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LC Determination of Deferasirox in Pharmaceutical Formulation

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ABSTRACT

An isocratic reverse phase liquid chromatography (RP-LC) method has been developed and subsequently validated for the determination of Deferasirox in pharmaceutical formulation. Separation was achieved with a Develosil ODS HG-5 (150 mmx4.6 mm I.D; particle size 5 µm) and Sodium dihydrogen phosphate monohydrate Buffer (pH adjusted to 3.0 with dilute orthophosphoric acid): Acetonitrile (55:45) as eluent at flow rate 2.0 mL/min. UV detection was performed at 245nm. The method is simple, rapid, and selective. The described method of Deferasirox is linear over a range of 11.999 µg/mL was 35.997 µg/mL. The method precision for the determination of assay was below 2.0%RSD. The percentage recoveries of active pharmaceutical ingredient (API) from dosage forms ranged from 100.5 to 101.0. The method is useful in the quality control of pharmaceutical formulations.

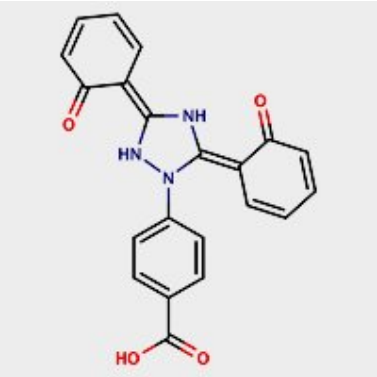
Key Words: LC Determination, Deferasirox

INTRODUCTION:

Deferasirox is an oral iron chelator. 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. It is the first oral medication approved in the USA for this purpose. Chemically 4-[(3Z,5E)-3,5-bis(6-oxo-1-

cyclohexa-2,4-dienylidene)-1,2,4-triazolidin-1-yl]benzoic acid.

Deferasirox is a white to off white crystalline powder with a Molecular weight 373.4. Deferasirox is freely soluble in Dimethyl formamide, Dimethyl sulfoxide, slightly soluble in methanol, practically insoluble in water. Its empirical formula is $C_{21}H_{15}N_3O_4$ and Chemical structure is given below[1-2]



Its melting point is 116 to 117°C. It is not official in any pharmacopoeia and till now, few liquid chromatographic procedures have been developed for the determination of Deferasirox. However there are no publications concerning the analysis of Deferasirox in pharmaceutical dosage forms[3-4]. So it is felt necessary to develolp a liquid chromatographic (LC) procedure which would serve as a rapid and reliable method for the determination of Deferasirox in pharmaceutical dosage forms. Finally the method was thoroughly validated for the assay determination of Deferasirox tablets (100,125, 250, 400,500mg).

EXPERIMENTAL[5-9]:

The waters LC system equipped with 2695pump and 2996 photodiode array detector was used. The output signal was monitored and integrated using waters Empower2 software.

Buffer preparation:

Accurately weigh and transfer about 2.72 grams of sodium dihydrogen phosphate monohydrate into 1000 mL of purified water. Adjust the pH of the solution to 3.0 with dilute orthophosphoric acid.

Mobile phase preparation:

Prepare a filtered and degassed mixture of Buffer and Acetonitrile in the ratio 550:450 v/v respectively.

Diluent preparation:

Mix Acetonitrile and Methanol in the ratio of 50:50 v/v respectively.

Standard preparation: (For Deferasirox Dispersible tablets 100mg, 125mg, 250mg, 400mg &500mg)

Accurately weigh and transfer about 24.0mg of Deferasirox working standard into a 100 mL volumetric flask, add 60 mL of diluent and sonicate to dissolve. Cool the solution to room temperature and dilute to volume with diluent.Transfer 5.0 mL of above solution into a 50 mL volumetric flask and dilute to volume with mobile phase.

Sample preparation: (For Deferasirox Dispersible tablets 100mg, 125mg, 250mg, 400mg &500mg)

Accurately weigh and finely powder not fewer than 20 tablets. Transfer an accurately weighed portion of the powered tablets, equivalent to about 500mg of Deferasirox into a 250 mL volumetric flask add about 180 mL of diluent, sonicate for 40minutes with occasional shakings. Cool the solution to room temperature and dilute to volume with diluent. Centrifuge the solution at 3000rpm for 5 minutes. Transfer 3.0 mL of the above supernatant solution into a 250 mL volumetric flask and dilute to volume with mobile phase.

Chromatographic conditions:

A Develosil ODS HG-5 (150 mmx4.6 mm I.D; particle size 5 µm) column was used for analysis at column temperature 40°C. The mobile phase was pumped through the column at a flow rate of 2.0mL/min. The sample injection volume was 10 µL. The photodiode array detector was set to a wavelength of 245nm for the detection and Chromatographic runtime was 10minutes.

RESULTS AND DISCUSSION:

Method development[6-11]:

To develop a suitable and robust LC method for the determination of Deferasirox in different mobile phases and columns were employed to achieve the best separation and resolution. The method development was started with Ammonium dihydrogen phosphate buffer (1.58gram in 1000ml of water) adjusted to pH 2.5 and Acetonitrile (55:45) is used as a mobile phase. In the above condition peak elution was very broad for Deferasirox. Instead of Develosil ODS HG-5 (250 mmx4.6 mm I.D; particle size 5 µm) is changed to Develosil ODS HG-5 (150 mmx4.6 mm I.D; particle size 5 µm) to reduce the runtime for Deferasirox. Deferasirox is a Low Solubility drug, initially Mobile phase was used as a diluent for extraction but extraction was not good and recovery was not found satisfactory so the diluent was changed to Methanol: Acetonitrile (50:50). Recovery was good and results are found satisfactory. The chromatogram of Deferasirox standard using the proposed method is shown in Fig-1. System suitability results of the method are presented in Table-1. Deferasirox show significant UV absorbance at Wavelength 245nm. Hence this wavelength has been chosen for detection in analysis of Deferasirox.

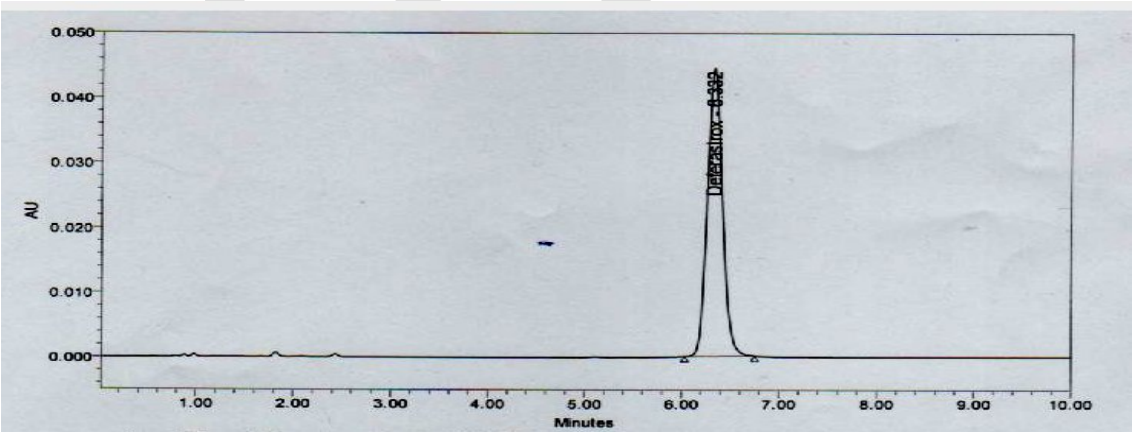


Fig-1. HPLC Chromatogram of Deferasirox Standard.

Table-1(SYSTEM SUITABILITY REPORT)

Compound	Retention Time *	Deferasirox area/response*	USP Tailing*	USP Plate count*	%RSD*
Deferasirox	6.328	485065	1.1	8100	0.23

*Number of standard injections analysed are six.

Column selection:

Based on the retention and better peak shape of the compound Develosil ODS HG-5 (150 mmx4.6 mm; 5 µm) column was selected as suitable column for analysis of Deferasirox.

Method validation[12-14]:

The developed LC method extensively validated for assay of Deferasirox using the following parameters.

Specificity- Blank and Placebo interference:

A study to establish the interference of placebo was conducted. Assay was performed on placebo in triplicate equivalent to about the weight of placebo in portion of test preparation as per test method. Chromatograms of Blank and Placebo solutions showed no peaks at the retention time of Deferasirox peak. This indicates that the excipients used in the formulation do not interfere in estimation of Deferasirox in Deferasirox Dispersible tablets.

The chromatogram of Deferasirox Blank and Placebo using the proposed method is shown in Fig- 2 & Fig-3.

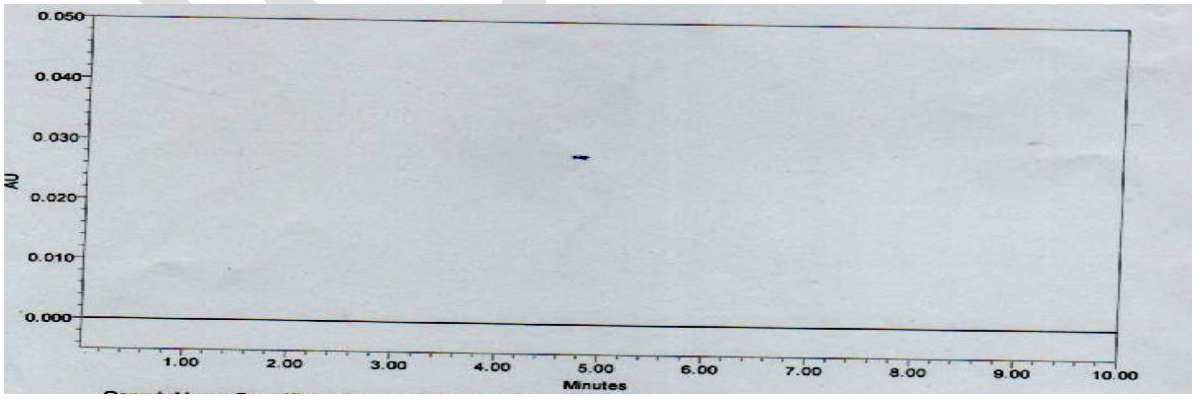


Fig-2. HPLC Chromatogram of Deferasirox Blank.

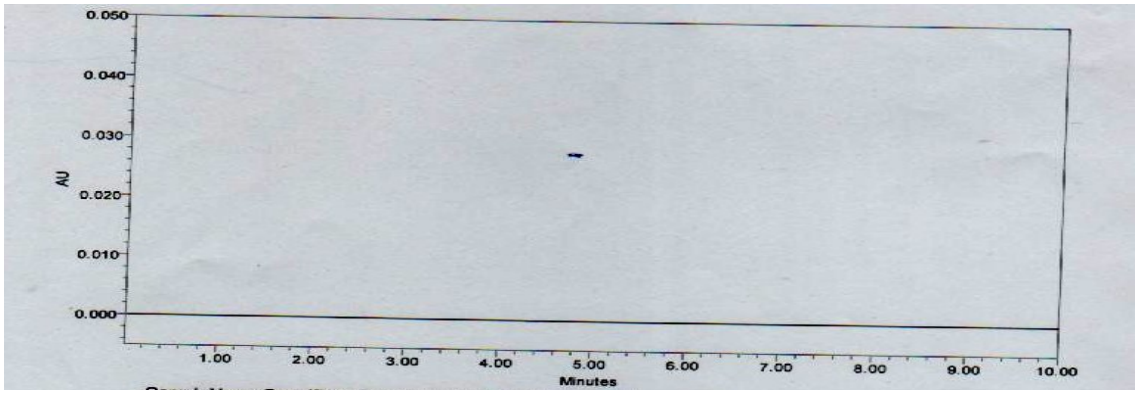


Fig-3. HPLC Chromatogram of Deferasirox placebo.

Linearity of detector response:

Linearity of detector response was established by plotting a graph to concentration *versus* average area and determining the correlation coefficient. A series of solutions of Deferasirox standard were prepared in the concentration range of about 11.999 µg/mL -35.997 µg/ mL. A graph was plotted to concentration in µg/mL on X-axis *versus* response/Area on Y-axis. The detector response was found to be linear with a correlation coefficient of 0.999. Linearity graph is shown in Fig-4. Linearity results of the method are presented in Table-2.

Table-2 (LINEARITY TABLE REPORT)

% Level	µg/mL (**ppm) (Concentration)**	**Area**	**y-Best fit**	**(Difference)^2**	Correlation Coefficient (R)=	0.9999
50	11.999	244591	244191	160067	Regression Coefficient (R^2)=	**0.9998**
80	19.198	393163	391000	4680362	y-Intercept=	-503.8
90	21.598	439235	439943	500707	Slope of Regression line=	20393
100	23.998	484985	488886	15214895	Residual Sum of squares=	21984778
110	26.398	538373	537829	296319	Minimum Con in mcg/mL =	11.999
120	28.797	587560	586751	654034	Maximum Con in mcg/mL =	35.997
150	35.997	734272	733580	478394	y-Intercept at 100 % level =	**-0.1**

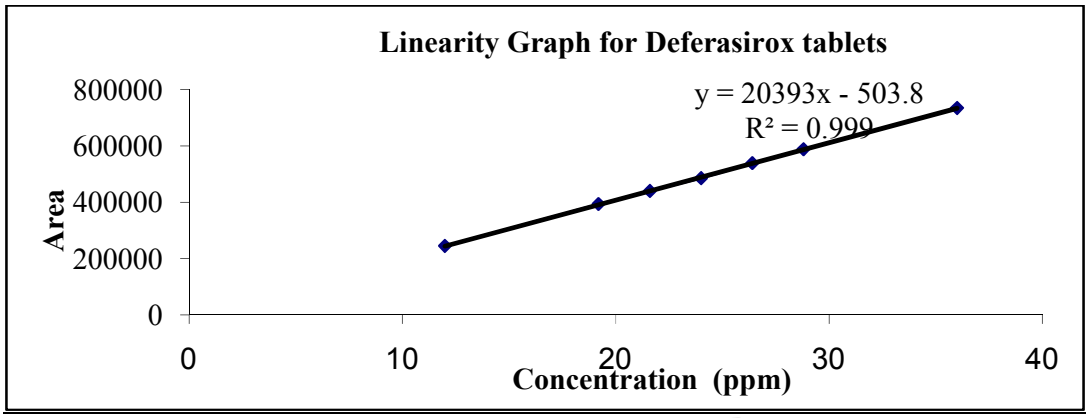


Fig-4. Linearity of detector response graph.

Precision of test Method:

The precision of test method was conducted by assay in six samples of Deferasirox dispersible tablets. The average % assay of Deferasirox in Deferasirox tablets was found to be 100.9, 102.2, 102.4, 101.1, 103.3 for 100, 125,250, 400,500 mg tablets respectively and the %RSD is 0.2, 0.4, 0.4, 0.3 and 0.2%. The results were given in Table-3.

A typical LC Chromatogram is shown in Fig-5.

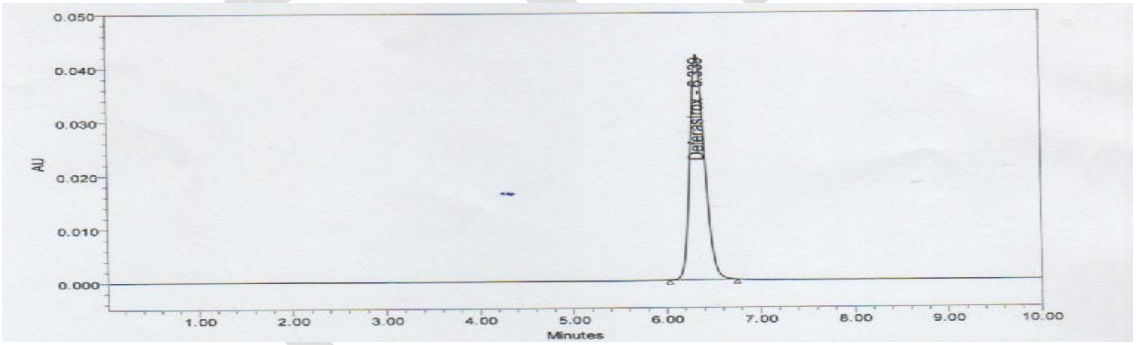


Fig-5. Typical LC chromatogram of formulated Deferasirox 500mg.

Accuracy:

A Study of recovery of Deferasirox from spiked placebo was conducted at five different spike levels i.e.50, 80, 100, 120 and 150%. Samples were prepared by mixing placebo with Deferasirox raw material equivalent to about the target initial concentration of Deferasirox. Sample solutions were prepared in triplicate for each spike level and assayed as per proposed method. The % recovery was given in Table-4. The mean recoveries of Deferasirox from spiked were found to be in the range of 100.5-101.0%.

Table-3(RESULTS FOR PRECISION OF TEST METHOD)

	%Assay				
Sample No	100mg	125mg	250mg	400mg	500mg
01	100.8	102.2	102.7	101.1	103.3
02	100.9	102.3	102.7	101.5	103.2
03	100.9	102.1	102.2	100.6	103.5
04	101.1	102.3	101.6	101.2	102.8
05	100.6	101.4	102.3	101.1	103.3
06	101.1	102.8	102.7	101.1	103.4
Average	100.9	102.2	102.4	101.1	103.3
SD	0.1892	0.4535	0.4367	0.2898	0.2429
% RSD	0.2	0.4	0.4	0.3	0.2

Table-4(ACCURACY IN THE ASSAY DETERMINATION OF DEFERASIROX)

Sample No.	Spike level	'mg/mL' added	'mg/mL' found (recovered)	% of Recovery	Mean % recovery
1.	50%	0.0120	0.0120	100.00	100.6
2.	50%	0.0120	0.0122	101.67	
3.	50%	0.0120	0.0120	100.00	
4.	80%	0.0192	0.0193	100.52	100.5
5.	80%	0.0192	0.0193	100.52	
6.	80%	0.0192	0.0193	100.52	
7.	100%	0.0241	0.0243	100.83	100.8
8.	100%	0.0240	0.0242	100.83	
9.	100%	0.0241	0.0243	100.83	
10.	120%	0.0289	0.0293	101.38	100.9
11.	120%	0.0290	0.0291	100.34	
12.	120%	0.0289	0.0292	101.04	
13.	150%	0.0359	0.0363	101.11	101.0
14.	150%	0.0360	0.0363	100.83	
15.	150%	0.0360	0.0364	101.11	

Ruggedness:

A study to establish the stability of Deferasirox in standard and test solutions were conducted on bench top and refrigerator at Initial, 1 day and 2 day. The assay of Deferasirox in standard and test solutions were estimated against freshly prepared standard each time. The difference in% assay of standard and test solutions from initial to 1 day and 2 days was calculated and given in Table-5. From the above study, it was established that the Standard and sample preparations are stable for a period of 48hours at room temperature (25°C±2°C) and at refrigerator condition (2°C-8°C).

TABLE- 5:

Bench top Stability of Deferasirox Test preparation and Standard Preparation:

Time	**% Assay of Standard preparation**	**Difference**	**% Assay of test preparation**		**Difference**	
			Test-1	Test-2	Test-1	Test-2
Initial	99.7®	NA*	103.1	103.2	NA	NA
After 24 hours	100.5	0.8	103.2	103.4	0.1	0.2
After 48 hours	100.7	1.0	103.8	103.8	0.7	0.6

NA*----Not Applicable

®--------Potency of Deferasirox on as is basis.

Refrigerator Stability of Deferasirox Test preparation and Standard Preparation:

Time	**% Assay of Standard preparation**	**Difference**	**% Assay of test preparation**		**Difference**	
			Test-1	Test-2	Test-1	Test-2
Initial	99.7®	NA*	103.1	103.2	NA	NA
After 24 hours	100.1	0.4	103.2	103.5	0.1	0.3
After 48 hours	100.3	0.6	103.8	103.7	0.7	0.5

NA*----Not Applicable

®--------Potency of Deferasirox on as is basis.

Robustness:

A study to establish the effect of variation in mobile phase composition, flow, temperature and pH of Buffer in mobile phase was conducted. Standard and test solutions prepared as per proposed method were injected into HPLC system. The system suitability parameters and % assay were evaluated. From the above study the proposed method was found to be robust.

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