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Research Article

DEVELOPMENT AND VALIDATION OF NEW SPETROPHOTOMETRIC METHODS FOR THE ESTIMATION OF DEFERASIROX IN BULK DRUG AND ITS FORMULATIONS USING 1, 10-PHENANTHROLINE AND MBTH REAGENTS

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ABSTRACT

Two simple, sensitive, accurate, precise and economical spectrophotometric methods (method A and B) are developed for the quantitative estimation of Deferasirox in bulk drug and pharmaceutical formulations. Method A, in this method 1,10Phenanthroline forms complex with ferric (III) ions followed by the oxidation of the drug which results in the formation of orange red colored chromogen. Drug shows maximum absorbance at 510nm and obeys Beer's law in the concentration range of 50- 400mcg/ml. Method B is based on oxidation of 3-methyl-2-benzothiazolinone hydrazone (MBTH) by Ferric (III) followed by its coupling with the drug in acidic medium forming an intense emerald green colored chromogen with absorbance maxima at 603nm and obeys Beer's law in the concentration range of 20 – 160mcg/ml .The results of analysis for both methods have been validated statistically and by recovery studies .We have developed simple , sensitive U.V method (in distilled water in our laboratory) and adopted it as a reference method , for comparing accuracy of the results obtained by the proposed methods .

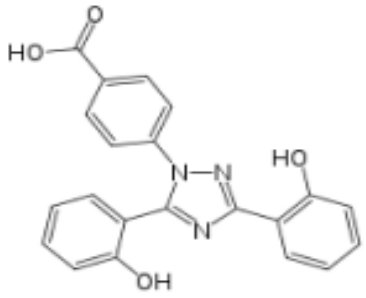
Keywords: Spectrophotometry, Deferasirox, 1, 10-Phenanthroline, 3-methyl-2-benzothiazolinone hydrazone.

INTRODUCTION

Deferasirox is an iron chelating agent. Deferasirox molecular formula is $C_{21}H_{15}N_3O_4$ and its molecular weight is 373.4. Deferasirox designated chemically as 4-[3,5-Bis (2-hydroxyphenyl)-1H-1,2,4-triazol-1-yl]-benzoic acid[1-2]. The Literature survey reveals that few methods like Validation of a novel Spectrophotometric method for estimation of Deferasirox[3].

Spectrophotometric determination of Deferasirox in formulations using Folin ciocalteu and ferric chloride reagents[4]. HPLC coupled with MS/MS[5] detection, LC[6], Terbium sensitized flourescence methods for the estimation of Deferasirox alone and electro catalytic oxidation method for determination of Deferasirox in combination with Deferiprone in the formulations[7-9] have been reported. The present investigation has been under taken to develop simple visible spectrophotometric methods for the estimation of Deferasirox in bulk drug and its formulation using 1, 10-Phenanthroline and MBTH reagents.

Chemical structure:



Deferasirox

MATERIALS AND METHODS

Equipment: A Shimadzu UV-Visible spectrophotometer model 1800 with 1cm matched quartz cell was used for the absorbance measurements. Systronics electronic balance was used for measuring the samples.

Reagents and solutions:

1. All chemicals employed were of analytical grade and double distilled water was used.
2. Deferasirox pure sample was obtained as a gift sample from Novartis pharma ltd, Mumbai, India.
3. 1,10Phenanthroline 0.3% w/v in Isopropanol.
4. Ferric chloride solution 0.5% w/v in double distilled water.
5. MBTH 0.5%w/v in double distilled water.
6. Ferric chloride 0.7%w/v in 0.5%v/v Hydrochloric acid.

Standard solutions:

In this method Deferasirox stock solution (1000 µg/ml) was prepared by dissolving 100mg of drug in 100ml of water. Working solution of the drug was prepared by dilution of the stock solution. The marketed tablet formulation of Deferasirox used in the determination was Asunra with a labelled strength of 100mg and manufactured by Novartis pharma ltd, Mumbai, India.

Analytical method development:

Method A:

Aliquots of Deferasirox ranging from 1mL - 5.5mL (1mL=1000mcg/mL) were transferred into a series of 10ml volumetric flasks. To each of the aliquots 0.5ml of (0.5 % w/v) $FeCl_3$ solution and 1ml of 1,10Phenanthroline (0.3%w/v) were added. The volumetric flask was heated on water bath for 20 min at 40°c then cooled to room temperature and diluted upto the mark with distilled water and the absorbance of each solution was measured at 510nm against the reagent blank (Fig 1) and the calibration curve was prepared (Fig 2).

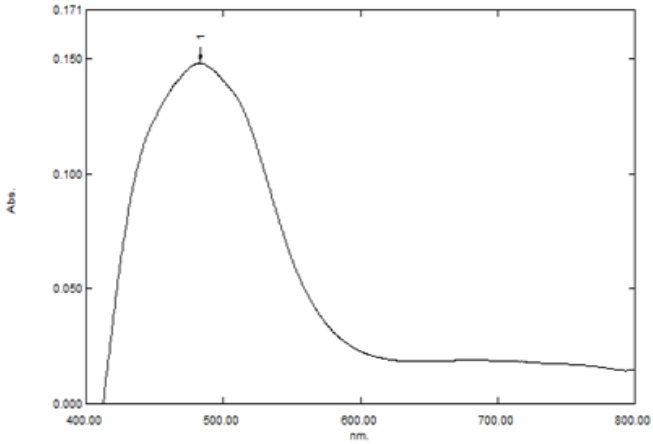


Figure 1: Absorption spectra of Deferasirox with 1, 10-Phenanthroline/Fe (III) system λmax at 510nm

Method B:

Aliquots of Deferasirox ranging from 0.2mL to 1.6mL (1mL=1000mcg/mL) were transferred into a series of 10ml volumetric flasks. To each 1ml of $FeCl_3$ (0.7%w/v) and 1ml of MBTH (0.5%w/v) were added and kept aside for 10min. The volume was made upto the mark with distilled water. The absorbance of the emerald green colored chromogen was measured at 603nm against the reagent blank (Fig 3) and obeyed Beer's law in the concentration range of 20 – 160 mcg/mL (Fig 4). The absorbance of reaction product at 603nm remained stable for more than 60 min. The amount of Deferasirox present in the sample solution was computed from its calibration curve.

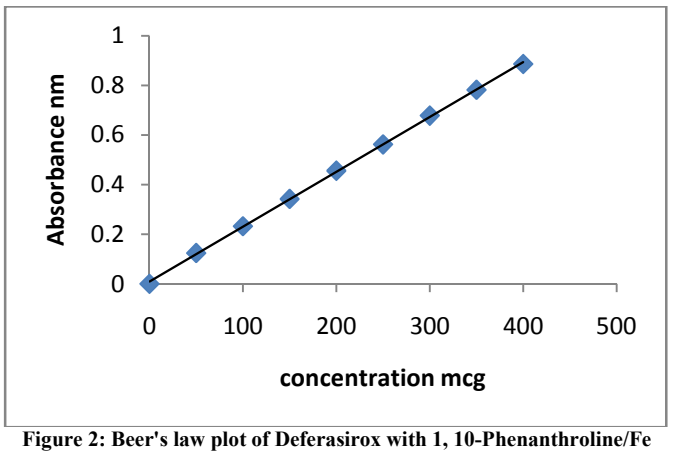


Figure 2: Beer's law plot of Deferasirox with 1, 10-Phenanthroline/Fe (III)

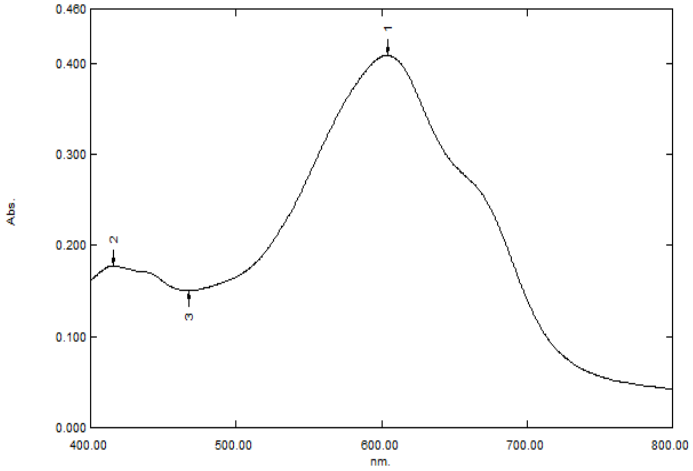


Figure 3: Absorption spectra of Deferasirox with MBTH reagent. λmax at 603 nm

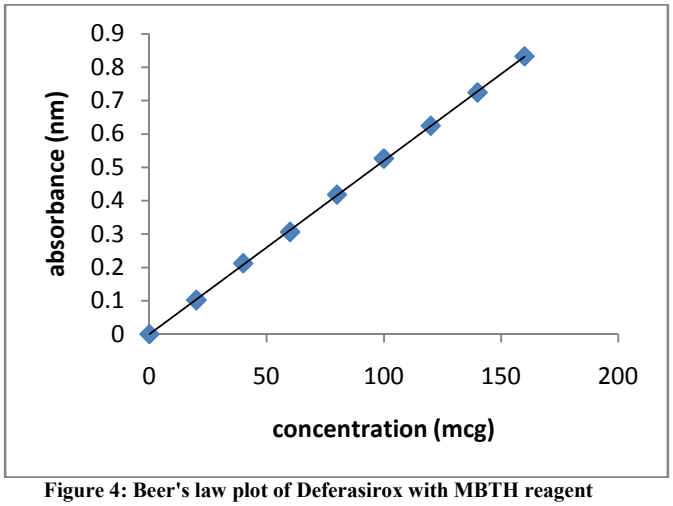
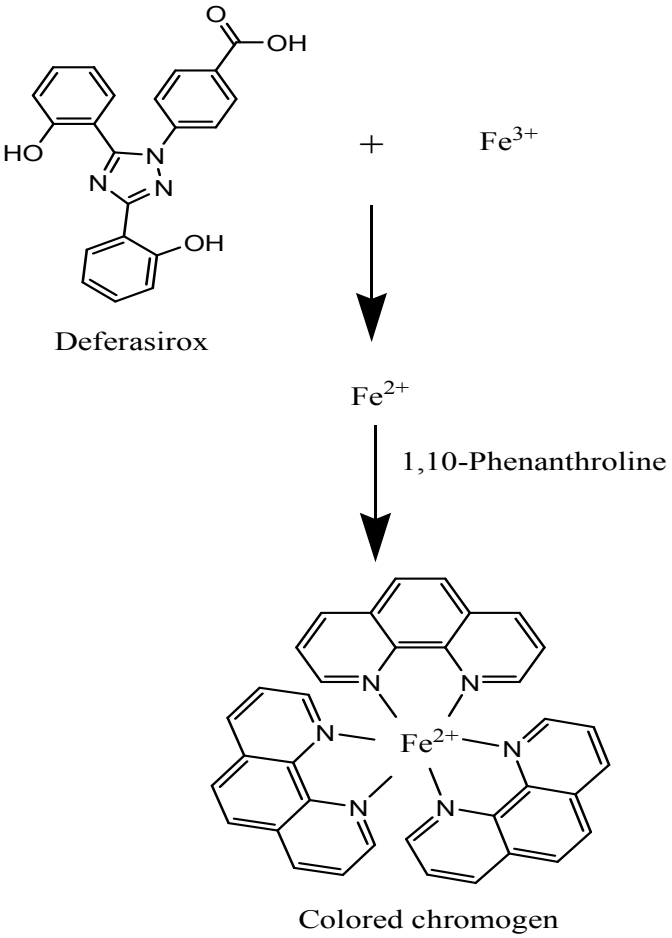


Figure 4: Beer's law plot of Deferasirox with MBTH reagent

Analysis of formulation:

Accurately weigh formulation powder equivalent to 100mg of Deferasirox was transferred to a 100ml volumetric flask .50ml of distilled water is added and sonicated for 15min and diluted to the mark with distilled water. The resulting solution was filtered through the whatmann filter paper. The assay of formulation was carried out with above mentioned procedure.

Scheme: 1



RESULTS AND DISCUSSION

Method A: In this method Deferasirox was made to react with excess of Ferric chloride under specified experimental conditions which converts into ferrous salt, the amount of conversion corresponds to the drug concentration. The ferrous ions thus formed in the reaction were made to react with 1,10Phenanthroline to form orange red colored chromogen (as shown in the scheme-1) due to the formation of six coordination bands between Ferrous ion and six nitrogen atoms of three molecules of 1,10Phenanthroline which have a lone pair of electron each, with absorption maxima λ_{max} at 510nm (Fig 1) and obeyed beer's law in the concentration range of 50-450mg/mL.

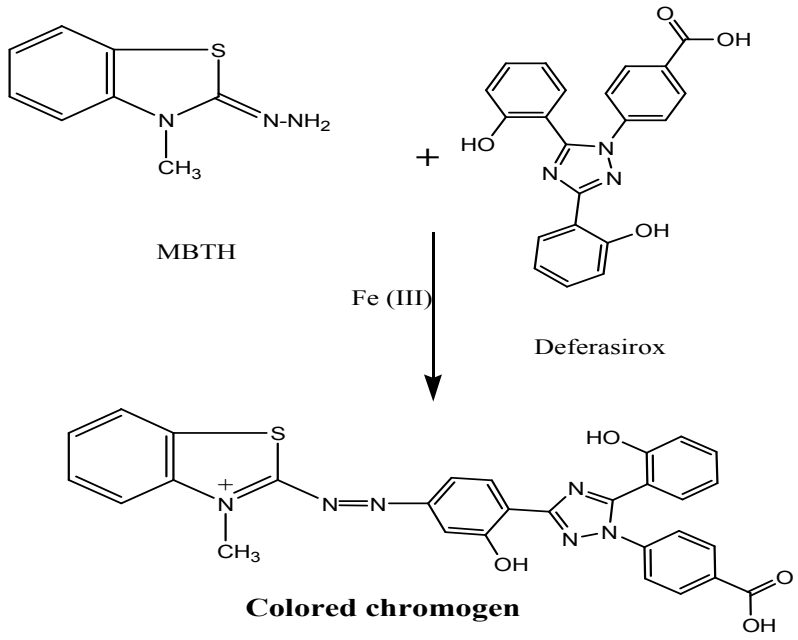
The first step of this method is the oxidation of Deferasirox with oxidant.

Deferasirox +Fe (III) ⟶ oxidation product + Fe (II)

The second step carries with the estimation of Fe (II) with 1, 10-Phenanthroline.

The method B is developed based on the formation of emerald green colour chromogen. The colour formation by MBTH was oxidized by the ferric chloride in acidic medium followed by its coupling with the drug to form emerald green colored complex. Actually, this is an iron catalysed oxidative coupling reaction of MBTH with the drug. Under reaction conditions, on oxidation, MBTH loses two electrons and one proton forming an electrophilic intermediate, which is the active coupling species reacts with the coupler (Deferasirox) by electrophilic attack on the most nucleophilic site of Deferasirox as shown in the scheme-2 with absorption maxima at 603nm (Fig 3) and beer's law obeyed in the concentration range of 20-160mg/mL (Fig 4).

Scheme: 2



The optical characteristics such as absorption maxima, Beer's law limits, Molar absorptivity and Sandell's sensitivity for both methods are presented in Table -1. The regression analysis using the method of least squares was made for the slope (a) and intercept (b) obtained from different concentrations are summarised in Table-1. The precision and accuracy were found by analysing six replicates sample containing known amounts of the drug and the results are summarized in Table-2 and Table-4. Recovery studies were conducted by analysing each pharmaceutical formulation in the first instance for the active ingredient by the proposed methods. Known amount of drug was once again determined by both proposed methods. After bringing the active ingredient concentration within the Beer's law limits. The results are reported in Table-3 and Table-5.

Table 1: optical characteristics, precision and accuracy of Proposed Methods

Parameters	Method A	Method B
$\lambda_{max\ (nm)}$	510	603
Beer's law limit (µg /ml)	50 - 400	20 – 160
Molar absorptivity (Litre . $mole^{-1}.cm^{-1}$)	0.8523×10^3	0.1953×10^3
Correlation coefficient	0.9998	0.9999
Stability of colour(hours)	50min	60 min
Sandell's sensitivity (µg/cm^2/.0.001 abs unit.)	0.043	0.0196
Regression equation (Y)		
Slope (b)	0.00219	0.00519
Intercept (a)	0.0150	0.0003
% RSD	± 0.4686	0.3131
% Range of errors (95 % confidence limit)		
±0.05 significance level	±0.00178	0.00109
±0.01 significance level	±0.00264	0.00161
Limit of detection (LOD/$mcgml^{-1}$)	3.221	0.831
Limit of Quantification (LOQ/ $mcgml^{-1}$)	9.757	2.518

Note:*Y=bC+a, where C is the concentration of Deferasirox in µg/ml and Y is the absorbance at the respective maximum absorbency. For eight replicates %RSD – Relative standard deviation.

Table 2: Assay results of Deferasirox in pharmaceutical formulations by 1, 10- Phenanthroline

Sample	Labelled amount (mg)	Amount found*(mg)		% Recovery of the proposed method
		Proposed method*	Reference method	
T_1	100	98.47	98.98	98.47
T_2	100	98.56	98.92	98.56

T_1 and T_2 are tablets of different manufacturers.
*** Mean of six determinations.**

Table 3: Results of Recovery studies of Deferasirox by 1, 10-Phenanthroline

Drug concentration(% at specification level)	Amount of Drug in formulation (mg)	Amount of drug added (mg)	Amount of drug recovered (mg)	% Recovery
80	98.47	80	178.29	99.77
100	98.56	100	198.37	99.81
120	98.62	120	218.46	99.86

Table 4: Assay results of Deferasirox in pharmaceutical formulations by MBTH

Sample	Labelled amount (mg)	Amount found*(mg)		% Recovery of the proposed method
		Proposed method*	Reference method	
T_1	100	98.73	98.98	98.73
T_2	100	98.69	98.92	99.69

T_1 and T_2 are tablets of different manufacturers.
*** Mean of six determinations.**

Table 5: Recovery studies of Deferasirox by Oxidative Coupling method (MBTH)

Drug concentration(% at specification level)	Amount of Drug in formulation (mg)	Amount of drug added (mg)	Amount of drug recovered (mg)	% Recovery
80	98.73	80	178.63	99.87
100	98.69	100	198.58	99.89
120	98.65	120	218.47	99.85

CONCLUSION

The proposed methods are simple, sensitive, economical, accurate, precise and reliable for the determination of Deferasirox in bulk drug and its formulations depending upon the needs of the situation.

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REFERENCES

1. Neil OMJ, editor. The Merck Index: An Encyclopedia of Chemicals, Drug and Biologicals.14th edn, Merck & Co. Inc, 2006; 483.
2. Sweetman SC, editor. Martindale: The Complete Drug Reference, 35th edn, Pharmaceutical Press: London (U.K), 2007; 1294.
3. Lalitha Manasa P, Shanmukh Kumar JV,Vijaya Saradhi S and Rajesh V. Validation of a novel Spectrophotometric methods for estimation of Deferasirox **.** IJPBR**,** 2011**;** Vol. 2(1): 1-3.
4. Sambashivarao, Vattikuti, Ashokkumar G. Spectrophotometric determination of Deferasirox in Formulations using Folin-Ciocalteu and Ferric chloride reagents IJRRPAS, 2011 ; 1 (2) : 62-71.
5. Chauzit Emmanuelle PharmD, Bouchet Stéphane PharmD. A Method to Measure Deferasirox in Plasma Using HPLC Coupled With MS/MS Detection and its Potential Application, The Drug Monit, 2010; 32(4):476-81.
6. Ravi kiran kaja; Surendranath; K.V. Radhakrishna; P. Satish. J; Satyanarayana P.V.V. A Stability Indicating LC Method for Deferasirox in Bulk Drugs and Pharmaceutical Dosage Forms Chromatographia, 2009; 72(5-6):441-446.
7. Jamshid L. Manzoori, Abolghasem Jouyban, Mohammad Amjadi, Vahid Panahi-Azar, Elnaz Tamiz, Jalil Vaez-Gharamaleki Terbium-sensitized fluorescence method for the determination of Deferasirox in biological fluids and tablet formulation . The journal of biological and chemical sciences, 2011; 63(3): 236-240.
8. Lough WJ, Wainer IW. High Performance Liquid Chromatography: fundamental principles and practice. Glasgow (UK): Blackie Academic & Professional, 1995: 2-28.
9. M. Hajjizadeh, A. Jabbari, H. Heli, A.A. Moosavi-Movahedi, A.Shafiee and Karimian Electrocatalytic oxidation and determination of Deferasirox and Deferiprone on a nickel oxyhydroxide-modified electrode. Anal Biochem, 2010; 373(2):337-48.

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