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Development And Validation Of Newer Analytical Methods For The Estimation Of Deferasirox In Bulk And In Tablet Dosage Form By Uv Spectroscopy And RP – HPLC

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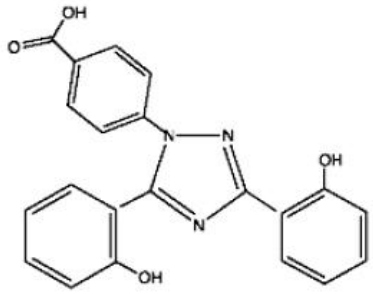
Abstract: Two simple, sensitive, specific UV- spectroscopic and RP-HPLC methods are developed for the estimation of Deferasirox in bulk and pharmaceutical formulation. The first method was based on UV – spectroscopic determination of the drug. It involves absorbance measurements at 319nm (λ max of Deferasirox) in 0.1M sodium hydroxide. Calibration curve was linear with the correlation coefficient was 0.9997 over a concentration range of 5 to 30µg/ml for the drug. The second method was based on HPLC separation of the drug in reverse phase mode using C_{18} column (150 mm × 4.6 mm i.d. 5µ). The mobile phase constituted of Acetonitrile: Water pH 3.5 adjusted with orthophosphoric acid (70:30 v/v) and flow rate 1.0ml/min. Detection was performed at 248nm. Separation completed with in 5minutes. Calibration curve was linear with the correlation coefficient was 0.9996 over a concentration range of 1 to 6µg/ml for the drug. The relative standard deviation (R.S.D) was found <2.0% for UV – spectroscopic and RP-HPLC methods. Both these methods have been successively applied to bulk and pharmaceutical formulation. The present methods were validated according to ICH guidelines.

Key words: Deferasirox, UV-spectroscopy, High performance liquid chromatography.

Introduction

Deferasirox belongs to the class Antidote. Chemical name is 4-[3,5-bis (2-hydroxyphenyl)-1H-1,2,4-triazol-1-yl]-benzoic acid. 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. Deferasirox is an orally active chelator that is selective for iron (as Fe^{3+}). It is a tridentate ligand that binds iron with high affinity in a 2:1 ratio [1]. It is not official in any of the pharmacopoeia. It is listed in the Merckindex 14th edition [2] and Martindale the complete drug reference 35th edition [3].



Literature survey revealed estimation of Deferasirox by several techniques such as a method to measure deferasirox in plasma using HPLC coupled with Ms/Ms detection and its potential application [4], Terbium - sensitized fluorescence method for the determination of deferasirox in biological fluids and tablet formulation [5], LC determination of deferasirox in pharmaceutical formulation [6], A stability indicating LC method for deferasirox in bulk drug and pharmaceutical dosage forms [7], Relative bioavailability of deferasirox tablets administered without dispersion and dispersed in various drinks [8], Pharmacokinetics, distribution, metabolism, and excretion of deferasirox and its iron complex in rats [9], were reported.

In this present work study an attempt was made to developed rapid and economical spectroscopy and RP-HPLC method for estimation of Deferasirox in bulk and pharmaceutical formulation with better sensitivity, precision and accuracy using C_{18} column and UV detector.

Materials And Methods

Deferasirox was procured from Natco pharma, Hyderabad, India. All chemicals and regents used were of HPLC grade and AR grade. Tablets were purchased from Indian market, containing Deferasirox 400mg/per tablet (Asunra 400mg, Novartis pharmaceutical corporation, Switzerland).

Shimadzu - 1700 Double Beam UV - Visible spectrophotometer with pair of 10mm matched quartz cells, Shimadzu HPLC system(LC – 10 ATvp solvent deliver module, SPD – 10 Avp UV - Visible detector,) using Phenomenax Luna C_{18} column (150 mm × 4.6 mm i.d. 5µ), apparatus was used for the analysis. The mobile phase constituted of Acetonitrile : Water pH adjusted to 3.5 with orthophosphoric acid (70:30 v/v) and flow rate was 1.0ml/min. Detection was performed at 248nm.

UV – spectroscopic method

Standard solution

Standard solution of Deferasirox was prepared by dissolving 25mg of the drug in 25ml of 0.1M sodium hydroxide. This solution contains 1mg/ ml concentration.

Selection of wavelength

The standard stock solution was further diluted with 0.1M sodium hydroxide to get the concentration of 10 µg/ ml and the solution was scanned between 200 and 400 nm using 0.1M sodium hydroxide as blank. From the spectra, λmax was found to be 319 nm and was selected as an analytical wavelength. The spectrum are **shown in fig** 1.

Preparation of calibration graph

Aliquots of standard stock solution (0.5 – 3ml) were taken and diluted with 0.1M sodium hydroxide to obtain series in the concentration range of 5-30µg/ml. The absorbances were measured at 319nm and calibration curve was plotted using absorbance Vs concentration. The value of slope and correlation coefficient were found to be 0.03033 and 0.9997respectively. The optical characteristics of the method is listed in table 1.

Quantification of raw material

1.5 ml of standard stock solution was taken in to series of six 100 ml standard flasks and the volume was made up to mark with 0.1M sodium hydroxide. The absorbance of these solutions was measured at 319 nm. The amount Deferasirox present in the raw material was determined by using slope and intercept values from calibration graph. The results are shown in table 2.

Assay of tablet formulation

Six tablets of Deferasirox were accurately weighed and average weight of tablet formulation was determined. The tablets were crushed, the tablet powder equivalent to 25 mg of Deferasirox was weighed and dissolved in to 25ml of 0.1M sodium hydroxide. The content of flask was sonicated for 15mintues centrifuged for another 15 minutes. The supernatant liquid was filtered through Whatmann filter paper No. 41. Further dilutions were made by diluting 1.5 ml into 100 ml with 0.1M sodium hydroxide to obtain15 µg/ml solution theoretically. Absorbance was measured at 319nm using 0.1M sodium hydroxide as a blank. The amount of Deferasirox per tablet was calculated using the calibration curve. The results are shown in table 3.

RP-HPLC method

Optimized Chromatographic Conditions

Mode of operation	-	Isocratic
Stationary phase	-	C_{18} column (150 mm × 4.6 mm i.d. 5µ)
Mobile phase	-	Acetonitrile: Water pH adjusted to 3.5 with orthophosphoric acid
Proportion of mobile phase	-	70: 30 % v/v
Detection wavelength	-	248 nm
Flow rate	-	1 ml/ min
Temperature	-	Ambient
Sample load	-	20 µl
Operating pressure	-	80 kgf
Method	-	External Standard Calibration method

The solution of Deferasirox was injected and the respective chromatogram was recorded.The chromatogram are **shown in fig 2.**

Table 1: Optical characteristics of Deferasirox by UV – spectroscopy and RP-HPLC method

METHOD	PARAMETERS	VALUES*
UV-spectrocopy	λmax (nm)	319
	Beer's law limit (µg/ ml)	5 – 30
	Sandell's sensitivity (µg/cm^2/0.001 A.U)	0.03301
	Molar absorptivity (L mol^{-1} cm^{-1})	1.1410×10^4
	Correlation coefficient (r)	0.99970
	Regression equation(y=mx+c)	Y = 0.03033x + 0.00865
	Slope(m)	0.03033
	Intercept(c)	0.00865
	Precision Inter day, intraday (RSD)	<2
	Accuracy	
	LOD (µg/ ml)	0.40116
	LOQ (µg/ ml)	1.21565
	Standard error	0.00852
RP-HPLC	λmax (nm)	248
	Beer's law limit (µg/ ml)	1 – 6
	Correlation coefficient (r)	0.99967
	Regression equation (y = mx + c)	Y = 970997.285x + 18000.285
	Slope (m)	970997.285
	Intercept (c)	18000.285
	LOD (µg/ ml)	0.10737
	LOQ (µg/ ml)	0.32537
	Standard error	58905.592

*Mean of six observations

Table 2: Quantification of raw material by UV method

Drug	Sample No.	Amount Found (mg/tab)*	Percentage Obtained*	Average (%)	S.D	%R.S.D	S.E
	1	15.3845	102.56				
	2	15.0779	100.51				
DEF	3	14.9163	99.44	99.19	1.3230	1.3338	0.0367
	4	14.6106	97.40				
	5	14.6761	97.84				
	6	14.9113	99.40				

*Mean of six observations

Table 3: Quantification of formulation - Asunra by UV method

Drug	Sample No	Labeled amount (mg/tab)	Amount found (mg/tab)*	Percentage Obtained*	Average (%)	S.D	% R.S.D	S.E
	1	400	401.7209	100.43				
	2	400	392.9806	98.25				
DEF	3	400	396.4314	99.11	99.07	0.7815	0.7888	0.0217
	4	400	396.6860	99.17				
	5	400	396.4224	99.11				
	6	400	393.4053	98.35				

*Mean of six observations

Fig1: UV spectrum for Deferasirox

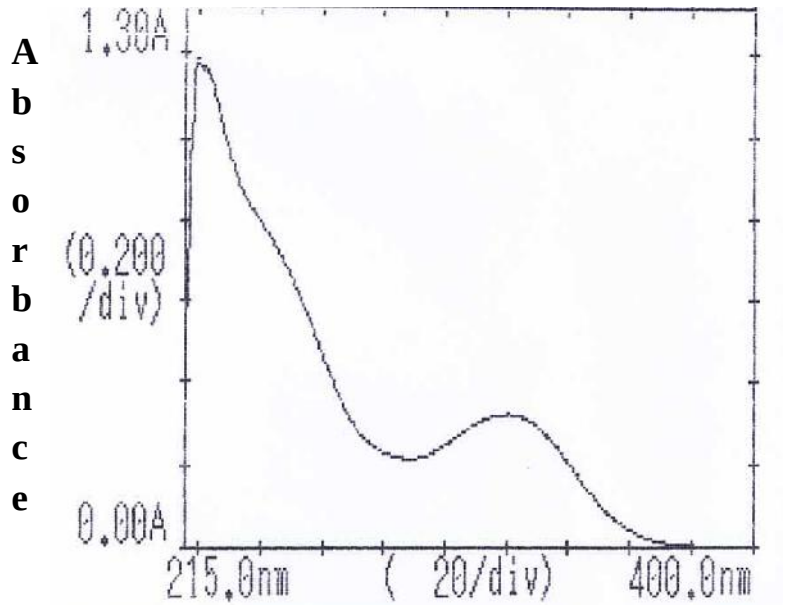
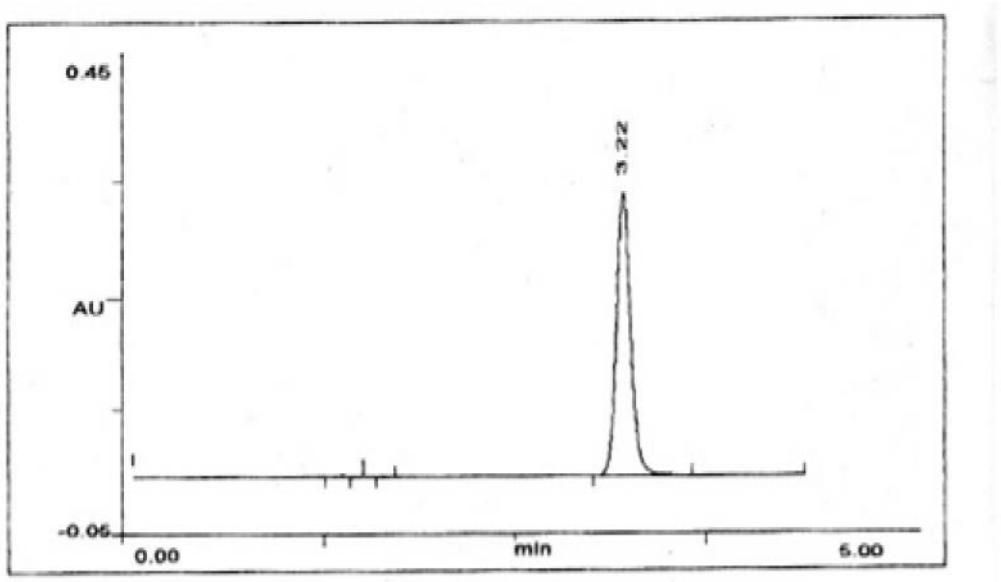


Fig 2: Chromatogram of Deferasirox



Standard solution

Standard stock solution of Deferasirox was prepared by dissolving 25mg of the drug in 50ml of methanol (HPLC grade). Further dilution was made by pipetting 1 ml of stock solution into 50 ml to get the concentration of 10 µg/ml with mobile phase (working standard solution). Aliquots of working standard solution (1 - 6ml) were taken and diluted with mobile phase to obtain series of solution in the concentration range of 1-6 µg/ml. All the solutions were injected and the chromatograms were recorded at 248 nm and calibration curve was plotted using peak area Vs concentration. The values of slope and correlation coefficient were found to be 970997.285 and 0.9996 respectively.

Assay of tablet formulation

Six tablets of Deferasirox were accurately weighed and average weight of tablet formulation was determined. The tablets were crushed, the tablet powder equivalent to 25 mg of Deferasirox was transferred to a 50ml volumetric flask. Dissolve the active ingredients and volume was made up to 50ml with methanol, the contents were sonicated for 15 minutes, centrifuged at 2000 rpm for 15 minutes and filtered through a 0.2µ membrane filter. From the clear solution, further dilutions were made by diluting 1ml into 50 ml with mobile phase to obtain 10 µg/ml concentration. This solution was used for further analysis. 3 ml of test solution was transferred into six 10 ml volumetric flasks and made up to the mark with mobile phase. A 20µl volume of each sample solution was injected into the sample injector of HPLC six times under the chromatographic conditions as described. The peak area was measured at 248nm. The amount of drug present in the sample solutions were determined using calibration curve of standard Deferasirox. The results are shown in table 4.

Table 4: Quantification of formulation - Asunra by RP – HPLC method

Drug	Sample No	Labeled amount (mg/tab)	Amount found (mg/tab)*	Percentage Obtained*	Average (%)	S.D	% R.S.D	S.E
DEF	1	400	394.5706	98.64	99.74	0.7661	0.7681	0.0213
	2	400	400.1234	100.03				
	3	400	401.7590	100.44				
	4	400	400.8110	100.20				
	5	400	400.9414	100.24				
	6	400	395.6412	98.91				

* Mean of six observations

Method Validation

Linearity

The plot of absorbance against concentration is shown in fig 3 and 4 for UV and HPLC methods, respectively. It can be seen that plot is linear over the concentration range of 5 to 30µg/ml for UV – Spectroscopy and for HPLC 1 - 6µg/ml Deferasirox with a correlation coefficient (r^2) 0.9997 and 0.9996, respectively.

Precision

Intra day and inter day precision was determined by repeating assay three times on the same day for intra day and on different days for inter day precision. The relative standard deviation for six replicates of sample solution was less than 2.0%, which met the acceptance criteria established for spectroscopic method. The obtained results were presented in table 5.

Accuracy

To check the accuracy of the proposed method, recovery studies were carried out at 80,100,120% of the test concentration as per ICH guidelines and low relative standard deviation value show the accuracy of the Spectroscopy and HPLC methods. The data were presented in table 6.

LOD and LOQ (sensitivity)

The LOD and LOQ were separately determined based on the standard deviation of response of the calibration curve. The relative standard deviation of the regression lines and slope of the calibration curve were used to calculate LOD and LOQ.

Standard and sample solution stability

Standard and sample solution stability was evaluated at room temperature for 48 hours. The relative standard deviation was found below 2.0%. It shows that standard and sample solution were stable up to 48 hours at room temperature.

Fig 3: Calibration curve of Deferasirox in 0.1m sodium hydroxide at 319 nm

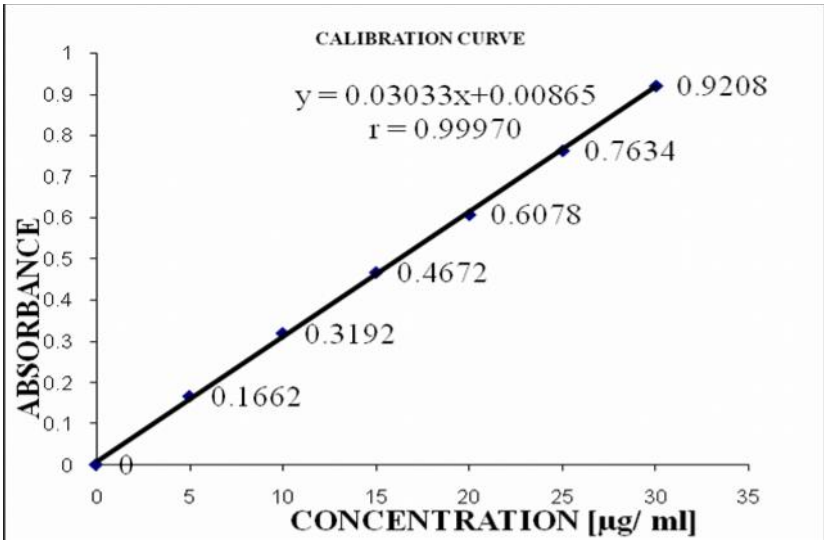


Fig 4: Calibration curve of Deferasirox by RP – HPLC method

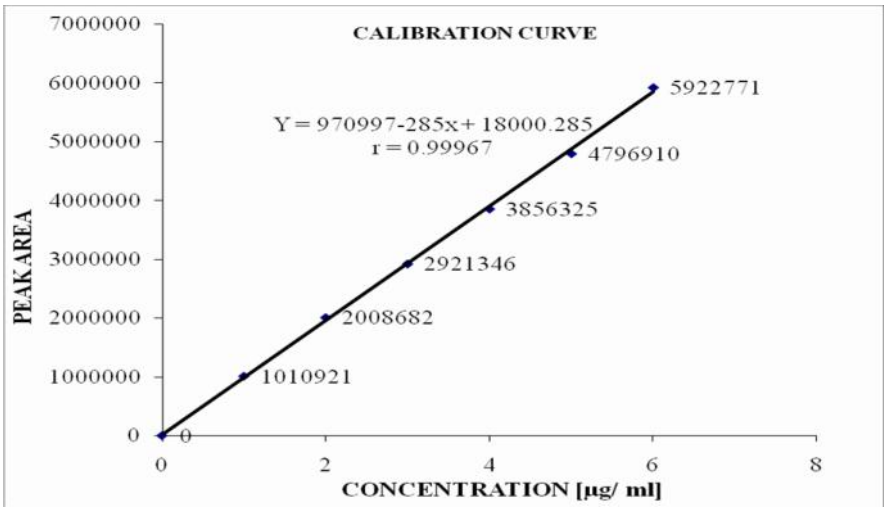


Table 5: Inter day and Intra day analysis of formulation – Asunra by UV method

Drug	condition	Sample No	Labeled amount (mg/tab)	Amount found (mg/tab)*	Percentage Obtained*	Average (%)	S.D	% R.S.D	S.E
DEF	Intra day	1	400	397.9455	99.49				
		2	400	406.0884	101.52	100.29	1.0811	1.0780	0.1201
		3	400	399.4248	99.86				
DEF	Inter day	1	400	395.4918	98.87				
		2	400	393.8593	98.45	98.92	0.4968	0.5023	0.0552
		3	400	397.7405	99.44				

* Mean of six observations

Table 6: Recovery analysis of formulation – Asunra by UV – spectroscopy and RP-HPLC method

Method	Level Deferasirox	Drug added mg	Drug recoverd mg	% Recovered ± S.D	%R.S.D
UV-spectroscopy	80	11.5667	11.3277	97.94 ± 1.4424	1.4728
	100	14.7889	14.9370	101.00 ± 0.6000	0.5941
	120	17.2350	17.4626	101.32 ± 0.9877	0.9749
RP-HPLC	80	2.3698	2.3677	99.91 ± 0.9920	0.9929
	100	2.9575	2.9488	99.71 ± 0.5024	0.5039
	120	3.4889	3.5091	101.58 ± 0.4618	0.4592

*Mean of three observations

Results And Discussion

In this study a simple, fast and reliable UV spectroscopy and HPLC methods were developed and validated for the determination of Deferasirox in bulk and pharmaceutical formulation. As these proposed methods have the lowest LOD values and wider linearity range is more sensitive method. From the results obtained, we conclude that the suggested methods showed high sensitivity, accuracy, reproducibility and specificity. Moreover, these methods were simple and in expensive and this can be employed for the routine quality control of Deferasirox in bulk and pharmaceutical formulation.

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