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HPLC METHOD FOR ESTIMATION OF DEFERASIROX IN PHARMACEUTIICAL FORMULATION AND ENVIRONMENTAL WATER SAMPLES

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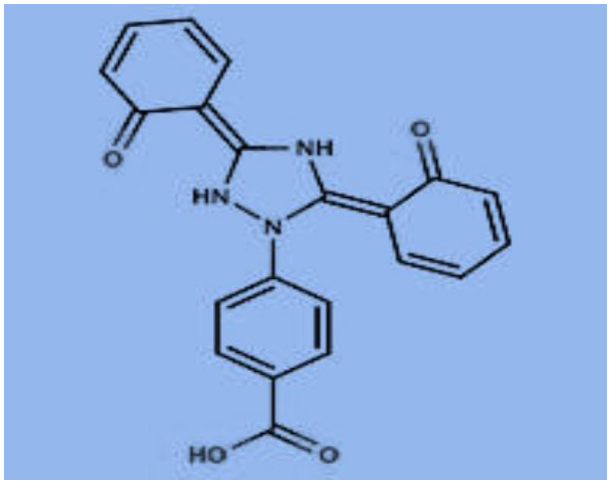
ABSTRACT

A simple, precise, rapid, and accurate high performance liquid chromatography method has been developed for the determination of Deferasirox in pure from, pharmaceutical formulations and environmental water samples. Chromatography was carried out on L7 (C_8) machereg-nagel column (15cm × 4.6mm), 5 microns, using a mixture of H_20: Ethanol (35:65 v/v) adjusted to pH2.5 by H_3PO_4 as a mobile phase at a flow rate of 1.5 ml.min^{-1}. Detection was performed at 250nm at ambient temperature. The retention time was found 21 minutes. The calibration curve was linear (r= 0.998) over a concentration range from 10 to 200 µg/ml. Limit of detection (LOD) and limit of quantitation (LOQ) were found 0.2377µg/ml and0.7845 µg/ml respectively. The method was validated for its linearity, precision and accuracy. The proposed method was successfully applied for the determination of Deferasirox in pure form, pharmaceutical formulations and in environmental water samples.

KEYWORD: HPLC, Deferasirox, Pharmaceutical Formulations, Environmental Water samples.

INTRODUCTION

Deferasirox belongs to the class Antidote. Chemical name is 4-[(3Z,5E)-3,5-bis(6-oxo- 1-cyclohexa-2,4-dienylidene)-1,2,triazolidin-1-yl] benzoic acid. (Figure.1)



Molecular weight=373.4 and molecular formula is $C_{21}H_{15}N_3O_4$

Figure 1: Chemical Structure of Deferasirox.

Deferasirox is an oral iron chelator. Its main use to reduce chronic iron overload in patients who are receiving long term blood transfusion for conditions such as beta-thalassemia and other chronic anemia's. It is first oral medication approved in the USA for this purpose.. Deferasirox is a white crystalline powder, freely soluble in Dimethyl form amide, Dimethyl. Its melting point is 116o to 117°C.[1-5], Deferasirox It is not official in any of the pharmacopoeia till now. Literature. survey revealed several method have been reported for the analysis of this drug using Spectrophotometric methods[6,7], HPLC methods[8-10], fluorescence method[11], Electro catalytic oxidation method[12], LC Method[13], and HPLC coupled With MS/MS Detection.[14] These methods are required expensive or sophisticated instruments and not simple for routine analysis. The present paper reports the development of a new high performance liquid chromatography (HPLC) method for determination of Deferasirox in pharmaceutical formulations (tablets) and environmental water samples.

MATERIALS AND METHODS

Apparatus

Chromatographic system consisted of an shimadzu HPLC model LC-20AD with UV detector model SPD-20A and C_8 column (15cm ×4.6mm),5µm particle size. HPLC condition were given in Table 1.

Table 1: HPLC conditions.

Column	Column machereg-nagel C_8 (15cm×4.6mm),5 µm
Wavelength	250 nm
Mobile phase	H_20 : Ethanol (35:65 v/v) adjusted to pH2.5 by H_3PO_4
Retention time	21 minutes
Flow rate	1.5 ml/min
Temperature	Ambient
Injection volume	20 µL

Reagents

All chemicals used were of analytical or pharmaceutical grade and HPLC grade. Ethanol were used throughout.

Standard stock solution of Deferasirox (0.25mg/ml). [Provided from Iraqi pharmaceutical industry co] This solution was prepared in ethanol. Working standard solutions in a range of (10-200 µg/ml) were prepared by dilution from standard stock solution.

HPLC method for determining Deferasirox

A series of standard solution containing 10-200 µg/ml of Deferasirox and the sample solution of pharmaceutical preparations were applied respectively. 20µl aliquot of each solution was injected into the column in a duplicate and the chromatograms were recorded. Calibration graph was constructed by plotting the mean peak area versus concentration of Deferasirox. The concentration of the unknown was calculated from the regression equation derived from the concentration and peak area data, or was read from calibration graph.

Procedures for pharmaceutical preparations(Tablets)

Weigh and powder 10 tablets(Ipijde-500mg). Transfer an accurately weighed portion of the powder equivalent to 25 mg Deferasirox was dissolved well in into 100 mL ethanol, mixed well for 20 min and then filtered by filter paper No.1. The filtrate was made up to 100mL with ethanol, The final concentration is 250µg /ml. This solution was subsequently diluted and analyzed, the amount of Deferasirox was determined by comparing the peak area of the assay preparation with the standard preparation at the same concentration.

Procedure for water samples

To demonstrate the practical applicability of the proposed method. The tap and river water samples were collected in polyethylene container cleaned with nitric acid, and filtered through Whatman No.1 filter paper. Filtered samples were stored at 4 C^0 until analyzed which shows negative results found to be free from of Deferasirox, synthetic samples were prepared by adding known concentration of Deferasirox to each samples prior analysis in the range from 20-70 ppm The determination of Deferasirox proceeded as described under recommended procedure. Calculate the percentage recovery using a calibration graph previously prepared.

RESULTS AND DISCUSSION

The development of HPLC methods for the determination of drugs has received considerable attention in recent years because of their importance in the quality control of drugs and pharmaceutical products.[15-18] The aim of this study was to develop accurate, sensitive, rapid, selective, and reproducible HPLC method for the determination of Deferasirox in pure from, its pharmaceutical formulations and water samples using the most commonly employed L7 column(C_8) with UV detection. The detection wavelength of 250nm was chosen in order to achieve a good sensitivity for quantitative determination of Deferasirox in tablets and water samples. The mobile phase consisting of H_2O (PH 2.5 by H_3po_4 : Ethanol (35:65 v/v) offered a good separation at ambient temperature under these conditions using a flow rate of 1.5 ml/min and retention time of 21 minutes as shown in the chromatogram, (Figure 2).

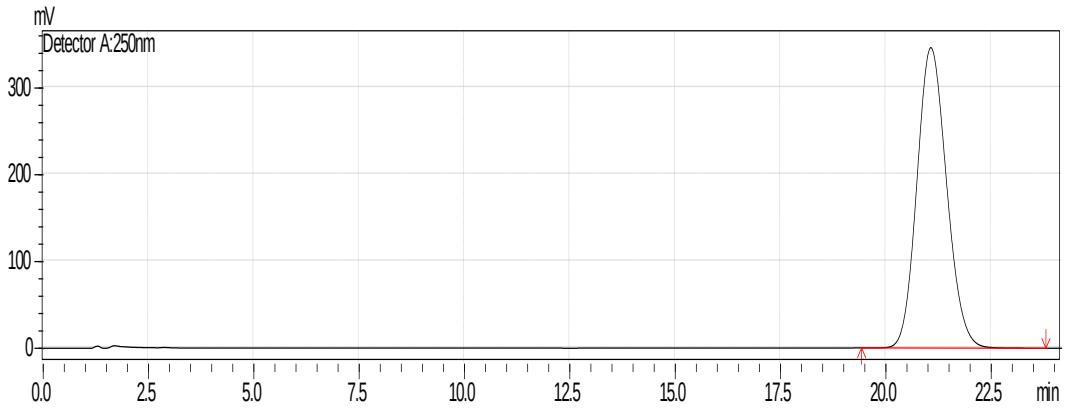


Figure 2: Typical chromatogram (Deferasirox (25 µg/ml).

Under the described experimental conditions, the analytic peak were well defined and free from tailing. Deferasirox was determined by measuring the peak area. A plot of peak area against concentration gave a linear relationship (r=0.998) over the concentration range 10-200 µg/ml. Using regression analysis, the linear equation Y=80367x-191052 was obtained where Y is the mean peak area and X is the concentration in µg/ml (Figure 3).

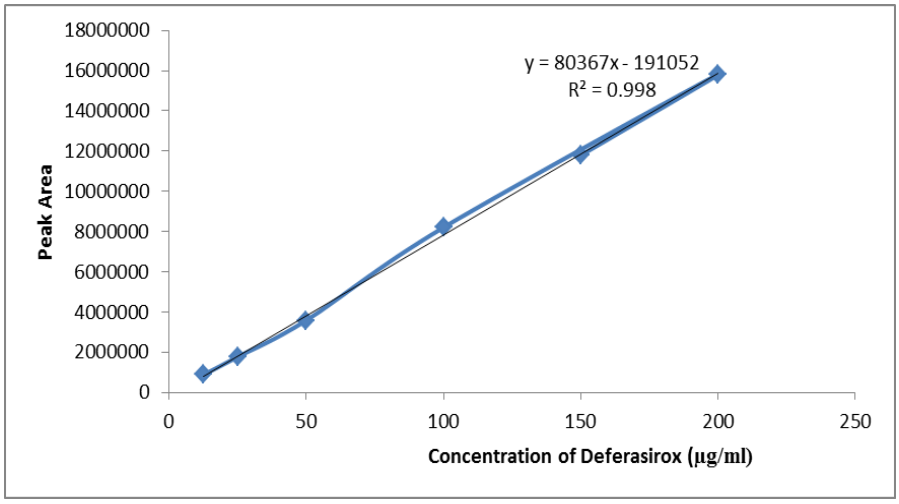


Figure 3: Calibration curve for Deferasirox.

Table 2: Some information about the calibration curve.

Linearity range	Intercept	Correlation coefficient	Slope*
10-200 µg/ml	191052	0.998	80367

*Slope: The ratio between the measured quantity in the analytical technique to the concentration of the substance to be determined quantity to the small change in concentration indicate a good sensitivity (high slope).[19]

Determination the limit of detection and limit of quantification (sensitivity)

The standard deviation at concentration zero was calculated and this value was used for the calculation of the limit of detection and limit of quantification. The limits of detection (LOD) and quantification (LOQ) were calculated using the following formulae: LOD= (3.3σ/s) and LOQ=(10σ/s) where σ is the standard deviation of the response and s is the slope of the regression line.[20] Limit of detection (LOD) and limit of quantification (LOQ) were found 0.2377µg/ml and0.7845 µg/ml respectively. The results indicate that the method was sensitive enough to detect a concentration of 240 ng/ml and able to quantify at a concentration of above 785 ng/ml.

Precision and accuracy

The precision of the method was established by carrying out the analysis of Deferasirox (n=10) using the proposed method. The low value of standard deviation showed that the method was precise. The results obtained are presented in Table. (3).To ensure the reliability and accuracy of the method recovery studies were carried out at five different levels. The results of recovery studies were found to be accurate, mean recoveries being 100.51±1.1 (n=10) as shown in Table 3.

Table 3: Method accuracy and precision.

Concentration of Deferasirox µg/ml	RSD %	Recovery %
10	0.72	101.6
20	0.65	100.5
40	1.08	101.0
80	1.06	99.95
100	0.70	99.5
Mean(n=10)	0.769	100.51

Analytical application

The proposed method was successfully applied to the assay of Deferasirox in pharmaceutical formulations (tablets) and water sample. The result of analysis for pharmaceutical formulation Table 4. This reveals that there was close agreement between the results obtained by the proposed method and label claim.

Table 4: Determination of Deferasirox in pharmaceutical formulations.

Pharmaceutical formulations	Label amount (mg)	Found by proposed method *mg	Recovery %
Tablets Ipijde-125mg Ipi company	125mg/Tablet	124.92	99.93
Tablets-Exjade-500mg Novartis company	500 mg/Tablet	499.6	99.92

*Mean value of ten determinations

The results of water samples Table 5. Show that the recovery values obtained were closed to 100%.

Table 5: Determination of Deferasirox in water samples.

Water samples	Deferasirox (µg.ml^{-1})		% Recovery(n=10)
	Found	Taken	
Tap water	1 9.94	20	99.7
	30.02	30	100.066
	69.99	70	99.988
River water	1 9.96	20	99.8
	29.95	30	99.83
	70.03	70	100.043

★Mean of ten determinations.

CONCLUSION

In this study, accurate, simple, and rapid HPLC method was developed and validated for the determination of Deferasirox in pharmaceutical formulations and water samples. The method was selective using L7(C_8) analytical column and applicable to pharmaceutical preparations. Thus the developed method was recommended for control throughout the entire manufacturing process of drugs as well as quality control of the finished product in view of its high recovery and precision.

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