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METHOD DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE DETREMINATION OF DEFERASIROX IN TABLETS

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ABSTRACT

A simple, accurate, precise and linear isocratic RP-HPLC has been developed and subsequently simultaneous for determination of Deferasirox in pharmaceutical formulations. Develosil ODS HG-S (150X4.6) 5µ with flow rate of 2mL/min. By using HPLC water PU-2695 pump and photodiode array detector-2996 at 245nm. The separation was carried out using a mobile phase consisting of mixture of sodium dihydrogen phosphate monohydrate buffer (pH3.0) and acetonitrile in the ratio of 55:45. The retention time of Deferasirox was found to be 6.31 min. The mean percentage recovery was found to be 99.55. The correlation coefficient was found to be 0.998. The percentage estimation of the drug was found near to 100% representing the accuracy of the method. The proposed method was also validated and applied for the analysis of the drug in tablet formulation.

KEYWORDS

RP-HPLC, Deferasirox, Method development, Validation.

INTRODUCTION

Deferasirox is chemically 4-{3, 5-Bis (2-hydroxyphenyl)-1H-1, 2, 4-triazo-1yl}-benzoic acid, is Iron chelating agents. It is indicated for treatment of chronic iron overload due to blood transfusions (transfusional hemosiderosis) in patients 2 years of age and older. It has been shown to reduce the liver iron concentration and serum ferritin levels [(1-5)]. Literature survey revealed several method have been reported for the analysis of this drug using Spectrophotometric, electro-chemical, HPLC and spectroflorometric methods in single and combination with other drugs. But there is no work in RP-HPLC method for the determination of Deferasirox [(6-20)]. The proposed method presented here is simple, fast, accurate and precise and can be used for the determination in tablet dosage forms. The method was validated as per ICH guidelines [(21-22)].

INSTRUMENT

Instrument used in present study was HPLC waters 2690. The pump was waters series pu-2695 pump. The samples were applied Develosil ODS-HG-S (150X4.6) 5µ column with Rhedyne injector. The sample was performed using photodiode array detector-29996 with flow rate 2mL/min. The Sartorius analytical weighing balance was used for weighing purpose.

MATERTIALS

Deferasirox raw material was supplied by Natco pharma limited, Hyderabed. Exjade (Novartis pharmaceutical UK LTD) was taken for study which contains Deferasirox 125mg. HPLC grade acetonitrile (Rankem, Loba PVT.LTD), sodium dihydrogen phosphate monohydrate GR grade (Merck LTD, Mumbai), methanol (Merck LTD, Mumbai), ortho phosphoric acid HPLC grade, HPLC grade water (Millipore, USA).

PREPARATION OF MOBILE PHASE

Sodium dihydrogen phosphate monohydrate buffer (pH3.0) and acetonitrile in the ratio of 55:45 were mixed, sonicated for 15 minutes and filtered through the membrane filter of micron 0.45µ.

PREPARATION OF STANDARD SOLUTION

24 mg of Deferasirox was dissolved in 60 mL of mobile phase and sonicated for 5 minutes and make upto 100mL. The working standard solution concentration was prepared 24µg/mL.

PREPARATON OF SAMPLE SOLUTION

Weigh accurately 20 tablets and powdered. Amount equivalent to 500 mg of Deferasirox from powdered formulation was accurately weighed and taken in 250 mL volumetric flask. Add 180mL of diluent (mixture of acetonitrile and methanol in the ratio of 50:50) and sonicated for 40 minutes and made upto the volume with mobile phase, centrifuged the solution at 300 rpm for 5 minutes. Transfer 3mL of the clear supernatant solution was diluted to 250 mL with mobile phase.

METHOD DEVELOPMENT

SELECTION OF WAVELENGTH

Stock solution of 100 mg/mL was prepared for Deferasirox and further diluted to get the concentration of 10µg/mL of Deferasirox was prepared with methanol. The wavelength was selected by scanning the above standard drug solution between 200 to 400nm. The scanned results showed that reasonable maximum absorbance was recorded at 245nm. Therefore 245nm was selected as the detection wavelength for the RP-HPLC investigation **Figure 1**.

METHOD

The sample was applied Develosil ODS-HG-S (150X4.6) 5µ column with Rheodyne injector in reverse saturation mode using sodium dihydrogen phosphate monohydrate buffer (pH3.0) and acetonitrile in the ratio of 55:45 as mobile phase. The sample was performed using photodiode array detector-2996 with flow rate of 2mL/min. The instrument computes accurate results with minimal time. The result of assay was reported in the **Table 1 and Figure 2**.

VALIDATION OF THE METHOD

ACCURACY

Accuracy was determined by tablet sample with different concentration of the drug (50%, 100% and 150%). Each concentration was injected in three times and assay was performed as per the developed method. From this percentage recovery and amount present (or) recovered were calculated. Result of recovery study was reported in the **Table 2**.

PRECISION

Standard solution of Deferasirox was prepared in same manner for the standard preparation. This solution containing 24µg/mL of Deferasirox. The repeatability was performed for six times. A result of precision was reported in the **Table 3**.

LINEARITY

Linearity was determined in the range of 50-150% (50, 75, 100, 125 and 150) targeted concentration of assay procedure. Five series of standard solution containing 11.99, 19.19, 23.99, 28.79 and 35.99 µg/mL of Deferasirox was injected. Linearity of each concentration and response ratio of concentration was found, linearity was reported in the **Table 4 and Graph 1**.

SYSTEM SUITABILITY

System suitability was analysed by giving six replicates and evaluated the chromatographic parameters like retention time, tailing factor, theoretical plates and peak area the results of system suitability was reported in the **Table 5**.

Table 1: Results of assay

Name of the drug	Amount present per tablet(mg)	% of assay
Deferasirox	125	99.55

Table 2: Results of % recovery studies

Name of the drug	Amount taken (µg)	Amount found (µg)	% recovery	% of mean recovery
Deferasirox	62.5	62.3	99.78	99.78
	125	124.5	99.55	99.55
	187.5	187.4	99.68	99.68

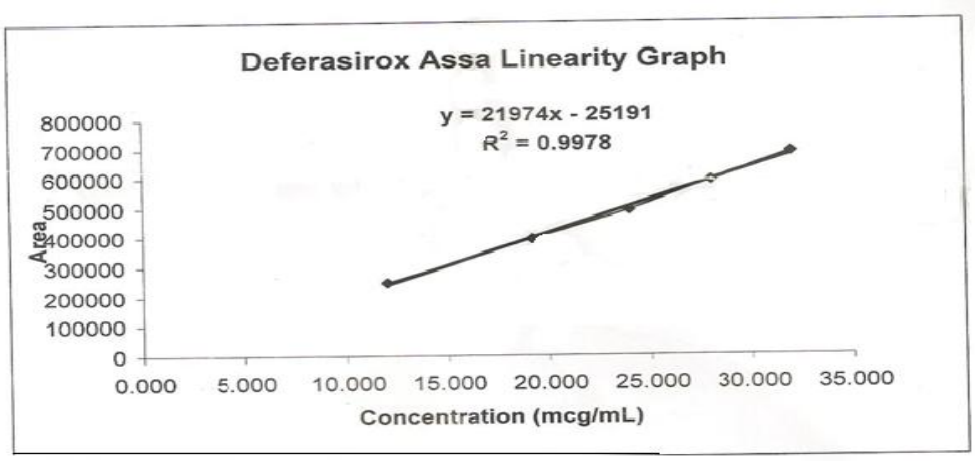
Table 3: Results of precision studies

Injection No.	Retention time	Peak area
1.	6.17	491623
2.	6.16	491323
3.	6.15	491124
4.	6.16	490626
5.	6.17	491126
6.	6.17	491842
Avg	6.16	491277.33
SD	0.007	426.864
%RSD	0.120	0.086

Table 4: Results of linearity studies of standard Defrasirox

Concentration in µg/mL	Standard peak area
11.99	245044
19.19	393897
23.99	491272
28.79	589366
35.99	686102

Graph 1: Linearity chart of standard Deferasirox



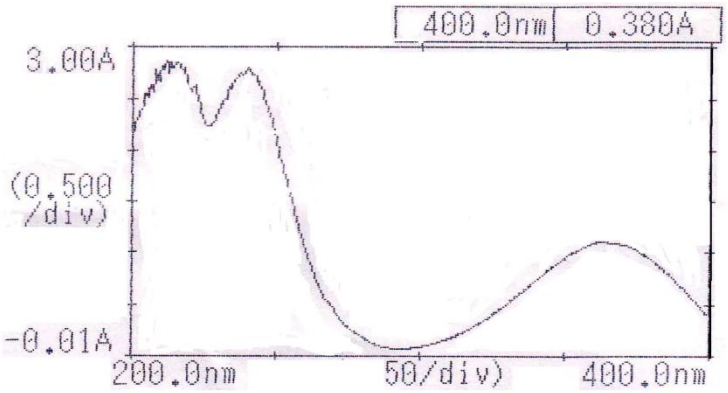


Figure 1: UV spectra of standards

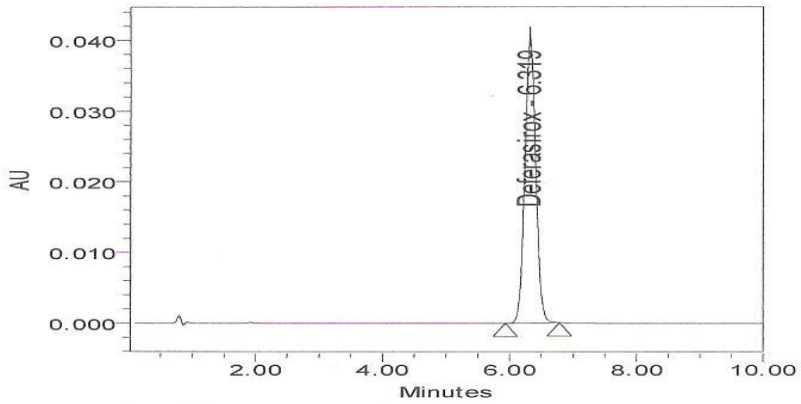


Figure 2: Assay chromatogram of Deferasirox

RESULTS AND DISCUSSION

The present study was aimed to developing an accurate, precise and linear RP-HPLC method for analysis of Deferasirox and in pharmaceutical formulations as per ICG guidelines. Deferasirox shown the linearity response over range 11.99, 19.19, 23.99, 28.79 and 35.99µg/mL and the correlation coefficient was found to be 0.998. The recovery studies of the drug were found to be 99.55% for Deferasirox. The precision %RSD was found to be 0.086 for Deferasirox. The system suitability was studied with six replicates standard solution of Deferasirox and results were found to be acceptance criteria.

CONCLUSION

The proposed method gives good resolution of Deferasirox within a short intervals time (10 minutes). The validation parameters are validated and the results are complied with in the ICG guidelines. The method is accurate, precise and linear for the determination of Deferasirox and pharmaceutical formulations. Therefore the method can be use in routine quality control analysis of the drug.

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