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RESEARCH ARTICLE

REVERSE PHASE HPLC METHOD FOR THE ANALYSIS OF DEFERASIROX IN BULK AND PHARMACEUTICAL FORMULATIONS

M. VIJAYA LAKSHMI[1], J.V.L.N. SESHAGIRI RAO[1] and A. LAKSHMANA RAO*

[1]A.U. College of Pharmaceutical Sciences, Andhra University, Visakhapatnam- 530 003, A.P., India.

*V.V. Institute of Pharmaceutical Sciences, Gudlavalleru- 521 356, A.P., India.

ABSTRACT

A rapid and sensitive high performance liquid chromatographic method is developed for the estimation of deferasirox in bulk and pharmaceutical formulations. Deferasirox was chromatographed on a reverse phase C_8 column in a mobile phase consisting of phosphate buffer (pH 3.0 adjusted with orthophosphoric acid) and acetonitrile in the ratio 40:60 v/v. The mobile phase was pumped at a flow rate of 1 ml/min. with detection at 295 nm. The detector response was linear in the concentration of 10-125 µg/ml. The limit of detection and limit of quantitation was found to be 0.01 and 0.05 µg/ml, respectively. The intra and inter day variation was found to be less than 1%. The mean recovery of the drug from the solution containing 50 µg/ml was 98.8%. The proposed method is simple, fast, accurate, precise and reproducible hence can be applied for routine quality control analysis of deferasirox in bulk and pharmaceutical formulations.

Keywords: *Deferasirox, RP-HPLC, Estimation, Tablets.*

INTRODUCTION

Deferasirox is an iron chelating agent[1]. It is a tridentate ligand that binds iron with high affinity in a 2:1 ratio. Its main use is to reduce chronic iron overload in patients who are receiving long-term blood transfusions for conditions such as beta-thalassemia and other chronic anemias. Deferasirox, chemically[2] designated as 4-[3,5-Bis(2-hydroxyphenyl)-1*H*-1,2,4-triazol-1yl]-benzoic acid

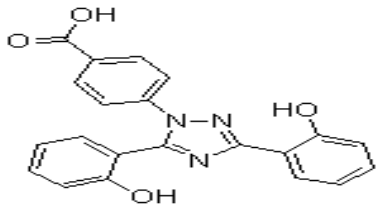

Fig.1: Chemical Structure of Deferasirox

A few HPLC[3-4] and LC-MS[5] methods were reported earlier for the determination of deferasirox in bulk and pharmaceutical dosage forms. In the present study the authors report a rapid, sensitive, accurate and precise HPLC method for the estimation of deferasirox in bulk samples and tablet dosage forms.

EXPERIMENTAL

Chemicals and reagents:

Methanol of HPLC grade was purchased from E.Merck (India) Ltd., Mumbai. Potassium dihydrogen phosphate and orthophosphoric acid of AR grade

were obtained from Qualigens Fine Chemicals Ltd., Mumbai. Deferasirox was a gift sample by Sun Pharmaceutical Industries Ltd., Baroda. The commercially available deferasirox tablets were procured from the local market.

Instrumentation:

The separation was carried out on HPLC system (Waters) with 2695 binary HPLC LC pump, with a 2487 UV-Visible dual absorbance detector, Empower software and RP-C_8 column (250mmx4.6mm I.D; particle size 5µm).

Chromatographic conditions:

The mobile phase consisting of phosphate buffer (pH 3.0 adjusted with orthophosphoric acid) and acetonitrile were filtered through 0.45µ membrane filter before use, degassed and were pumped from the solvent reservoir in the ratio of 40:60 v/v was pumped into the column at a flow rate of 1 ml/min. The detection was monitored at 295 nm and the run time was 10 min. The volume of injection loop was 20 µl prior to injection of the drug solution the column was equilibrated for at least 30 min. with the mobile phase flowing through the system. The column and the HPLC system were kept in ambient temperature.

Procedure:

Stock solution of deferasirox was prepared by dissolving 24.8 mg of deferasirox in 100 ml standard volumetric flask containing 25 ml of mobile phase and the solution was sonicated for 20 min. and then made upto the mark with mobile phase to get a concentration of 250 µg/ml. Subsequent dilutions of this solution were made with mobile phase to get concentration of 10-125 µg/ml. The standard solutions prepared as above were injected into the 20 µl loop and the chromatogram was recorded in Fig. 2.

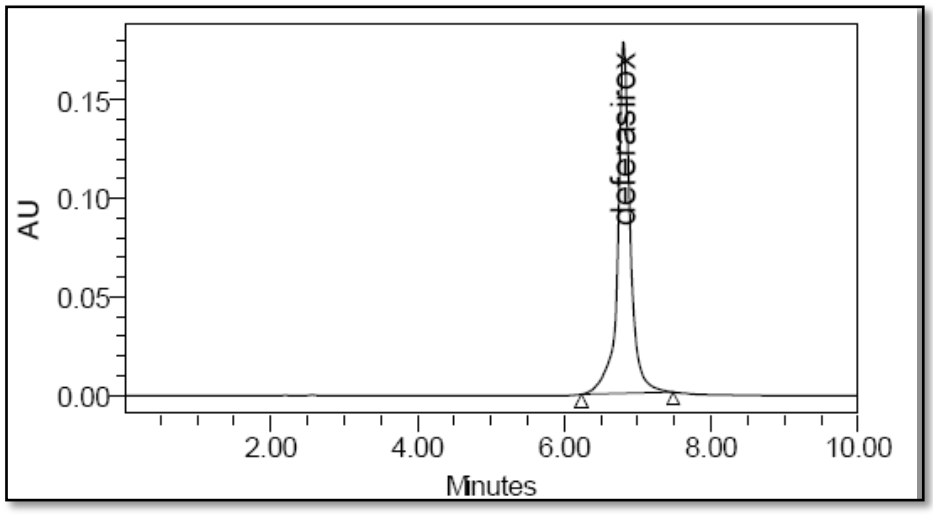

Fig.2. Typical chromatogram of deferasirox

The retention time of deferasirox was found to be 6.80 min. The calibration curve was constructed by plotting concentration vs peak area ratio. The amount of deferasirox present in sample was calculated

through the standard calibration curve. The linearity experiment was carried out in triplicate to ascertain accuracy and precision of the method. The peak area ratios of the drug vs concentration were found to be linear and the results are furnished in Table 1.

Table 1. Calibration data of the method

Concentration (µg/ml)	Peak area (n=6)
10	437046
25	1106803
50	2081654
75	3392822
100	4320548
125	5453041

Assay:

Two commercial brands of tablets were chosen for testing suitability of the proposed method to estimate deferasirox in pharmaceutical formulations. Twenty tablets each containing 100 mg were weighed accurately and powdered. A quantity equivalent to 25 mg of deferasirox was weighed accurately and transferred to 100 ml volumetric flask with mobile phase. The contents were sonicated for 20 min. and made upto the mark with the mobile phase. The resulting solution is filtered through a membrane filter. The solution obtained was diluted with the mobile phase so as to obtain a concentration in the range of linearity previously for the pure drug determined. Sample solution was injected under the chromatographic conditions and the chromatogram was recorded. The amount of deferasirox present in tablet formulation was determined by comparing the peak area from the standard. The results are furnished in Table 2.

Table 2. Assay of deferasirox

Formulation	Label claim (mg)	Amount found (mg)	% Amount found
Brand-1	100	100.02	100.02
Brand-2	100	99.80	99.80

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Validation of proposed method:

Selectivity of the method was assessed on the basis of elution of deferasirox using the above mentioned chromatographic conditions. To study the specificity, linearity, precision, accuracy, limit of detection, limit of quantitation, robustness and system suitability parameters has been validated for the determination of deferasirox. The results are furnished in Table 3.

Specificity:

The specificity was established by preparing a deferasirox standard at 0.5% level of test concentration and injected 5 times into HPLC system as per the test procedure.

Linearity:

The standard curve was obtained in the concentration range of 10-125 µg/ml. The linearity was evaluated by linear regression analysis using the least square method. It was found that correlation coefficient and regression analysis are within the limits.

Precision:

The precision of the assay was determined in terms of intra-day and inter-day precision. The intra-day and inter-day variation in the peak area of drug solution was calculated in terms of coefficient of variation (C.V.) obtained by multiplying the ratio of standard deviation to mean with 100. The results are furnished in Table 4.

Concentration of Deferasirox (µg/ml)	Measured concentration of deferasirox (µg/ml)			
	Intra-day		Inter-day	
	Mean (n=3)	% C.V.	Mean (n=3)	%C.V.
25	25.8	0.146	24.9	0.181
50	49.6	0.037	49.96	0.348
75	76.6	0.052	74.76	0.171

Table 4. Precision of the proposed HPLC method

Limit of detection (LOD) and limit of quantitation (LOD):

The LOD and LOQ for deferasirox were predicted basing on the parameters of standard error of estimate and slope, calculated from linearity of the response data of deferasirox.

Robustness:

The robustness was checked by changing the temperature to 30^{0}and 35^{0}C and the method suits best.

Accuracy:

The accuracy of the HPLC method was assessed by adding known amount of drug solution to a drug solution of known concentration and subjecting the samples to the proposed HPLC method. The recovery studies were replicated 3 times. The accuracy was expressed in terms of recovery and calculated by multiplying the ratio of measured drug concentration to the expected drug concentration with 50 so as to give the percentage recovery. The results are furnished in Table 5.

Table 5. Recovery studies of the proposed HPLC method

Concentration	Amount added (µg)	Mean (±SD) amount found (µg)	Mean % of recovery	Mean
50%	25	12.8(±0.046)	99.2	98.8
100 %	50	24.3(±0.037)	98	
150%	75	38(±0.052)	99.2	

RESULTS AND DISCUSSION

By applying the proposed method, the run time of the method was set at 10 min. and deferasirox appeared on the typical chromatogram at 6.80 min., which indicates a good base line. When the same drug solution was injected 5 times, the retention time of the drug was same. Linearity range was observed in concentration range of 10-125 µg/ml. The regression equation of deferasirox concentration over its peak area ratio was found to be Y=-4138.88+43679.86X (r=0.999) where Y is the peak area ratio and X is the concentration of deferasirox (µg/ml). The proposed HPLC method was also validated for intra-day and inter-day variation. The coefficient of variation in the peak area of the drug for 5 replicate injections was found to be less than 1%. The asymmetry factor was found to be 1.260, which indicated asymmetric nature of peak. The number of theoretical plates was found to be 10464, which indicates efficient performance of the column. The limit of detection and limit of quantitation was found to be 0.01 µg/ml and 0.05 µg/ml, indicates the sensitivity of the method. To optimize the chromatographic conditions, various combinations of phosphate buffer and acetonitrile were tested. The use of phosphate buffer and acetonitrile in the ratio of 40:60 v/v resulted in peak with good shape and resolution. The high percentage of recovery of deferasirox ranging from 98 to 99.2 indicates that the proposed method is highly accurate. No interfering peaks were found in the chromatogram indicating that excipients used in tablet formulation did not interfere with the estimation of the drug by proposed HPLC method.

CONCLUSION

The proposed HPLC method was found to be simple, rapid, precise, accurate and sensitive for the determination of deferasirox in pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine analysis of deferasirox in pure and its pharmaceutical formulations.

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REFERENCES

1. www.rxlist.com
2. www.chemblink.com
3. M.C. Rouan, F. Marfil, P. Mangoni, R. Sechaud, H. Humbert and G. Maurer, J. Chromatogr. B: Biomed. Sci. Appl., 2001, 755, 203-213.
4. M.C. Rouan, C. Buffet, L. Masson, F. Marfil, H. Humbert and G. Maurer, J. Chromatogr. B: Biomed. Sci. Appl., 2001, 754, 45-55.
5. C. Emmanuelle, B. Stephane, M. Marguerite, M. Francois Xavier, M. Nicholas, T. Karine and M. Mathieu, Therap. Drug Monitor., 2010, 32, 476-481.

ADDRESS FOR CORRESPONDENCE

dralrao@gmail.com