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Research Article

DEVELOPMENT AND VALIDATION OF NEWER ANALYTICAL METHODS FOR THE ESTIMATION OF DEFERASIROX IN BULK AND IN TABLET DOSAGE FORM BY CALORIMETRIC METHOD

VENKATA S RAO SOMISETTY[1*], PREM KUMAR BICHALA[2], CH M M PRASADA RAO[1], V.KIRANKUMAR[3]

[1]Department of Pharmaceutical Analysis, QIS College of Pharmacy, Ongole 523272, [2]Department of Pharmaceutical Analysis, Chaitanya College of Pharmacy, Markapur 523316, [3]Department of Pharmaceutical Analysis, Unity College of Pharmacy, Raigir bhongir, Nalgonda 508116, A.P, India. Email: subbuanalysis@gmail.com.

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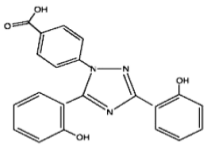
ABSTRACT

A simple, sensitive, accurate and rapid caloremetric method were developed for the estimation of Deferasirox in pure and tablet dosage forms. Deferasirox react with Gibb's reagent to form a stable greenish blue coloured complex. Deferasirox contain para free phenolic group that reacts with this reagent and formed a coloured complex which can be estimated by colourimetrically. The positive colour is obtained in alkaline pH. The pH maintained by using alkaline borate buffer (pH 9.4). The colour of the solutions were measured the absorbance at 658 nm. Sandell's sensitivity, molar extinction coefficient, slope, intercept, LOD and LOQ were determined. The percentage recovery was found to be 100.443 ± 0.6595. The proposed method was accurate, precise, reproducible and economical for the routine analysis of Deferasirox in bulk drug and in formulation.

Keywords: Deferasirox, Gibb's reagent, Alkaline borate buffer (pH-9.4), UV- visible spectrophotometry.

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 calorimetric method for estimation of Deferasirox in bulk and pharmaceutical formulation with better sensitivity, precision and accuracy using UV spectroscopy.

MATERIALS AND METHODS

A colorimetric method was developed for deferasirox by using Gibb`s reagent as agent in presence of alkaline borate buffer (pH 9.4).

Experimental

Instrumentation

A Shimadzu – 1700 Double Beam UV- Visible Spectrophotometer with 1 cm matched Quartz cells were used for all absorbance measurements. deferasirox pure drug samples and tablet formulation was generously gifted by Natco pharma, Hyderabad, India. The tablet formulation Asunra contains 100 mg of deferasirox. All the chemicals and reagents used were of analytical grade and procured from Qualigens India Ltd., Loba Chemicals Ltd.

Preparation of Gibb`s reagents

0.1% Gibb`s reagent was prepared by weighing 100 mg of Gibb`s reagent into 100 ml volumetric flask and added minimum quantity of methanol to dissolve the substance and made up to mark with methanol.

Preparation of standard stock solution

10 mg of Deferasirox raw material was weighed accurately and transferred in to 50 ml volumetric flask, dissolved in methanol and made up to the volume with more methanol. The solution contains 200 µg/ ml concentration.

Selection of wavelength for estimation and stability studies

5 ml of standard stock solution of Deferasirox was pipetted out in to 100 ml standard flask. To this added 20 ml of alkaline borate buffer, 5 ml of (0.1%) Gibb's reagent were added and kept for 15 minutes. The volume was made up to the mark with distilled water to get concentration of 10 µg/ ml. The greenish blue coloured chromogen was scanned in visible region (400 – 800 nm) against reagent blank. From the spectra the wavelength (λmax) selected was 658 nm.

The stability was performed by measuring the absorbance of same solution at different time intervals. It was observed that Deferasirox was stable for up to one hour at the selected wavelength.

Optimization of reagents

The absorbance of Deferasirox in different volumes of alkaline borate buffer, different strengths and volumes of Gibb`s reagent were optimized to get steady absorbance.

A solution of 10 µg/ ml was prepared from the stock solution and added 20 ml of alkaline borate buffer (pH 9.4) and 5 ml of 0.1% Gibb's reagent and the solution was made up to the volume with distilled water. The intensity of the coloured solution was scanned in the visible region of 400 - 800 nm. The spectrum was recorded. The recorded spectra showed that at 658 nm Deferasirox has the maximum absorbance. Hence this was selected as an analytical wavelength. This is shown in figure 1. The optimization of the reagents were done by measuring the absorbance of drug solution by adding different volumes of alkaline borate buffer, different concentrations of Gibb's reagent and different volumes of Gibb's reagent. The absorbance was plotted against different volumes of alkaline borate buffer, different concentrations of reagent and different volumes of reagent. These are shown in figures 2, 3 and 4, respectively.

Calibration graph

The standard stock solutions of Deferasirox (0.5 – 3 ml) were transferred into a series of 100 ml volumetric flasks. To each flask 20 ml of alkaline borate buffer and 5 ml of 0.1% of Gibb`s reagent were added and made up to the volume with distilled water. The absorbance of different concentration solutions were measured at 658 nm. The calibration curve was plotted. Deferasirox was linear with the concentration range of 1 – 6 µg/ ml at 658 nm. Calibration graph was shown in the figure 5.

Analysis of formulation

Six tablets of formulation (Asunra containing Deferasirox equivalent to 400 mg) were weighed accurately and the average weight of each tablet was found. The tablets were ground to a fine powder. The tablet powder equivalent to 10 mg of Deferasirox was weighed and transferred into 50 ml volumetric flask, added a minimum quantity of methanol to dissolve the substance and made up to the volume with the same (200 µg/ ml). The solution was sonicated for 15 minutes, centrifuged for another 15 minutes at 2000 rpm and filtered through Whatmann filter paper No. 41. From the clear solution pipette out 1.5 ml into a series of six 100 ml volumetric flasks, added 20 ml of alkaline borate buffer, 5 ml of 0.1% Gibb`s reagent and volume was made up to the mark with distilled water. The absorbance of solutions was measured at 658 nm. This procedure was repeated for six times the results were shown in the table no 3.

Recovery studies

The recovery experiment was done by adding known concentrations of raw material stock solution of Deferasirox to the preanalyzed formulation. added 2 ml, 2.5 ml, and 3 ml of standard stock solution in to a series of 50 ml standard flasks, dissolved in methanol and made up to the mark with same. The solution was sonicated for 15 minutes. After sonication, centrifuged for 15 minutes at 2000 rpm and the solution was filtered through a Whatmann filter paper No.41. 1.5 ml of the clear solution was transferred in to 100 ml volumetric flask, added 20 ml of alkaline borate buffer, 5 ml of 0.1% Gibb`s reagent and volume was made up to the mark with distilled water. The absorbance of solutions was measured at 658 nm. This procedure was repeated for three times.

Repeatability

Repeatability is given by interday and intraday precision. The assay and recovery procedures were repeated for three times, on the same day and once for three successive days for both the methods.

Ruggedness

The degree of reproducibility of the test results obtained in this method was detected by analyzing the drug sample under the variety of test conditions like different analyst and different instruments is ruggedness. The procedure was repeated under the above conditions.

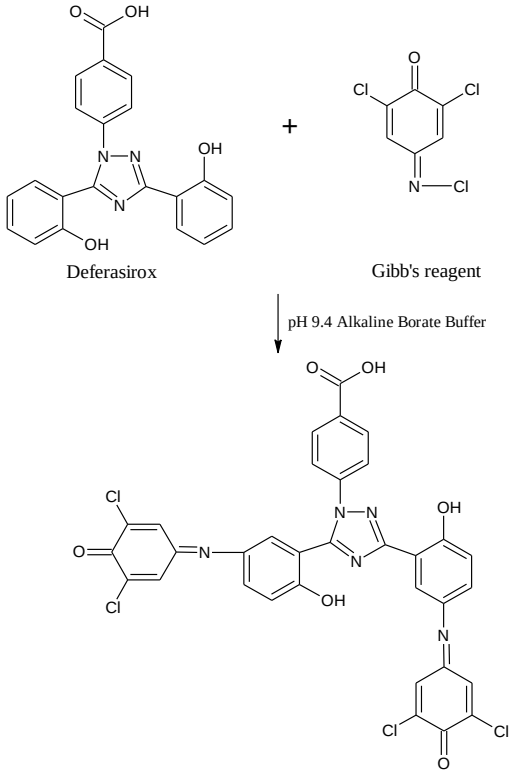
RESULTS AND DISCUSSION

Simple, precise and accurate visible spectrophotometric method was reported for the estimation of DEF in bulk and in tablet formulation. In this Method Deferasirox react with Gibb's reagent gives greenish blue coloured complex at alkaline pH 9.4 and it was measured at 658 nm. The optical characteristics such as Beer's law limit, molar extinction coefficient, Sandell's sensitivity, correlation coefficient, slope and intercept values were calculated and shown in Table-1. The spectrum was shown in figure 1. Deferasirox was found to obeyed Beer's law in the concentration range of 1-6 µg/ml. The correlation coefficient for was found to be 0.9995. The formulation Deferasirox was selected for analysis and the percentage purity was found to be 100.41± 0.7044. The procedure was repeated for six times to validate the methods. The developed methods were validated according to ICH[10,11] Guidelines.

The percentage RSD was found to be less than 2%, which indicates that method had good precision. The results of the analysis are shown in Table 2.Further the precision of the developed methods is confirmed by interday and intraday analysis. The results show good agreement with the label claim of the formulation which is shown in Table 3.The accuracy of the method was confirmed by the recovery studies. To the pre-analysed formulation, a different concentration of the raw material was added and the amount of the drug recovered was calculated. The percentage recovery was found to be 100.4433± 0.6595. The procedure was repeated for three times. The % RSD value is 0.6566. The % RSD value indicates that there is no interference due to the excipients used in the formulation. Thus the developed methods are found to be accurate, which is shown in Table 5. Both the methods were validated for ruggedness. The result confirmed the ruggedness of the developed methods. This is shown in table 4.

CONCLUSION

The UV-Visible Spectrophotometric methods developed for DEF shows good precision and accuracy. The low percentage RSD values in the recovery studies, indicates that there is no interference due excipients used in the formulation. Hence it is concluded that the developed methods are simple, precise, accurate and rapid for the analysis of Deferasirox in pure and in tablet dosage form. Thus the developed methods can be adopted for the routine analysis of Deferasirox in bulk and in tablet dosage form.



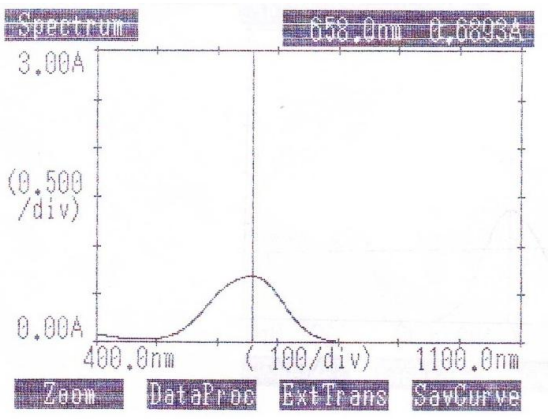


Fig. 1: Visible spectrum of Deferasirox by colorimetric method

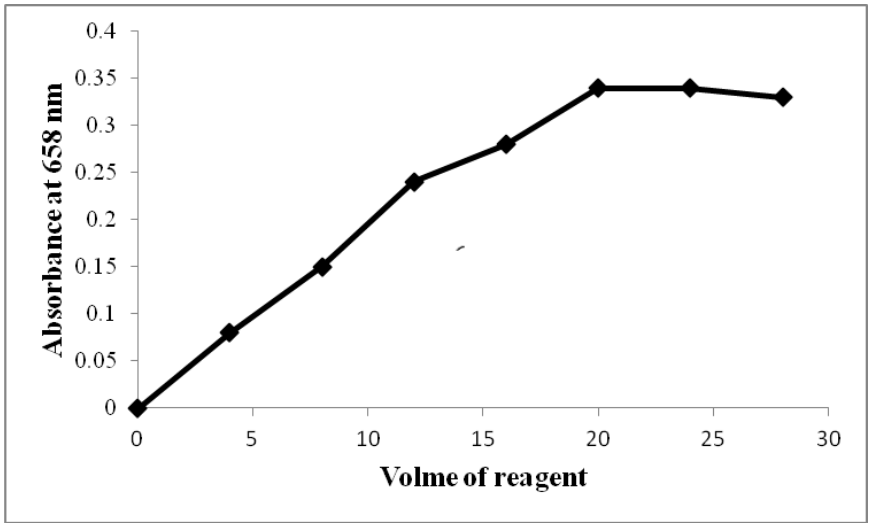


Fig. 2: Optimization of alkaline borate buffer with different volumes.

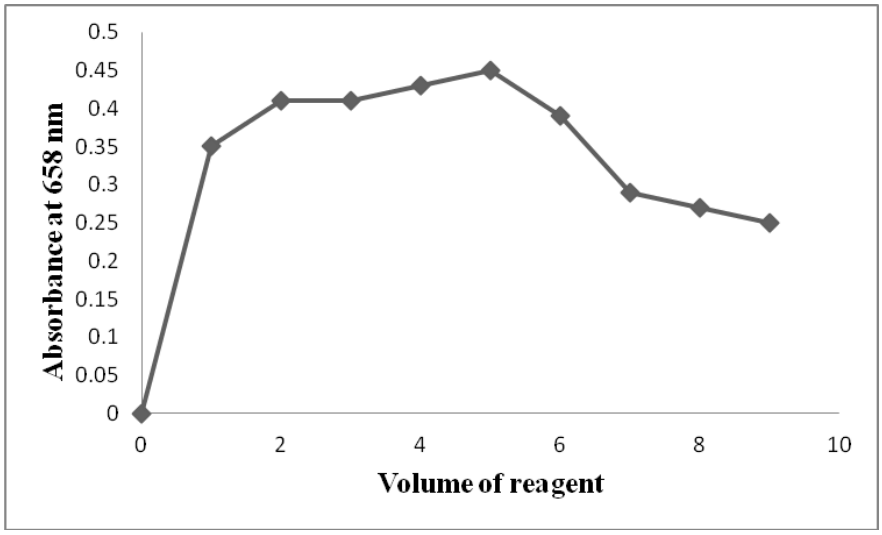


Fig. 3: Optimization of 0.1% Gibb's Reagent with different volumes.

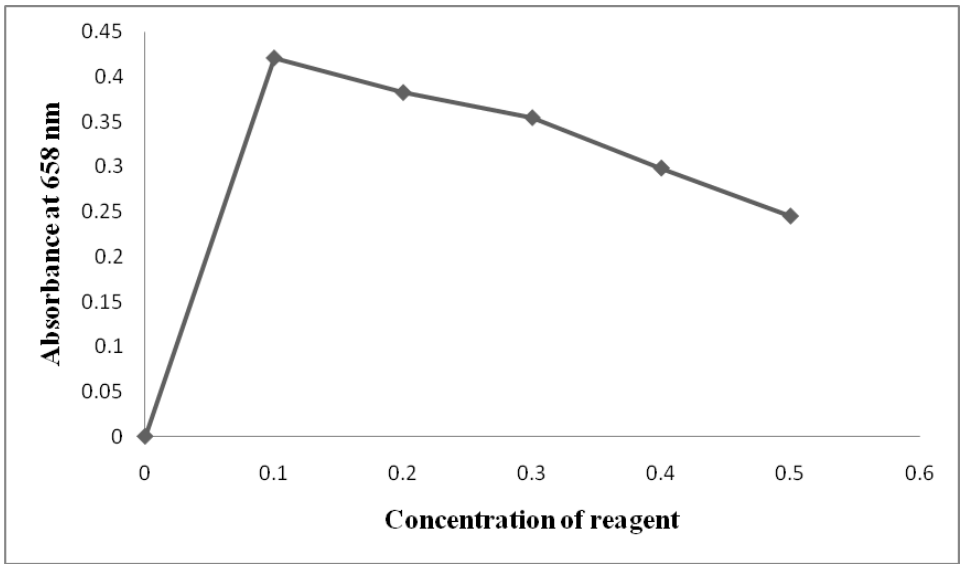


Fig. 4: Optimization of Gibb's Reagent with different concentrations

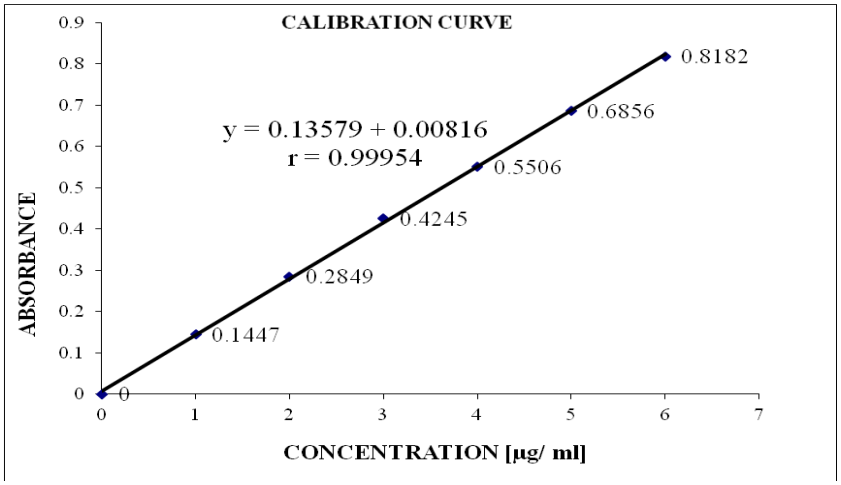


Fig. 5: Calibration curve of Deferasirox by colorimetric method

Table 1: Optical characteristics of deferasirox by colorimetric method

Parameters	Valves*
λmax (nm)	658
Beer's law limit (µg/ ml)	1 - 6
Sandell's sensitivity (µg/cm²/0.001 A.U)	0.00737
Correlation coefficient (r)	0.99954
Regression equation (y = mx + c)	Y = 0.13579x + 0.00816
Slope (m)	0.13579
Intercept (c)	0.00816
LOD (µg/ ml)	0.10840
LOQ (µg/ ml)	1.21564
Standard error	0.32849

*Mean of six observations

Table 2: Quantification of formulation - Asunra by Colorimetric method

Drug	Sample No	Labeled amount (mg/tab)	Amount found (mg/tab)*	Percentage Obtained*	Average (%)	S.D.	% R.S.D.	S.E
	1	400	402.4878	100.62	100.41	0.7044	0.7015	0.0196
DEF	2	400	402.3744	100.59				
	3	400	399.9746	99.99				
	4	400	406.4196	101.61				
	5	400	398.3380	99.59				
	6	400	400.2978	100.07				

*Mean of six observations

Table 3: Intra day and inter day analysis of formulation-Asunra by colorimetric method

Drug	Sample No.	Labeled amount (mg/tab)	Percentage obtained*		± S.D		% R.S.D.	
			Intra day	Inter day	Intra day	Inter day	Intra day	Inter day
	1	400	101.61	100.62	1.0554	0.3554	1.0509	0.3540
DEF	2	400	99.59	100.59				
	3	400	100.07	99.99				
Mean			100.4233	100.4				

* Mean of Three Observations.

Table 4: Ruggedness study of formulation-asunra by colorimetric method

Drug	Condition	Sample No	Labeled Amount (mg/tab)	Amount found (mg/tab)*	Percentage Obtained*	Average (%)	S.D	%R.S.D	S.E
DEF	Analyst 1	1	400	406.2436	101.56				
	Analyst 2	2	400	401.9826	100.49	100.63	0.6123	0.6085	0.0170
		3	400	401.0031	100.25				
		1	400	401.1304	100.28				
		2	400	399.9843	99.99				
		3	400	404.7840	101.19				
DEF	Instrument 1	1	400	402.4878	100.62				
	Instrument 2	2	400	402.3744	100.59	100.41	0.7044	0.7015	0.0196
		3	400	399.9746	99.99				
		1	400	406.4196	101.61				
		2	400	398.3380	99.59				
		3	400	400.2978	100.07				

* Mean of Three Observations.

Table 5: Recovery analysis of formulation – Asunra By colorimetric method

	Sample No	Amount present (µg/ml)	Amount added (µg/ml)	Amount found* (µg/ml)	Amount recovered (µg/ml)	% Recovered	S.D	%R.S.D	S.E
	1	3.0196	2.3650	5.4130	2.3934	101.20	0.6595	0.6566	0.2692
DEF	2	3.0196	2.9948	6.0187	2.9947	100.14			
	3	3.0196	3.6121	6.6312	3.6116	99.99			
					Mean	100.4433			

* Mean of Three Observations.

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