to mark. Resulting solution was scanned over UV range (200-400nm), maximum absorbance was found at λ_{max} 254.00 nm.

Selection of Separation Variable

Standard drug solution of Fexinidazole was prepared in different mobile phase and chromatograph was recorded by using different column (5µm) at different chromatographic condition like different flow rate and temperature. Considering the theoretical facts and after several trials separation variables were selected which were constant during whole experiment.

System Suitability Parameters

Separation variables were set and mobile phase was allowed to saturate the column at 1.00 ml/min. After complete saturation of column, three replicates of working standard of Fexinidazole 10µg/ml was injected separately. Peak report and column performance report were recorded for all chromatogram.

Preparation of Standard Stock Solution

10mg of Fexinidazole was weighed accurately and transferred to separate 10ml volumetric flask, and the volume was adjusted to the mark with the methanol to give a stock solution of 1000ppm.

Preparation of Working Standard Solution

From stock solutions of Fexinidazole 1 ml was taken and diluted up to 10 ml from this solution 0.5, 1.0, 1.5, 2.0, 2.5 ml solutions were transferred to 10ml volumetric flasks and make up the volume up to 100 ml with methanol, gives standard drug solution of 5, 10, 15, 20, 25 µg/ ml concentration.

Preparation of the Calibration Curves of the Drug

Standard drug solutions were injected 3 times and the mean peak area of drug was calculated and plotted against the concentration of the drug. The regression equation was found out by using this curve.

Analysis of Tablet Formulation

For analysis of the tablet formulation, weight equivalent to weight 10mg of Fexinidazole was transferred to 10ml volumetric flask and dissolved in mobile phase. The solution was shaking vigorously for 20mins and filtered through Whattman filter paper no. 41, then volume was made up to mark with acetonitrile. From the above solution 1ml of solution was taken and diluted to 10 ml with mobile phase to get a solution containing 100µg/ml. From the above solution 1ml of solution was taken and diluted to 10ml with methanol to get a solution containing 10µg/ml of Fexinidazole. The amounts of Fexinidazole in tablet formulation were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated six times with tablet formulation.

Validation

Linearity

Linearity of analytical procedure is its ability (within a given range) to obtain test, which are directly proportional to area of analyte in the sample. The calibration plot was contracted after analysis of five different (from 5 to 25µg/ml) concentrations and areas for each concentration were recorded three times, and mean area was calculated. From the mean of AUC observed and respective concentration value, the response ratio (response factor) was found by dividing the AUC with respective concentration.

Accuracy

Recovery studies were performed to validate the accuracy of developed method. To preanalysed sample solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed.

Precision

(A) Repeatability

Standard dilutions were prepared and three replicates of each dilution were analyzed in same day for repeatability and results were subjected to statistical analysis. Standard dilutions were prepared and three replicates of each dilution were analyzed in different days and by different analysts. Statistical analysis was carried out.

(B) Intermediate Precision

(a) Day to Day

The statistical analysis method was carried out.

(b) Analyst to Analyst

The intermediate precision expresses with in laboratories variation (different days, different analysts, different equipment etc). The standard dilution was prepared and three replicate of each dilution were analyzed by different analysts for all the developed methods.

Robustness

As per ICH norms, small, but deliberate variations, by altering the pH and concentration of the mobile phase were made to check the method capacity to remain unaffected. The effect of change in pH of mobile phase, flow rate, mobile phase ratio on the retention time, theoretical plates, area under curve and percentage content of Fexinidazole was studied.

Detection Limit and Quantitation Limit

The LOD and LOQ of developed method were calculated based on the standard deviation of response and slope of the linearity curve.

Analysis of both the drug in Tablet Sample

Twenty tablets were accurately weighed and their mean weight was determined. The tablets were grinded to fine powder, an accurately weighed quantity of powder equivalent to 10mg of Fexinidazole was transferred to 10 ml volumetric flask containing methanol. The solution was sonicated for 25 min and the final volume was made with mobile phase. The mixture was then filtered through a 0.45 µm filter. The stock solution was further diluted sufficiently with methanol to get sample solution of drug concentration of 10µg/mL for Fexinidazole.

Forced degradation studies

In order to determine whether the method is stability indicating, forced degradation studies were conducted on drug powder and the analysis was carried out by HPLC with a U.V. detector. 20µl of each of forced degradation samples were injected.

Acid degradation:

50mg of the drug sample was taken into a 50 ml separate round bottom flask, 50 ml of 0.1 N HCl solution was added and contents were mixed well and kept for constant stirring for 8h at 80ºC. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drug.

Alkaline hydrolysis:

50mg of the drug sample was taken into a 50 ml separate round bottom flask, 50 ml of 0.1 M NaOH solution was added and contents were mixed well and kept for constant stirring for 8 h at 80ºC. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drug.

Oxidative degradation:

50mg of the drug sample was taken into a 50 ml separate round bottom flask, 50 ml of 3% hydrogen peroxide solution was added, and contents were mixed well and kept for constant stirring for 24 hr at room temperature. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drug.

Thermal degradation:

50mg of the drug sample was taken in to a petri dish and kept in oven at 50ºC for 4 weeks. Samples were withdrawn and diluted to get 10 µg/ml subjected to HPLC and calculate the percentage degradation using calibration curve of drug.

Results and Discussion

The RP-HPLC method was developed for estimation of Fexinidazole in combined formulation by isocratically using 15mM KH_2PO_4 and Acetonitrile in the ratio of 20:80v/v as mobile phase, Thermo C-18 column (4.6 x 250mm, 5µparticle size) column as stationary phase and chromatogram was recorded at 254nm. Then developed method was validated by using various parameters.

The results from the forced degradation stability study using HPLC for the estimation of fexinidazole in marketed formulations provide comprehensive insights into the stability and robustness of the developed method. The system suitability parameters (Table 1) indicate that the HPLC method is precise and reliable, with a high number of theoretical plates (2547.840±8.397), a favorable tailing factor (1.177±0.025), and a consistent retention time (4.576±0.012). These parameters suggest that the method is well-optimized for the analysis of fexinidazole.

The linearity study (Table 2) demonstrates excellent correlation for fexinidazole within the concentration range of 5-25 µg/ml, with a correlation coefficient (r^2) of 0.999. This high level of correlation, along with the slope (504.3) and intercept (5.381), confirms the method's suitability for quantitative analysis.

The recovery study (Table 3) indicates that the method is accurate, with mean recovery rates close to 100% across different concentration levels (80%, 100%, and 120%). The results, 99.08±0.937, 98.87±0.447, and 99.07±0.878 respectively, validate the method's accuracy and consistency.

Precision and robustness tests (Table 4) reveal that the method is both repeatable and robust. The repeatability shows a mean of 98.955±0.095, and intermediate precision tests, including day-to-day and analyst-to-analyst precision, show values of

99.039±0.081 and 98.804±0.051, respectively. Robustness testing indicates a mean of 99.053±0.081, confirming the method's reliability under varied conditions.

The limits of detection (LOD) and quantification (LOQ) for fexinidazole (Table 5) are 0.15 mg/ml and 0.45 mg/ml, respectively. These low values indicate the method's high sensitivity, which is crucial for detecting even minimal amounts of degradation products.

Analysis of the tablet sample (Table 6) shows that the method accurately quantifies fexinidazole in marketed formulations, with a percentage found of 598.85 mg against a label claim of 600 mg, resulting in an assay of 99.80% and a relative standard deviation (RSD) of 0.125%. This high accuracy and low variability underline the method's effectiveness for routine quality control.The forced degradation studies (Table 7) highlight the stability of fexinidazole under various stress conditions. The standard drug showed negligible degradation (0%), while acidic hydrolysis, alkaline hydrolysis, oxidative degradation, and thermal degradation resulted in 13.2%, 16.53%, 9.71%, and 6.49% decomposition, respectively. These results indicate that fexinidazole is relatively stable under oxidative and thermal conditions but shows significant degradation under acidic and alkaline conditions.

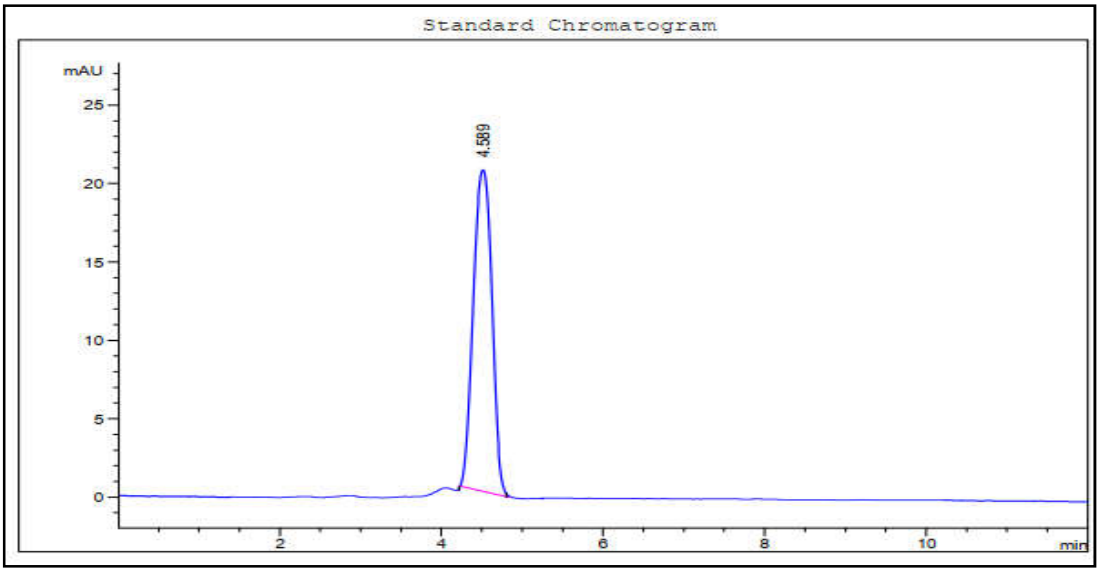

Figure 1: Chromatogram of Standard

Table 1: Results of system suitability parameters

Parameters	% MEAN±SD*
No. of Theoretical Plates	2547.840±8.397
Tailing Factor	1.177±0.025
Retention time	4.576±0.012

Table 2: Results of linearity of Fexinidazole

Parameter	Fexinidazole
Concentration (µg/ml)	5-25
Correlation Coefficient (r^2)*	0.999
Slope (m)*	504.3
Intercept (c)*	5.381

*value of six replicate

Table 3: Results of recovery study

% Level	% MEAN±SD*
80%	99.08±0.937
100%	98.87±0.447
120%	99.07±0.878

* Value of three replicate and three concentrations

Table 4: Results of precision and Robustness

Parameter	% MEAN±SD*
Repeatability	98.955±0.095
Intermediate precision	
Day to day precision	99.039±0.081
Analyst to Analyst	98.804±0.051
Robustness	99.053±0.081

* Value of five replicate and five concentrations

Table 5: Results of LOD and LOQ

Name	LOD (µg/ml)	LOQ (µg/ml)
Fexinidazole	0.15	0.45

* Value of five replicate and five concentrations

Table 6: Analysis of tablet sample

	Fexinidazole
Label Claim (mg)	**600mg**
% Found (mg)	598.85
% Assay	99.80
% RSD	0.125

*Average of three determination

Table 7: Results of Forced degradation studies of Fexinidazole

Stress conditions	Drug recovered (%)	Drug decomposed (%)
Standard drug	99.85	0
Acidic hydrolysis	86.65	13.2
Alkaline hydrolysis	83.32	16.53
Oxidative degradation	90.14	9.71
Thermal degradation	93.36	6.49

CONCLUSION

The method was effective in analyzing fexinidazole in tablet formulations, accurately quantifying the active ingredient with minimal variability. Forced degradation studies revealed that fexinidazole is relatively stable under oxidative and thermal conditions but susceptible to degradation under acidic and alkaline conditions.

In conclusion, this HPLC method is highly effective for the stability testing of fexinidazole in marketed formulations. It provides valuable insights into the degradation behavior of the drug, ensuring its safety, efficacy, and regulatory compliance throughout its shelf life. This method can be confidently applied in routine quality control and stability testing, contributing significantly to the development and assurance of fexinidazole's pharmaceutical quality.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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