IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Method Developement And Validation For Stability Studies In Tablet Dosage Form Of Deferasirox By Rp-Hplc

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Abstract:

Objective: The primary aim of this study was to develop a simple, fast, isocratic and stability indicating reverse phase high-performance liquid chromatography method for Deferasirox in its tablet dosage form.

Method: The chromatographic separation was achieved on C18 column (250mm × 4.6mm, 5µm particle size) by using simple mobile phase containing Acetonitrile (ACN): 0.1% Triethylamine (TEA) in the ratio of 70: 30 % (v/v). Diluent used was Acetonitrile: water in the ratio of 50:50 %v/v. The flow rate used was 1.0ml/min and detection were carried out at 248 nm.

Results: The Retention time for method was about 5.06 min. The reliability and analytical performance of proposed HPLC method was statistically validated with respect to linearity, ranges, precision, accuracy, robustness, detection and quantification limits. The method was linear in the range of 5.2-80 µg/ml, with correlation coefficient 0.9998. The limit of detection and limit of quantification was found to be 2.6545and 8.0442 µg/ml, respectively. The forced degradation studies were performed by exposing the drug to various conditions like acidic, alkaline, thermal, hydrolytic, and photolytic degradation conditions. The obtained results showed that the method was specific with no interferences due to excipients as well as degradation products. This method can be used for routine quality control of Deferasirox in tablet dosage form.

Conclusion: Degradation was performed under various stress conditions. The results obtained by acid, peroxide, photolytic, thermal, hydrolytic degradation were found within acceptable limits. Base degradation was not found to be within the acceptable limits. Compared to various existing methods, this method was found to be better in all the parameters.

Index Terms - Forced degradation studies, Deferasirox, method development/ Validation, RP-HPLC, Stability, Triethylamine.

I. INTRODUCTION

The Antidote class includes Deferasirox (DFS). It is chemically known as 4- [3, 5 bis (2-hydroxyphenyl)-1H-1, 2, 4-triazol-1-yl]-benzoic acid. DFS is an iron chelator that can be swallowed by mouth. The main application of DFS is to reduce severe iron overload in patients who need long-term blood exchange for disorders like beta-thalassemia and other severe anemias[1]. Chronic iron overload (IO) is a serious consequence of blood transfusions in patients affected by myelodysplastic syndromes, thalassemia, and sickle cell disease, regardless of age. In patients affected by hematological malignancies or who have undergone a hematopoietic stem cell transplantation (HSCT), IO is considered multifactorial. The advent of specific iron chelators such

as Deferasirox has represented an effective treatment to lessen the iron content in the body and to prevent subsequent tissue damage. Although the drug has a good tolerability, Deferasirox is characterized by non-negligible risks and toxicities that may require the temporary discontinuation of drug administration or another supportive therapies[7-9]. Deferasirox is a white crystalline powder with a molecular weight of 373.4 g/mol and has good tolerability. Deferasirox displays good solubility in organic solvents such as Ethanol, DMSO (Dimethyl Sulfoxide) and Dimethyl formamide, and is sparingly soluble in aqueous. The melting point is between 116 °C and 117 °C2. In pediatric patients, gastrointestinal disturbances, as well as diarrhea, nausea, vomiting, and liver toxicities are common adverse events. Deferasirox is highly bound to plasma proteins (>99%), and it undergoes hepatic biotransformation through glucuronidation (CYP biotransformation is a minor route of metabolism), with the following excretion into the feces as parent drug (60%) and metabolites (8.3%)[7].

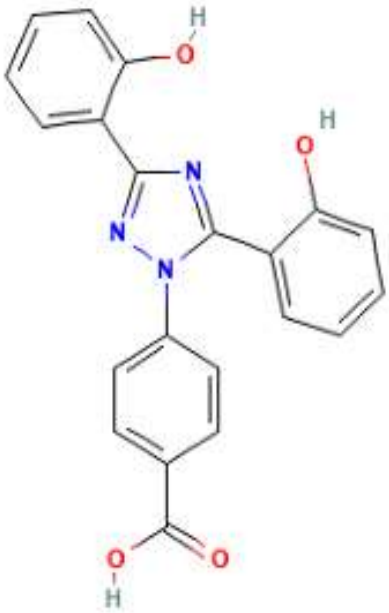

Figure no. 1: Chemical Structure of Deferasirox[3]

II.EXPERIMENTS

Chemicals and reagents: Deferasirox Standard (lek pharmaceuticals, Sandoz company) was obtained with defined purity of 100% from Central Drugs Testing Laboratory. Deferasirox dispersible tablets (Defer Ajaj) were manufactured by Bajaj Pharmaceuticals. Analytical reagents used were Acetonitrile, HPLC grade water, Triethylamine. HPLC grade water was prepared by MerckmilliQ direct water purification system.

Instrumentation: For all Chromatographic analysis Perkin Elmer TotalChrom v.6.2.0.0.1 was used. The LC system was equipped with Flexar solvent manager, Flexar UV/Vis detector, Flexar column oven, Flexar LC pump. The LC system was running on software TotalChrom Navigator version 6.3.4. The HPLC column used for analysis was InertSustain C18 (250mm*4.6mm, 5µm) manufactured by GL Life sciences.

Chromatographic conditions: The optimised mobile phase was prepared by mixing Acetonitrile and 0.1% TEA in the ratio of 70:30 %v/v. Prior to use the mobile phase was filtered using a 0.45 µm membrane filter and was degassed by sonicator prior to use. The wavelength of 248nm was finalized because at this wavelength maximum absorption was seen (UV visible spectroscopy was used to find out lambda Max). The flow rate was 1.0 ml/min, injection volume was of 10µL was used, the temperature of the column was 25 °C and the chromatographic run time was 15 minutes.

Preparation of diluent: Considering the chemical nature and solubility of the drug, Acetonitrile and water in the ratio of 50 : 50 % v/v was selected as diluent.

Preparation of standard solution: The standard solution for drug was prepared by dissolving exactly 10 mg of Deferasirox into 100 ml of diluent (ACN:Water) in 100 ml of volumetric flask. Sonication and further dilutions were made to get a concentration of 50 µg/ml of Deferasirox using diluent (ACN:Water).

Preparation of sample solution: Five tablets were taken and crushed in to fine powder. Crushed powder equivalent to 100mg of Deferasirox in 100ml of volumetric flask was taken. 60 ml of diluent (ACN: Water) was added and sonicated for 10 minutes with intermediate shaking and finally made up to 100ml with diluent. Further dilution was made to get a concentration about 50 ug/ml of sample in diluent.

Method development and optimization: The main objective of the study was to develop RP-HPLC method for Deferasirox in tablet dosage form and for its degradation products. The method that is developed had to be simple enough for use in routine quality control laboratory. The chemical structure of Deferasirox reveals that it is nonpolar molecule, therefore the selection of column and mobile phase was done according to nature of molecule. Diluent were selected based on solubility of the drug. Deferasirox spectra showed sufficient absorption at 248nm which was therefore chosen for the entire study. Finally, the following chromatographic conditions were chosen and they are summarized in the Table 1.

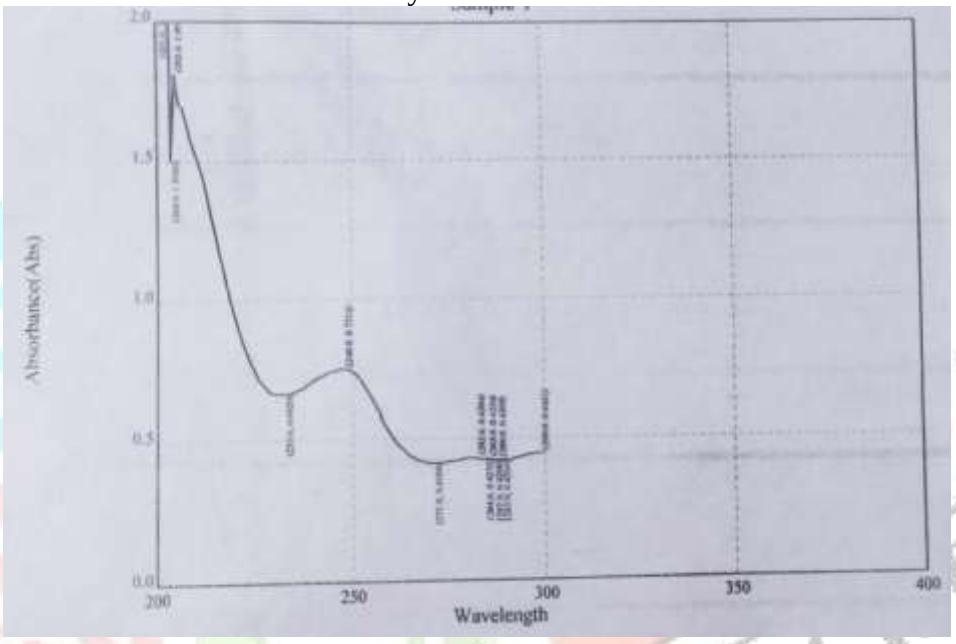

Figure no. 2: UV Spectra of Deferasirox

Table 1: Chromatographic conditions:

Parameters	Chromatographic Conditions
Column	InertSustain C18 (250MM*4.6MM, 5µm)
Mobile phase	ACN: 0.1% TEA buffer (70:30 v/v)
Flow rate	1.0ml/min
Run time	15 mins
Column temperature	25°C
Injection volume	10 ul
Detection wavelength	248nm

III. METHOD VALIDATION

The present and optimized RP-HPLC method was developed6 validated according to ICH Q2 R1(International conference on harmonization) guideline4 and ICH Q2 R2 guideline5 to determine parameters like specificity, linearity, precision, accuracy, robustness, limit of detection (LOD) and limit of quantitation (LOQ).

Precision:

Method precision (Repeatability) was carried out by injecting replicates of Deferasirox with concentration of 50 ppm and % RSD and % assay for Deferasirox was calculated.

Intermediate Precision was evaluated in terms of intraday and interday precision by analyzing different conditions like three different time intervals, different analyst, or on three different days by three different analysts as shown in table 2.

Robustness:

The robustness of the present method was examined by analysis of both reference and sample under variety of experimental conditions. For HPLC method, robustness was established by making deliberate modification including different wavelength (± 2 nm), different mobile phase composition (± 5%), different flow rate (± 0.1 %) and different column (Agilent Zorbax C18 column 250*4.6mm 5um), and results were calculated as shown in table 3.

Linearity:

The linearity of the drug was carried out by preparing appropriate aliquots from standard stock solution of Deferasirox to obtain concentration in the range of 5.2 80 ppm. The linear calibration was constructed by analyzing the concentration over the selected range versus peak area of standard solution. The results were estimated by least-squares regression analysis, and the correlation coefficient and calibration equation were calculated and mentioned in table 4 and figure 16.

Accuracy:

Accuracy was performed by standard addition method (recovery study) of known amounts of Deferasirox standard solution added to sample solution. Method was established at three different levels, 110 %, 120% and 130 %. The results of mean recovery well within the acceptable limits and shown in table 5.

Limit of detection (LOD) and Limit Of Quantitation (LOQ):

The limit of detection (LOD) and Limit of quantification (LOQ) of the optimized method were determined using the calibration curve. Samples of Deferasirox were prepared and injected 6 times. The LOD and LOQ were determined by using the following formula and is shown in table 6.

LOD = $3.3 \times \sigma/S$ (σ = standard deviation, S = slope of calibration curve)

LOQ = $10 \times \sigma/S$ (σ = standard deviations, S = slope of calibration curve)

Specificity:

Forced degradation was performed to establish degradation nature of drug substance and to establish stability indicating nature of a developed method, the drug was subjected to different conditions such as acidic, basic, water, photolytic, thermal and peroxide conditions. Degraded samples were analyzed using UV/VIS detector (Ultraviolet and visible) system to analyze the peaks at different time intervals and to determine the peak purity.

IV. RESULTS (CHROMATOGRAM)

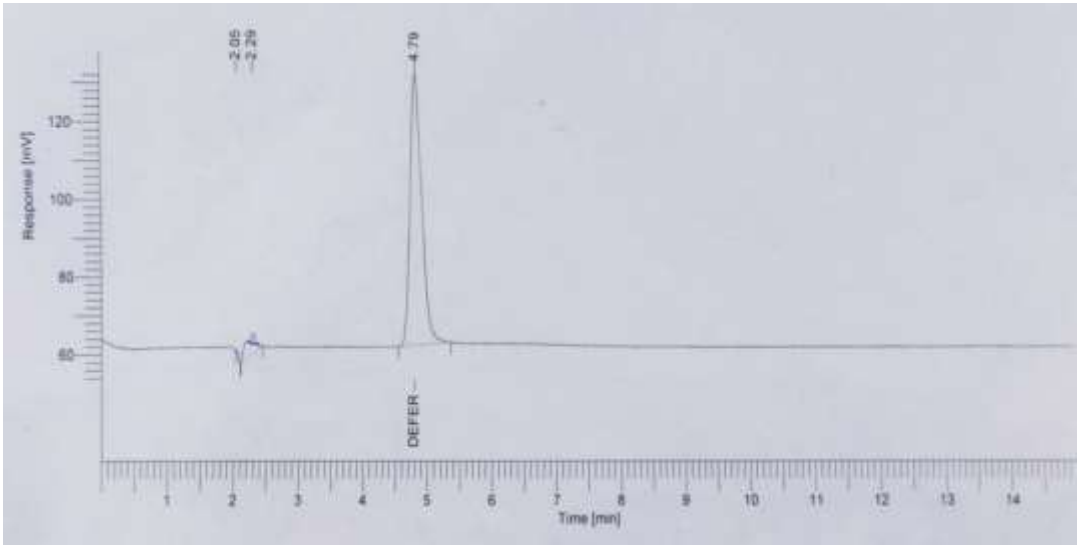

Figure no. 3: 0.1N NaOH

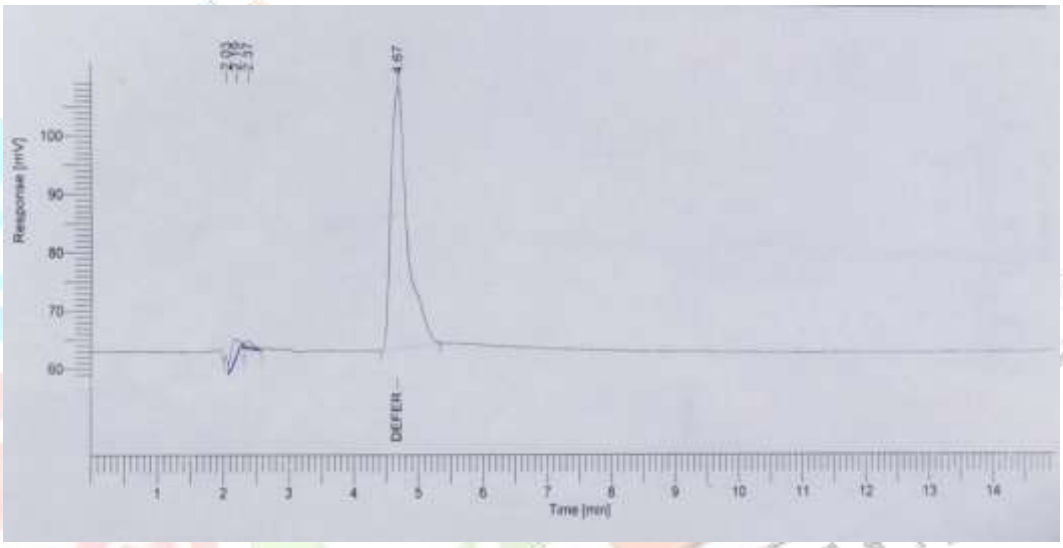

Figure no. 4: 0.5N NaOH

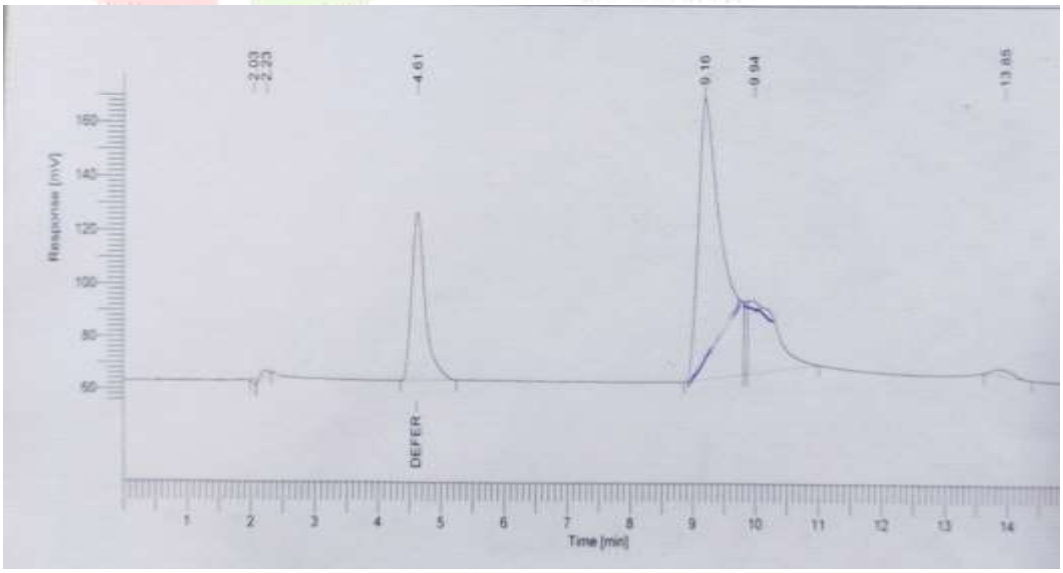

Figure no. 5: 1N NaOH

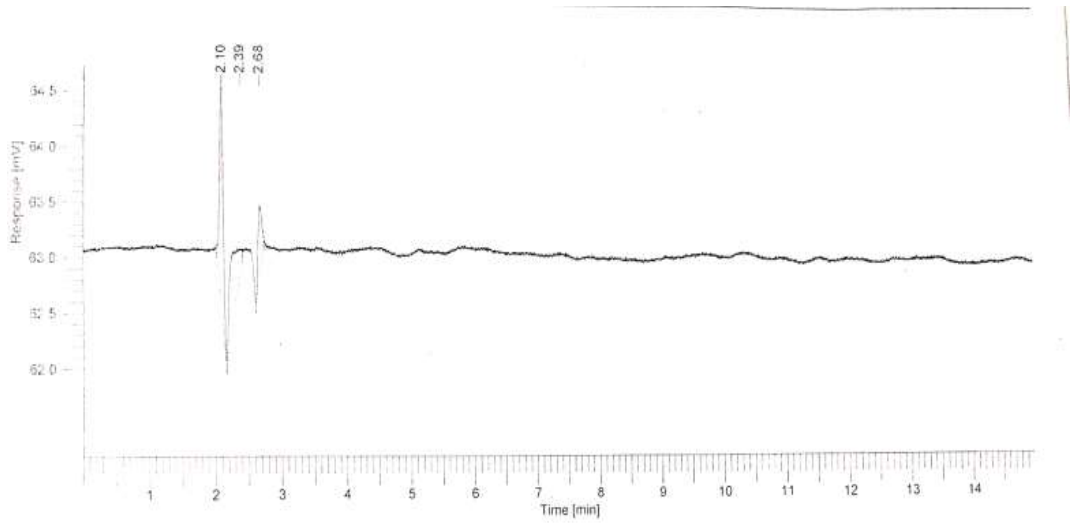

Figure no. 6: Blank

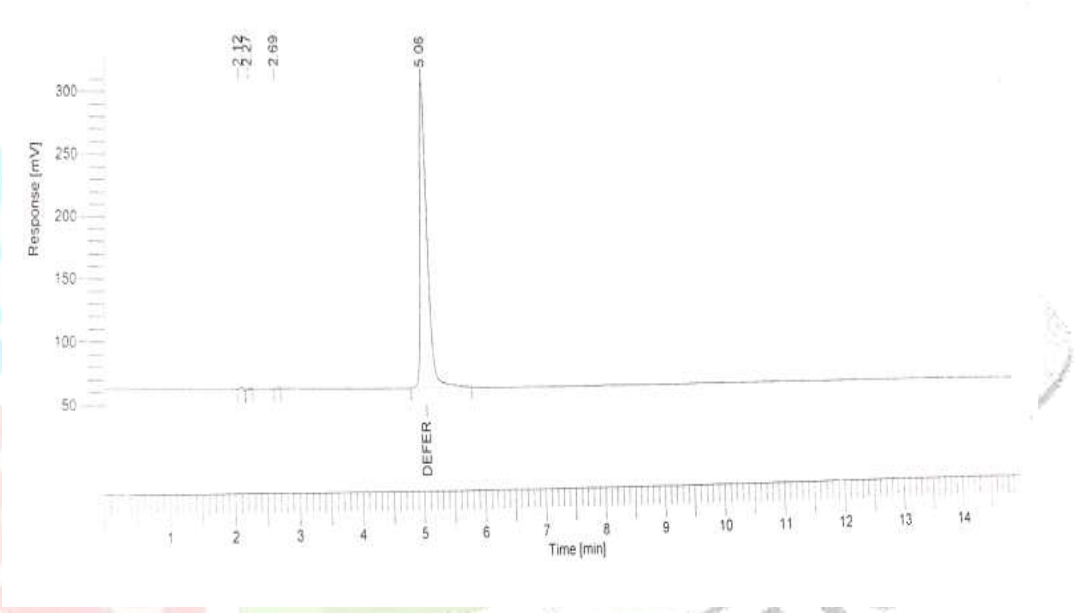

Figure no. 7: Standard

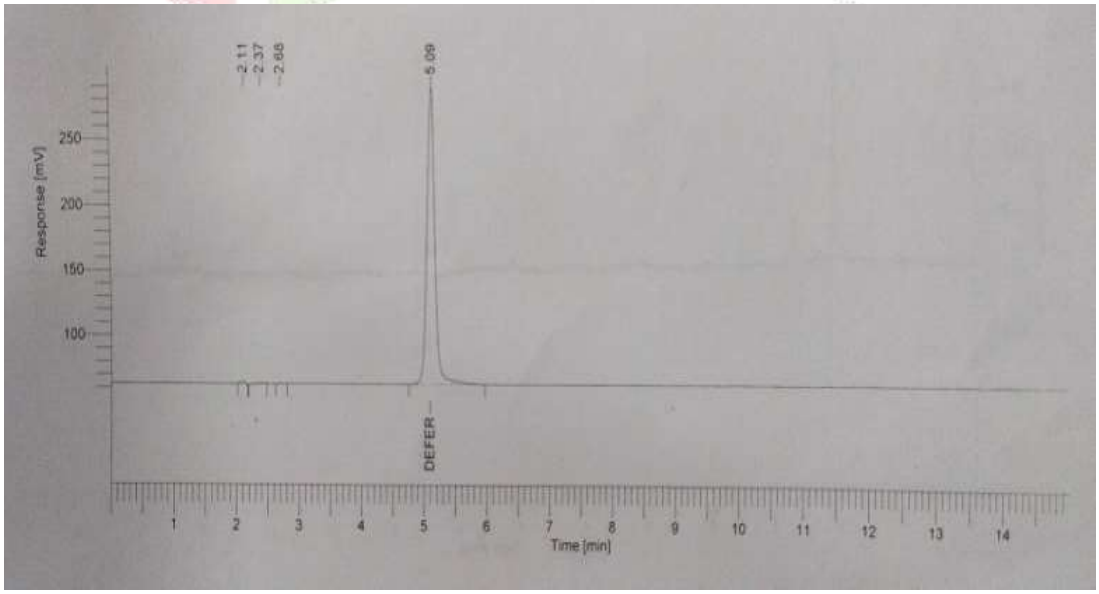

Figure no. 8: Sample

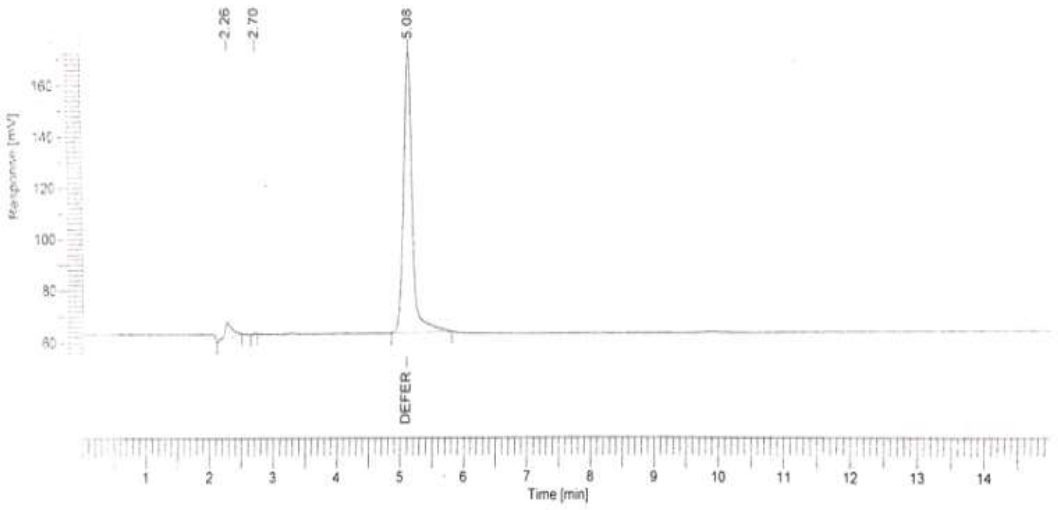

Figure no. 9: 0.1N HCl

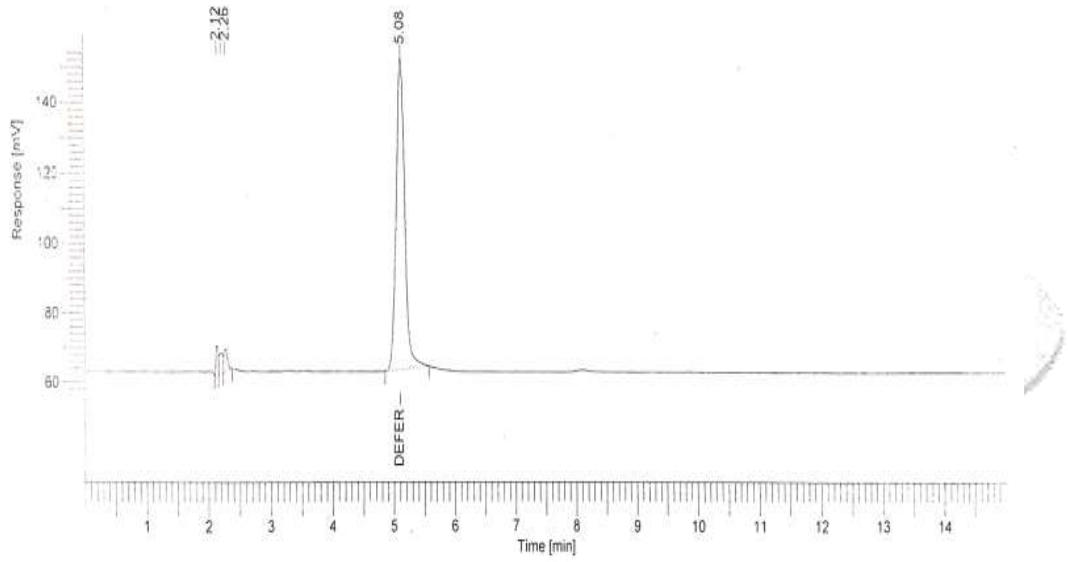

Figure no. 10: 0.5N HCl

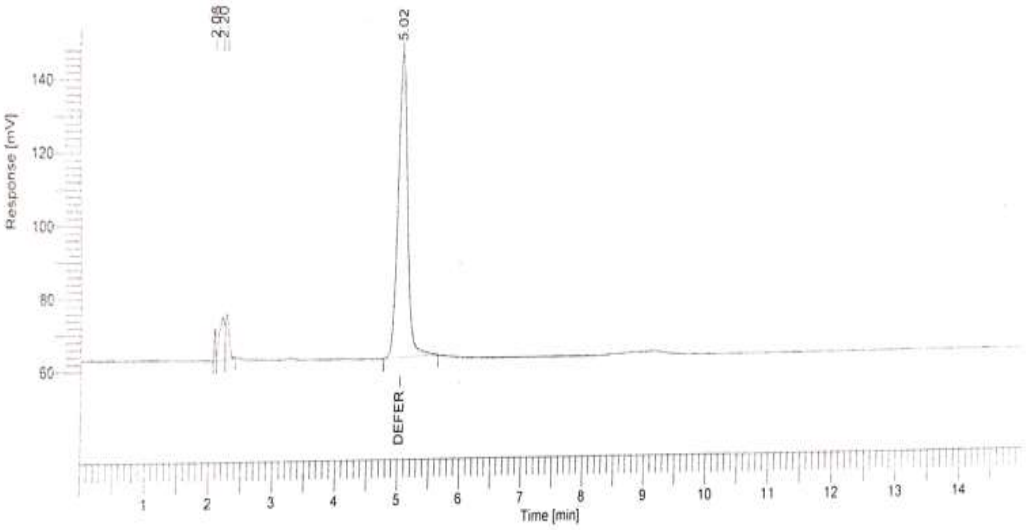

Figure no. 11: 1N HCL

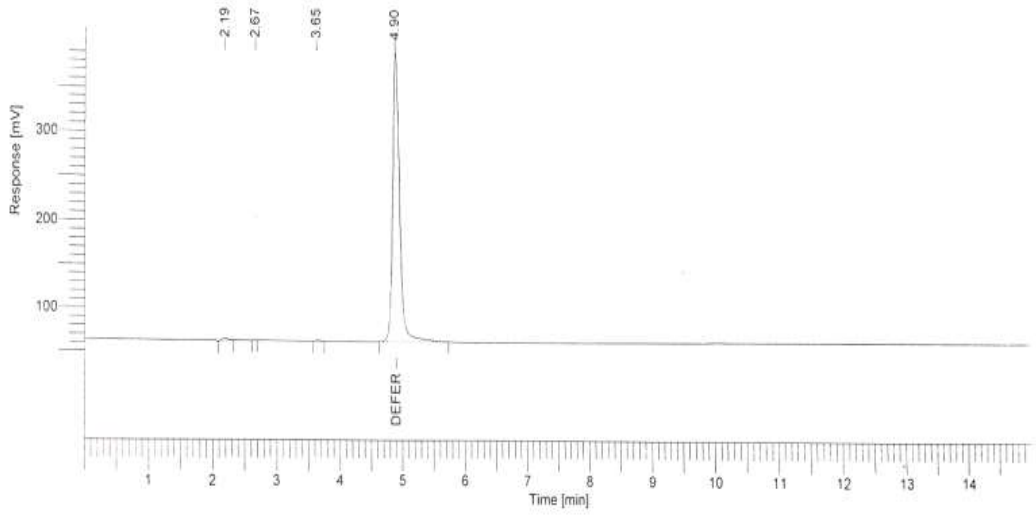

Figure no. 12: Photolytic standard

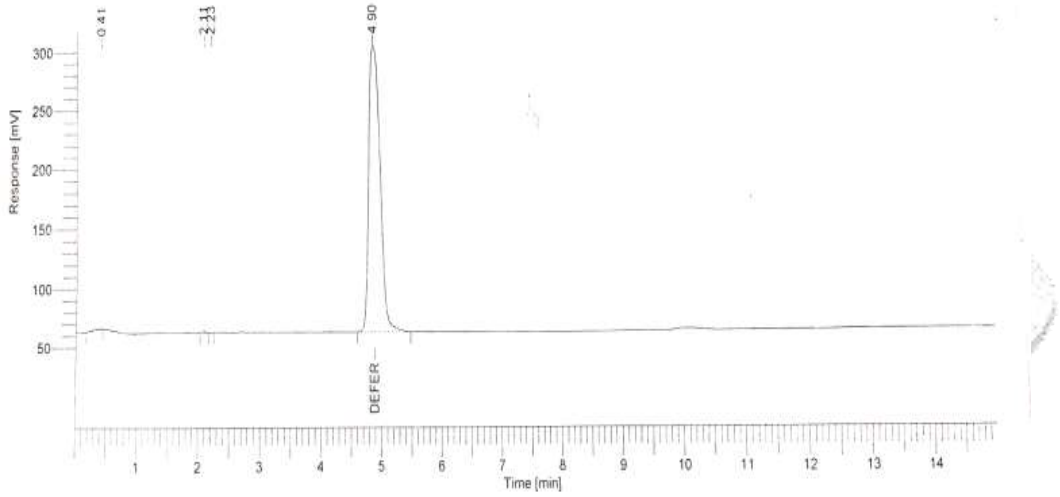

Figure no. 13: Photolytic sample

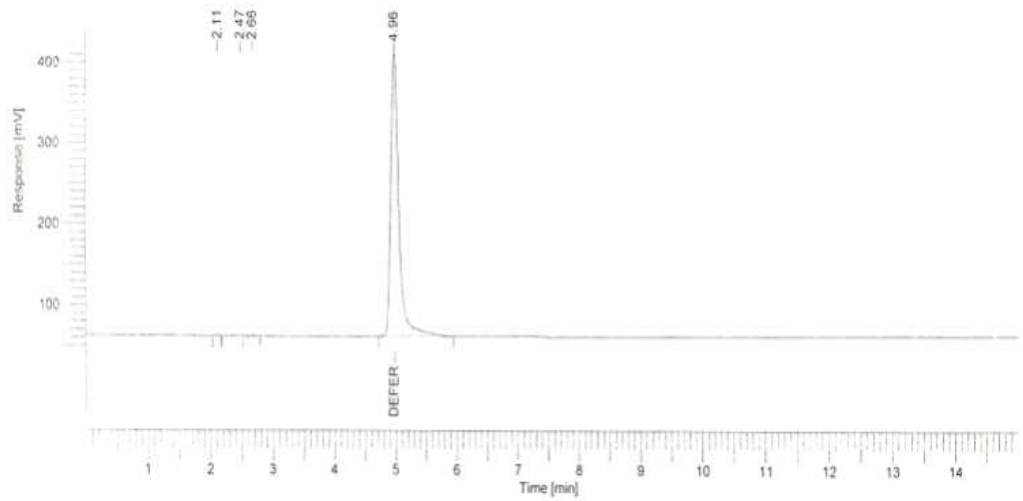

Figure no. 14: Thermal degradation of sample

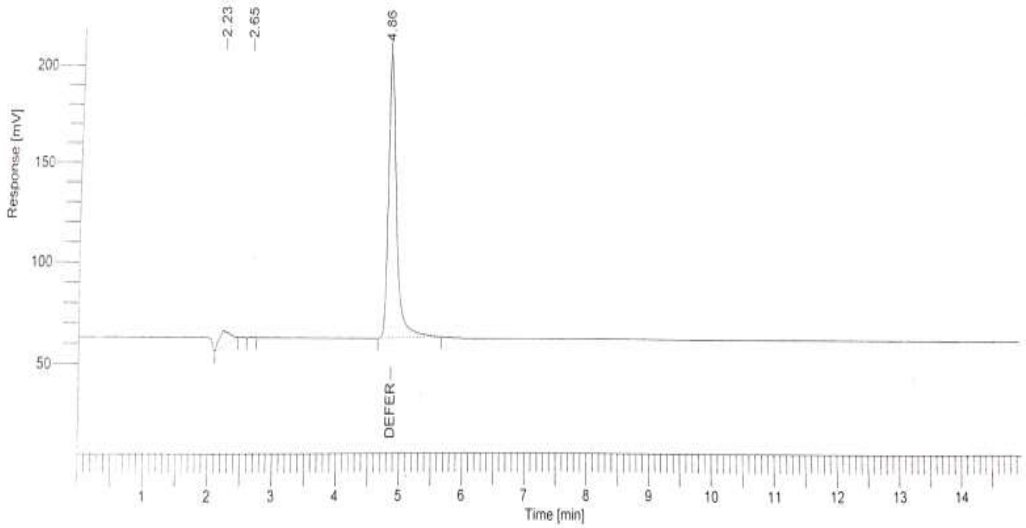

Figure no. 15: Hydrolytic degradation of sample

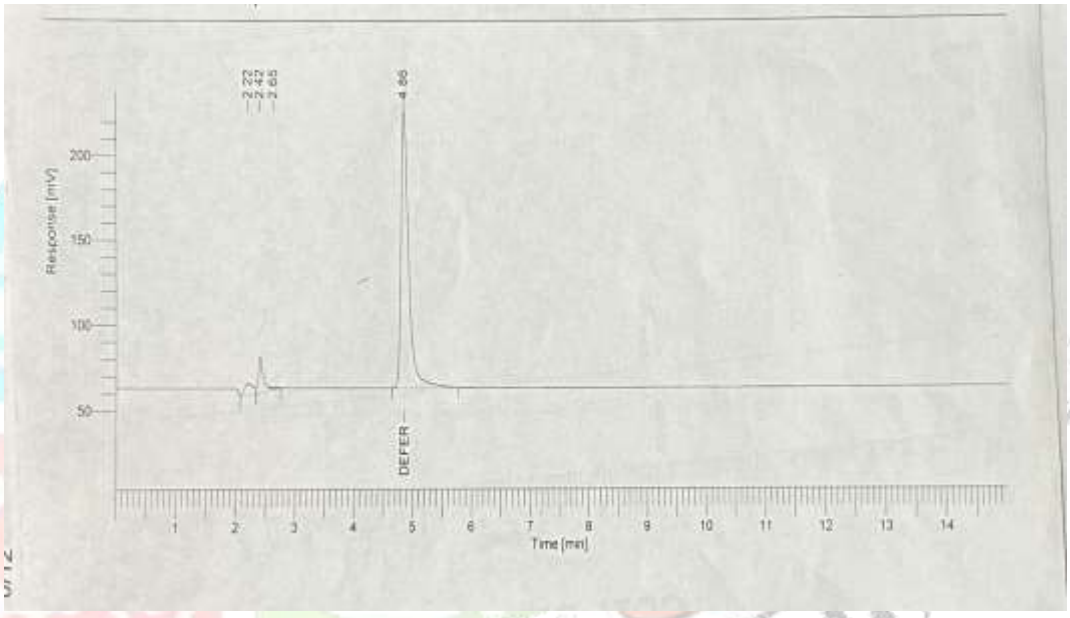

Figure no. 16: Peroxide degradation of sample

Table 2: Method precision (Assay repeatability) of Deferasirox:

Sr. No.	Peak Area
1	1908166
2	1905384
3	1918050
4	1908429
5	1983891
6	1953702
Mean	1929603.67
SD	32111.8787
%RSD	1.664

Table 3: Robustness data for Deferasirox:

Parameter	Change in Parameter	% Estimation	Mean	SD	% RSD
Wavelength	**246nm**	103.78	2077658.67	19512.6418	0.939
	250nm	107.01	2014402.33	11327.8041	0.562
Flow rate	0.9 ml/min	105.71	2224158.00	41751.1157	1.877
	1.1 ml/min	103.45	1808656.33	12108.0690	0.699
Mobile phase ratio	65:35	103.54	2002693.00	9262.3252	0.462
	75:25	104.05	1995442.00	4025.8220	0.202
Column change	Agilent Eclipse Plus C18 column	103.51	2005387.00	17630.2900	0.879

Table 4: Linearity data for Deferasirox:

Sr. no.	Conc. (PPM)	Mean AUC
1.	5.2	226252.7
2.	10	381706.3
3.	20	783708
4.	40	1591747
5.	50	2004204
6.	60	2415286
7.	70	2776731
8.	80	3189865

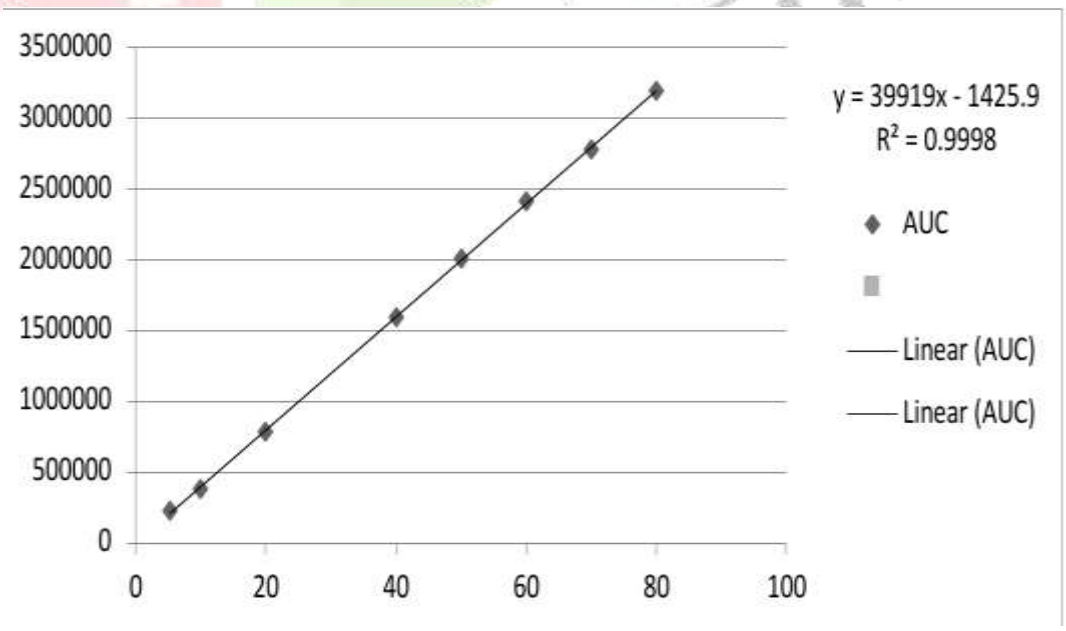

Figure no. 16: Calibration curve of Deferasirox

Table 5: Average recoveries:

% level	Standard added (ml)	% Found	% recovery	Standard deviations	% RSD
110 %	2	111.30 %	101.18 %	34004.071	1.687
120 %	4	120.24 %	100.20 %	34004.0741	1.687
130 %	6	131.58 %	101.22 %	34004.0741	1.687

Table 6: Limit of detection and quantification:

Sr. no.	Area
1.	1908166
2.	1905384
3.	1918050
4.	1908429
5.	1983891
6.	1953702
Average	1929603.67
SD	32111.8787
%RSD	1.664
Regression coefficient	0.9998
Slope	39919.1736
LOD	2.6545
LOQ	8.0442

Stability studies: Table

1)Acid degradation:

Acid degradation was performed, sample was prepared by adding 1:1 ratio of 50ppm sample and different concentrations of HCl- 0.1N, 0.5N, 1N respectively. This acid degradation sample was allowed to stand for 30 minutes each before each injection, and the percentage degradation was found to be 4.59% for 0.1N, 8.2% for 0.5N, 12.12% for 1N. shown in figure 9, figure 10, figure 11, respectively.

2)Base degradation:

Base degradation was performed, sample was prepared by adding 1:1 ratio of 50ppm sample and different concentrations of NaOH- 0.1N, 0.5N, 1N respectively. This base degradation sample was allowed to stand for 30 minutes each before each injection, and the percentage degradation was found to be 6.38 % for 0.1N, 7.07% for 0.5N, 79.8% for 1N. shown in figure no. 3, figure no. 4, figure no.5, respectively.

Photolytic degradation:

Photolytic degradation was performed by exposing 50ppm sample and 50ppm standard to UV light for 72 hours, and the sample and standard was injected, and it was observed that there was no degradation in both sample and standard shown in figure no.13, figure no.12, respectively.

Hydrolytic degradation:

Hydrolytic degradation was performed by adding 5mg sample in 100ml water and it was allowed to stand for 30 minutes, and then injected. The %degradation was found to be 5.17% shown in figure no. 15.

Peroxide degradation:

Peroxide degradation was performed by treating 50ppm sample with 0.1% of peroxide and the %degradation was found to be 11.22%. shown in figure no. 16.

Thermal degradation:

Thermal degradation was performed by exposing the 50ppm sample to 30°C for 30 minutes and injected. There was 0.16% degradation observed and shown in figure no. 14.

Table 7: Stability studies:

Sr. no.	Stress Condition	Duration	Retention time	% Degradation
1.	Acid degradation i) 0.1N ii) 0.5N iii) 1N	30 mins	5.02	 4.59 8.2 12.12
2.	Base degradation i)0.1N ii)0.5N iii) 1N	30 mins	 4.79 4.67 4.61	 6.38 7.07 79.8
3.	Photolytic degradation	72 hours	4.90	0.77
4.	Hydrolytic degradation	30 mins	4.86	5.17
5.	Peroxide degradation	30 mins	4.86	11.25
6.	Thermal degradation	30 mins	4.96	0.16

IV. RESULTS AND DISCUSSION

A simple, isocratic and stability-indicating RP-HPLC method has been developed for determination of Deferasirox in tablet dosage form. The optimized chromatographic conditions were predicted by chemical structure of Deferasirox shown in figure 1. Optimization of the method done on selection/choice of column, mobile phase, composition, injection volume and detection wavelength. The obtained chromatogram of Deferasirox reference Standard were well resolved and sharp with retention time 5.06 min given in Figure no.7.

The system suitability parameters were applied to standard chromatograms and RSD of peak area, retention times were determined. The plates and tailing factors were also determined and found well within specified limits.

The concentration obtained was linear in range between 5.2-80 µg/ml. The linear calibration was constructed by analyzing the concentration over the selected range versus peak area of standard solution and regression coefficient was found to be 0.9998 from calibration curve shown in figure no. 16.

The average of percent mean recovery at all three levels was found to be 101.18%, 100.20%, 101.22% respectively and mean percent RSD value found to be 1.687%which found to be within limits and shown in Table.5

For method precision percent RSD result found to be 1.664% for Deferasirox. Intermediate precision was evaluated in terms of intraday precision by analyzing two different day intervals.

Robustness was performed by keeping one parameter constant and other was kept altered. The concern variations on chromatographic method are compile and results obtained.

The optimized method can determine and quantify the analyte at a lower concentration. LOD and LOQ of Deferasirox are determined by using the mentioned formula and shown in Table 6.

The obtained assay results showed that percent assay of Deferasirox in tablet dosage form was within defined limits. These results clearly state that the developed method can be successfully applied for assay of tablet dosage form of Deferasirox.

DISCUSSION

The RP-HPLC method developed for determination of Deferasirox was found to be cost effective after reviewing the existing methods. From the data for precision, it can be concluded that the method is reproducible and reliable. As this method uses less solvent, reagents are easily available and the retention time is also within 6 minutes therefore the solvent consumption is less, therefore this method is superior.

CONCLUSION

A simple isocratic and stability indicating RP-HPLC method has been developed for determination of deferasirox in tablet dosage form. This method is accurate for conducting the stability studies of deferasirox in tablet dosage form as this method has been validated. The results for stability for deferasirox determined by this method were found within the acceptable limits according to the ICH Q2(R2) guideline. Compared to the previous method this method provides better results with respect to stability of deferasirox in tablet dosage form. Complete method validation was performed with respect to ICH Q2(R2) guideline. Retention time of deferasirox by this method was 5.06 min therefore this method is quick and consumes less mobile phase.

ACKNOWLEDGMENT

The authors are thankful to the Director of Central Drugs Testing Laboratory, Mumbai and Guru Nanak Khalsa College of Arts, Commerce and Science, Mating East for the guidance and support. Special thanks to Dr. Vijay Kumar and Dr. Naomita Dhume. The Authors are extremely grateful to Mrs. S.U. Warde, Mr. Shravan Kumar Singh for their valuable guidance and support.

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