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Research Article

STABILITY INDICATING RP-HPLC METHOD FOR THE DETERMINATION OF DEFERASIROX IN BULK DRUG AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A simple, selective, precise and stability indicating RP-HPLC method for analysis of Deferasirox in bulk drug and pharmaceutical dosage forms was developed and validated. The chromatographic conditions comprised of a reversed phase C_{18} column (hypersil 150mm×4.6, 5µm particle size), with a mobile phase composed of mixture of Methanol and Sodium Dihydrogenorthophosphate buffer (i.e, prepared by diluting 7gms of Sodium Dihydrogen Orthophosphate dissolved in 500 ml of water and pH was adjusted to 7) in the ratio of 30:70 v/v respectively. Flow rate was adjusted to 1.0ml/min. Detection was carried out at 215nm. The retention time of Deferasirox was found to be 2.390 min. The linear regression analysis data for the calibration plots showed good linear relationship within the concentration range 5-15µg/ml. The value of correlation coefficient was found to be 0.9995. The drug undergoes degradation under thermal, acidic, basic, peroxide and robustness conditions. All the peaks of degraded products were resolved from the active pharmaceutical ingredient with significantly different retention time. As the method could effectively separate the drug from its degradation product, it can be employed as a stability indicating one.

Keywords: Deferasirox, RP-HPLC, stability indicating Degradation studies

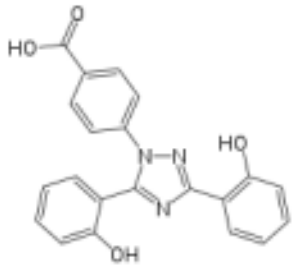
INTRODUCTION

Deferasirox is an iron chelating agent. Deferasirox molecular formula is $C_{21}H_{15}N_3O_4$ and its molecular weight is 373.4. Deferasirox designated chemically as 4-[3,5-Bis (2-hydroxyphenyl)-1H-1,2,4-triazol-1-yl]-benzoic acid[1-2]. The Literature survey reveals that few methods like Validation of a novel spectrophotometric methods for estimation of Deferasirox[3]. Spectrophotometric determination of Deferasirox in formulations using Folin ciocalteu and ferric chloride reagents[4]. HPLC coupled with a MS/MS[5] detection, LC, Terbium sensitized florescence methods for the estimation of Deferasirox alone and electro catalytic oxidation method for determination of Deferasirox in combination with Deferiprone in the formulations[6-8] have been reported. In view of these points an attempt was made to develop a simple, accurate and validated stability indicating RP-HPLC method for estimation of Deferasirox in bulk drug and pharmaceutical dosage forms.

EXPERMENTAL PROCEDURE

Instrumentation:
Stability indicating RP-HPLC determination was performed on Waters e 2695 separation modules equipped with a Hypersil C_{18} reverse phase column (150mm x 4.6mm. 5µ), a binary pump and Waters2998 variable wavelength programmable Photodiode array detector was employed in this study.

Chemical structure:



Deferasirox

Drugs and Chemicals:
Bulk drug of Deferasirox was obtained as a gift sample from Novartis Pharma pvt. Ltd, Mumbai,India. Formulations of Deferasirox used for the study was Asunra (Novartis Pharma pvt. Ltd) contaning 100mg was procured from local market. Methanol used as HPLC grade.

Chromatographic Condition:
The mobile phase used in this study was a mixture of Methanol and Sodium dihydrogen orthophosphate in the ratio

of 30:70v/v. The mobile phase was filtered before use through a 0.45 µ membrane and degassed with helium and purge for 30 min. The injection volume of 10µl was used. The mobile phase was pumped from the solvent reservoir to the column at a flow rate of 1.0 ml/min. The column temperature was maintained at 30°C. The eluents were monitored at 215 nm.

Preparation of the standard solution:

Weigh accurately about 100mg of Deferasirox and transfer it into a 100ml volumetric flask and add 50ml of mobile phase and sonicate for 10min and make up with mobile phase to get a 1mg/ml solution. This solution was suitably diluted by taking 5ml of above solution in 100ml volumetric flask and then the volume was made up with mobile phase to get working standard solution of 100µg/ml of Deferasirox.

Preparation of the sample solution:

A tablet powder equivalent to 100 mg was weighed accurately and transferred in to 100 ml volumetric flask containing 50 ml mobile phase, the flask was sonicated for 20 min, the volume was made up to mark with mobile phase, and the solution was filtered through 0.45µm filter, from the above stock solution, working standard solution of 100µg/ml were prepared by further dilution with mobile phase.

Assay procedure:

Two brands of commercially available tablets were taken, 20 tablets each weighing 100mg were weighed and powered. A tablet powder equivalent to 100 mg was weighed accurately and transferred in to 100 ml volumetric flask containing 50 ml mobile phase, the flask was sonicated for 20 min, the volume was made up to mark with mobile phase, and the solution was filtered through 0.45µm filter, from the above stock solution, working standard solution of 100µg/ml were prepared by further dilution with mobile phase. Subsequent dilutions of Deferasirox in the range of 5-15µg/ml were prepared from the above solution. The retention time of Deferasirox in bulk drug was found to be 2.391(Fig 1) and retention time of Deferasirox in Pharmaceutical formulation was found to be 2.388 (Fig 2).

METHOD VALIDATION:

Linearity:

The standard curve was obtained in the concentration range of 5-15 µg/ml. The linearity of the method was evaluated by regression analysis using the least squares method. The results are presented in Table No.1 and the linearity graph shown in Fig.3.

The precision of the method was ascertained separately from the peak area ratios obtained by actual determination of six replicates of a fixed amount of drug. The percent relative standard deviation and percent range of errors (at 0.05 and 0.01 confidence limits) were calculated for Deferasirox and are presented in Table No. 2. The precision of the assay was also determined in terms of repeatability and intra and interday variations in the peak areas for a set of drug solutions was calculated in terms of %RSD and the results are presented in Table No.2 and Table No.3.

Accuracy:

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts of bulk sample Deferasirox to the pre-analysed formulation. The results are recorded Table No.4 and Table No.5.

Robustness:

As part of the Robustness, deliberate change in the Flow rate, Temperature and P^H was made to evaluate the impact on the method.

Standard Solution 100µg/ml was prepared and analysed using varied flow rates along with method flow rate.

a. The flow rate was varied at 0.8ml/min and 1.2ml/min. By small increase or decrease the flow rate, there will be change in the retention time. The same value of the peak area indicated the robustness of this method. The results are summarised in Table No.6

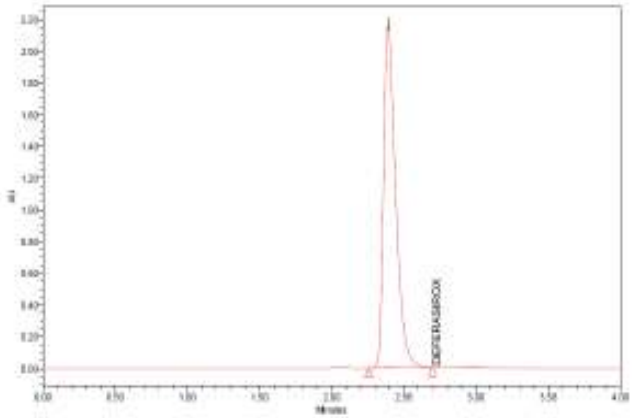


Figure 1: A Typical Chromatogram of Deferasirox Standard drug

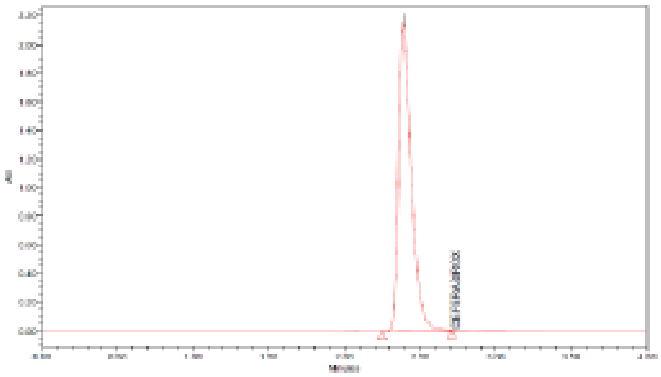


Figure 2: A Typical Chromatogram of Deferasirox Formulation

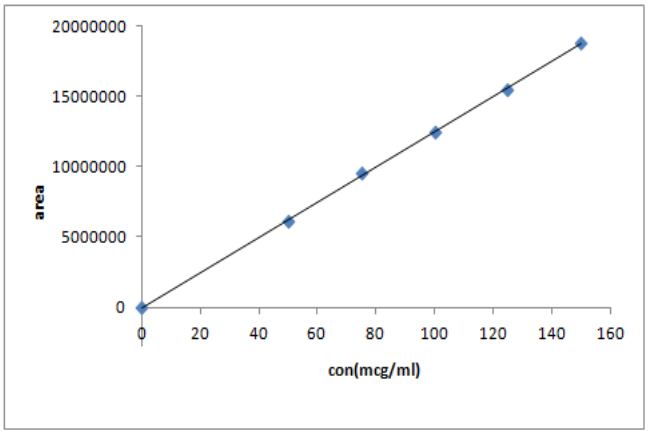


Figure 3: Calibration graph of Deferasirox 5-15 µg/ml Precision

Table 1: Calibration curve of Deferasirox 5-15µg/ml

Sl. No	Concentration of Deferasirox (µg/ml)	Mean Peak area
1	5	6175171
2	7.5	9532614
3	10.0	12440191
4	12.5	15555543
5	15	18875994

Table 2: Regression characteristics and precision of the proposed method for Deferasirox

Parameters	HPLC
Linearity Range	5-15µg/ml
Regression equation(Y*)	
Slope (a)	125698
Intercept (b)	-53927
Correlation coefficient (r)	0.9995
Standard deviation	1.195
% RSD	0.9606×10^{-5}
No. of Theoretical plates	4025
LOD	3.13×10^{-4}
LOQ	9.5×10^{-4}
HETP	3.7×10^{-5}
Range of errors**	
Confidence limits with 0.05 level Confidence limits with 0.01 level	0.9992 1.4787

***Y=bC+a, where Y is the absorbance unit and C is the concentration of Deferasirox in µg/ml,**
****Average of six determinations.**

Table 3: Intra and Inter-day Precision for Deferasirox Assay in Pharmaceutical dosage forms by the proposed method for Deferasirox

Concentration of Deferasirox (µg/ml)	Intra-day Precision			Inter-day Precision		
	Mean amount of drug found(n=3)	Percent amount of drug found	Percent RSD	Mean amount of drug found(n=3)	Percent amount of drug found	Percent RSD

5	4.99	99.80	0.15	4.92	98.4	0.203
10	9.98	99.83	0.03	9.94	99.4	0.266
15	14.99	99.94	0.02	14.96	99.62	0.063

Table 4: Evaluation of Deferasirox in Tablet Dosage formulations

	Label Claim (mg)	Amount of drug obtained by proposed methods (mg)	% Recovery*
T_1	100	99.94	99.40
T_2	100	99.92	99.20

T_1, T_2 are the tablets from different manufacturers.
*mean of six determinations.

Table 5: Recovery of Deferasirox using the proposed method

Drug concentration(% at specification level)	Amount of Drug in formulation (mg)	Amount of drug added (mg)	Amount of drug recovered (mg)	% Recovery
80	99.94	80	179.82	99.85
100	99.89	100	199.82	99.93
120	99.92	120	219.79	99.89

Table 6: Effect of different Flow rate on chromatogram of Deferasirox

Sl. No	Flow rate ml/min	Retention time(min)	Peak area
1	0.8	1.922	12513804
2	**1.0(actual)**	2.390	12514807
3	1.2	3.181	12515706

b. By introducing small deliberate changes in the Temperature, there will be change in the Retention time. The same value of the Peak area indicated the robustness of this method. The results are summarized in Table No.7.

c. By introducing small deliberate changes in the P^H, there will be change in the peak shape. The results are summarized in Table No.8.

Table 8: Effect of Different P^H on chromatogram of Deferasirox

pH	Observation
4.2	Fronting peak
7.0(Actual)	Good symmetrical Peak
8.5	Asymmetrical peak

Forced degradation studies: Forced degradation studies were conducted to evaluate the stability and specificity of the method.

Table 7: Effect of different Temperature on chromatogram of Deferasirox

S.No	Temperature(°C)	Retention time	Peak area
1.	25	2.506	12514302
2.	**30(Actual)**	2.390	12514807
3.	35	2.104	12514208

a. Thermal degradation: Thermal degradation studies were observed for 0, 2, 4, 8, 16, 24 hrs. There was no significant change in the peak. The drug was stable for 24hrs even after heating upto 80°C.

b. Acidic conditions:

For acidic decomposition study, Deferasirox was dissolved in 0.1N HCl and the solution was left in dark at 80^0c for 30min and 60min. No significant degradation of Deferasirox was observed when the drug was subjected to acidic conditions. There was no significant change in the peak as shown in Fig. 5. The results were represented in the Table No.9.

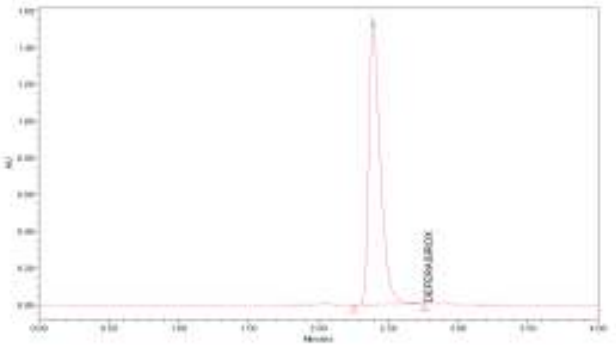


Figure 4: Thermal degradation

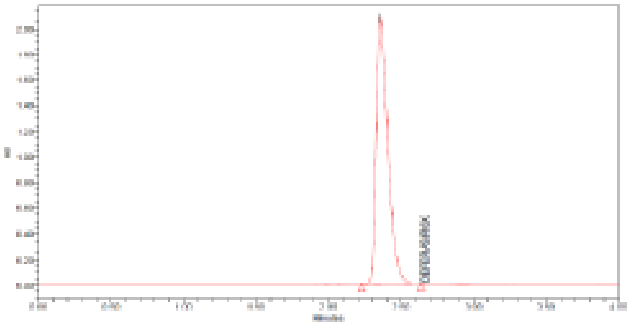


Figure 5: Model chromatogram of Deferasirox (Acidic conditions)

Table: 9 Acid Stress Study (0.1N HCl)

Conc. (µg/ml)	Time, min	Deferasirox (Rt)	Rt of degraded product
100µg/ml	0	2.390	-
	30	2.387	-
	60	2.384	2.377

c. Basic conditions:

For basic decomposition study, Deferasirox was dissolved in 0.1N NaOH and the solution was kept in hot air oven at 80^0c for 60min. No significant degradation of Deferasirox was observed when the drug was subjected to basic conditions. There was no significant change in the peak as shown in Fig. 6. The results were represented in the Table No. 10

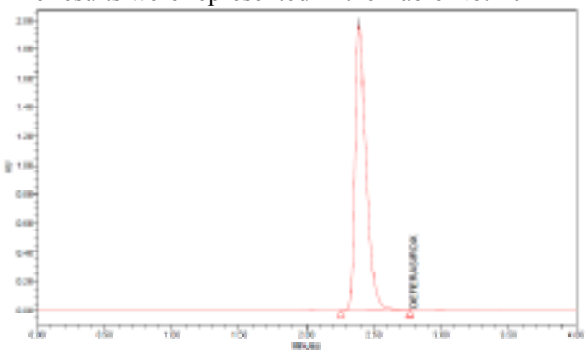


Figure 6: Model chromatogram of Deferasirox (Basic Conditions)

Table 10: Base Stress Study

Conc. (µg/ml)	Time, min	Deferasirox (Rt)	Rt of degraded product
100µg/ml	0	2.390	-
	30	2.385	-
	60	2.389	2.382

Table 11: Peroxide stress study(3%H_2O_2)

Conc. (µg/ml)	Time (min)	% of Hydrogen peroxide	Deferasirox(Rt)	Rt of degraded product
100µg/ml	0	3%	2.390	-
	30	3%	2.388	-
	60	3%	2.389	2.05

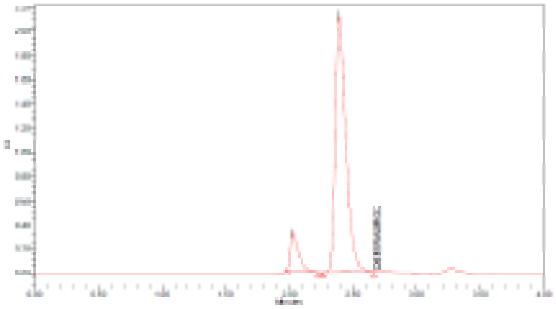


Figure 7: Model chromatogram of Deferasirox (3% H_2O_2)

Oxidation conditions:

For oxidation decomposition study, Deferasirox was dissolved in 3% Hydrogen peroxide and the solution was kept in hot air oven at 80^0c for 30min and 60min respectively. A significant degradation of Deferasirox was observed when the drug was subjected to 3% Hydrogen peroxide. The peaks were found at retention time of 2.05 respectively as shown in Fig. 7. The results were represented in the Table No.11.

RESULTS AND DISCUSSION

The critical evaluation of the method was performed. The different method validation parameters were validated and the results are shown in Table No.2. The good percentage recovery in the tablet formulation suggests that the excipients present in the formulation have no interference in the estimation of Deferasirox. The %RSD is less than 1% showed high degree of precision of the proposed method. The developed method was also specific as it was capable of determining Deferasirox in presence of its degradation products. The forced degradation study of Deferasirox shows that the drug degrades in order of 3%H_2O_2 >0.1N HCl> 0.1N NaOH and thermal with the above fact the developed method can be accepted as a novel stability indicating RP-HPLC method which uses Methanol : Sodium Dihydrogen orthophosphate buffer as the mobile phase which are not used previously, as per literature survey .

CONCLUSION

It can be concluded that the developed RP-HPLC method is stability indicating rapid, simple, specific, accurate and precise. That can be employed sucessfully for the determination of Deferasirox in bulk drug and pharmaceutical dosage forms.

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