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Research article

Stability indicating method development and validation of Deferiprone in pharmaceutical dosage form by RP-HPLC

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Abstract

The objective of this present is to develop a simple, precise, accurate stability indicating method for the estimation of deferiprone in formulation by using RP-HPLC. The separation was achieved on Inertsil ODS C18, 250x 4.6mm, 5µm i.d. column using 60 volumes of Mixed Phosphate buffer(KH_2PO_4+K_2HPO_4) pH 3.0 and 40 volumes of methanol as mobile phase and at a flow rate of 1.0 mL/min. Detection was carried out using a PDA detector at 280nm. The method was validated for accuracy, precision, specificity, linearity and sensitivity. The total chromatographic analysis time per sample was about 5 min with deferiprone eluting at retention time of about 4.980 min. The method was validated as per ICH guide lines. Stability studies reported absence of impurities at the peak retention time. The drug was stable to different conditions like acidic, alkali, thermal, oxidative and photolytic conditions. Validation studies demonstrated that the proposed HPLC method is simple, specific, rapid, reliable and reproducible. The standard curves were linear over the concentration range of 75-125µg/mL. The LOD and LOQ values for deferiprone were 3.91 and 11.8 µg/mL, respectively. The percentage recovery was found to be 97.3 to 98.0 and the %RSD for precision was found to be 0.5. The high recovery and low relative standard deviation confirm the suitability of the proposed method for the determination of deferiprone in bulk and capsule dosage forms.

Introduction

Deferiprone binds to iron in the blood. It helps to prevent and treat too much iron in the blood. It helps to prevent and treat too much iron in the blood caused by blood transfusions. Deferiprone is chemically 3-hydroxy-1,2-dimethylpyridin-4-one with molecular formula $C_7H_9NO_2$. Deferiprone is an oral iron chelating agent used to treat transfusion related, chronic iron overload. Deferiprone has been linked to a low rate of transient serum amino transferase elevations during therapy and to rare instances of clinically apparent liver injury. Deferiprone is a heavy metal antagonist that chelates iron. It is indicated for the treatment of patients with transfusional iron overload due to thalassemia syndromes when current chelating therapy is inadequate. On literature survey, it was found that only RP-HPLC [1] method have been reported for estimation of Deferiprone in human plasma and urine and no method is available in pharmacopoeias. In the view of the need for a suitable method for routine analysis, attempts are being made to develop simple precise and accurate analytical methods for the estimation of titled drug and extend it for determination in formulation. As chromatographic methods of analysis is a pre-requisite for the marketing of most of formulations. One stability indicating RP-HPLC, HPTLC along with spectrophotometric methods namely zero order, first order, second order derivative, and area under the curve methods are planned to develop and validate for the estimation of titled drug. In the present work an attempt has been made to develop a simple, accurate, sensitive, rapid and economic a RP-HPLC method for the quantitative estimation of deferiprone in bulk and pharmaceutical formulation.

Experimental

Materials, chemicals and reagents

Deferiprone standard was provided by Glenmark Pharmaceuticals, deferiprone capsules containing 5 mg of deferiprone were procured from the local market. Analytical grade sodium di-hydrogen ortho phosphate, potassium di hydrogen orthophosphate, ammonium acetate was purchased from S.S. fine chemicals, Hyderabad. HPLC grade tetrahydrofuran, methanol, acetonitrile and water were obtained from Merck, Mumbai.

Instrumentation

The chromatographic system used to perform development and validation of this assay method was comprised of a LC-10STvp binary pump, a SPD –M10 Avp photo diode array

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detector and a rheodyne manual injector model 7725i with 20µl loop connected to a multi –instrument data acquisition and data processing system, spinchrome software.(Class-VP 6.13SP2, Shimadzu).

Mobile phase preparation

The mobile phase consisted of a mixture of 60 volumes of mixed phosphate buffer (KH_2PO_4+ K_2HPO_4) pH 3.0, and 40 volumes of methanol. To prepare buffer 1.625 gm of potassium dihydrogen phosphate (KH_2PO_4) and 0.3 g of dipotassium hydrogen phosphate was weighed and dissolved in 100ml of water and volume was made up to 600ml with water. Adjust the pH to 3.0 using ortho phosphoric acid. The buffer was filtered through 0.45µ filters to remove all fine particles.

Wave length detection

An accurately weighed amount of 10mg of deferiprone was transferred in to 10ml volumetric flask and dissolved in mobile phase and then make up to the mark with mobile phase and prepare 10 µg /ml of solution by diluting 0.1ml to 10ml with mobile phase. The wavelength of maximum absorption (λ_{max}) of the drug, 10 µg/ml solution of the drugs in methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank. The absorption curve shows characteristic absorption maxima at 280 nm for tolcapone.

Chromatographic conditions

Chromatographic analysis was performed on a Inertsil ODS C_{18}, 250x 4.6mm ,5µm column. The mobile consisted of 60 volumes of Mixed Phosphate buffer (KH_2PO_4+K_2HPO_4) pH 3.0, and 40 volumes of methanol. The flow rate of the mobile phase was adjusted to 1.0 ml/min and the injection volume was 20 µl. Detection was performed at 280 nm.

Assay

Standard preparation

An accurately weighed amount of 250 mg of deferiprone was transferred to 100 ml of volumetric flask and dissolved in mobile phase and made up the volume with mobile phase. From above stock solution, pipette out 1ml and make up to 10ml to get 250 µg/ml of deferiprone.

Sample preparation

Twenty capsules of deferiprone were weighed separately and average weight was determined. Capsule powder equivalent to 250 mg was accurately weighed and transferred to 100 ml volumetric flask, dissolved in 50ml of mobile phase and sonicated for 15 min make up to the mark with mobile phase further dilutions were prepared by pipette out 1 ml and make up to 10 ml to get 250 µg/ml of deferiprone.

Table 1. Assay of deferiprone

Assay		
Deferiprone		
Standard Area	1	3323.905
	2	3320.771
	3	3293.678
	4	3274.549
	5	3193.689
	Average	3312.785
Sample area	1	3315.153
	2	3258.634
	3	3399.478
	4	3304.543
	5	3167.163
	Average	3288.994
Standard weight		250 mg
Sample weight		301.32 mg
Label claim		250 mg
std.purity		99.8
Assay in mg		249.1 mg
%Assay		**99.08**

The amount of deferiprone present in the formulation by using the formula given below, and results shown in above table:

$$\%\ \text{Assay} = \frac{\text{Area of Test}}{\text{Area of Standard}} \times \frac{\text{Weight of Standard}}{\text{Dilutions of Standard}} \times \frac{\text{Dilutions of Test}}{\text{Weight of Test}} \times \frac{\text{Potency}}{100} \times \frac{\text{Average Weight}}{\text{Label Claim}} \times 100$$

Forced Degradation Study

The degradation samples were prepared by transferring intact capsules [2] were employed for acidic, alkaline and oxidant media and also for thermal and photolytic conditions. After the degradation treatments were completed, the stress content solutions were diluted with diluents to attain about 100µg/ml concentration. Specific conditions were described as follows.

Acidic degradation

Acidic degradation study was performed by refluxing the drug content in 30 ml of 1N HCl at about 1 hr and after cooling to room temperature it was neutralized with 1N NaOH solution. Further solution was diluted to achieve concentration 100 µg/ml.

Alkali degradation

Alkali degradation study was performed by heating the drug content in 30 ml of 1N NaOH at around 100^0 C for 1hr and then the mixture was neutralized with 1N HCl. It was further diluted with diluents to achieve 100 µg/ml.

Oxidative degradation
Oxidative degradation study was performed by refluxing the drug content in 3% v/v of 10 ml of H_2O_2 at 100^0C for 1 hr then diluted to 100 µg/ml with diluents.

Thermal degradation
Thermal degradation study was performed by keeping powdered drug content at around 80^0 C for 72 hrs. After this it was allowed to come at room temperature

Photolytic degradation
Photolytic degradation study was performed by exposing the drug content in UV light (200 Watts hours per sq.m) for 72 hrs, further it was diluted to 100 µg/ml using mobile phase.

Results & Discussion

Method Validation
Specificity
The specificity of the method was determined by checking the interference of the placebo with the analyte and the proposed method was eluted by checking the peak purity of deferiprone during the analysis [3].
There is no interference of mobile phase, solvent and placebo with the analyte peak and also the peak purity of analyte peak which indicate that the method is specific for the analysis of analytes in their dosage form.

Method Precision
Precision study was established by evaluating method precision and system precision study. Method precision of the analytical method was determined by analyzing six sets of sample solution preparation. Assay of all six replicate sample preparations was determined and mean assay value, standard deviation and % relative standard deviation for the same was calculated. System precision of the analytical method was carried out to ensure that the analytical system was working properly. Standard solution was injected six times in to system and chromatograms were recorded.
The area responses are consistent as evidenced by the values of relative standard deviation. Hence it can be concluded that the system parameter meets the requirement of method validation.

Table 2. Method precision of Deferiprone

Sample No	%Assay
1	98.1
2	99.5
3	99.7
4	100.1
5	98.4
6	99.7
Mean (N=6)	99.2
%RSD(N=6)	0.7

Table 3. System precision of Deferiprone

System Precision		
Deferiprone		
S. No.	Rt	Area
1	4.987	3259.295
2	4.973	3291.558
3	4.950	3314.065
4	4.983	3293.678
5	4.98	3315.153
6	4.987	3267.163
Avg	4.9767	3290.152
St. Dev	0.0141	23.203
% RSD	0.28	0.70

Linearity
The linearity plot was prepared with five concentration levels (50, 75, 100,125 and 150µg/ml of deferiprone). These concentration levels were respectively corresponding to 50, 75, 100, 125, 150% of standard solution concentration. The peak area versus concentration data were evaluated by linear regression analysis. 250 µg/ml of standard stock solution of deferiprone was prepared and further diluted to attain concentration of about 50, 75, 100, 125 and 150µg/ml of standard solution concentration. From standard stock solution of 250 µg/ml accurately pipette out exact 5, 7.5, 10, 12.5 and 15 ml and dilute it up to 10 ml each with diluents to achieve 50-150 µg/ml concentration range. Coefficient of determination of the linearity study was found to R^2=0.994 with linear regression equation y = 33.40x + 325.2, which proves the method is highly linear over the working range.

LOD & LOQ
LOD is the lowest amount of the drug content which can be detected by the proposed method while LOQ is the lowest amount which can be quantified by the method. The guideline suggest minimum signal to noise ratio (S/N) more than 3.3 for LOD and more than 10 for LOQ. The LOD for this method was found to be 3.9 µg /ml .The LOQ for this method was found to be 11.8 µg/ml for deferiprone.

Accuracy
This experiment can be performed by the recovery test. Recovery of the method was evaluated at three different concentration levels by addition of known amounts of standard placebo preparation. For each concentration level three sets were prepared and injected as duplicate [4]. Accuracy of the method was determined by recovery studies. To the formulation (pre analyzed sample), the reference standards of the drugs were added at the level of 75%, 100%, 125%. The % recovery is within the limit of 75 to 125% this is in the limit of acceptance criteria and % RSD value of % recovery of replicate set is below 2%. Hence this suggests that proposed method is highly accurate.

Table 4. Linearity preparations of deferiprone

Sr. no	Volume from standard stock transferred in ml	Volume made up in ml (with mobile phase)	Conc. of solution (µg /ml)	Area
1	5	10	50	1904.438
2	7.5	10	75	2901.665
3	10	10	100	3680.717
4	12.5	10	125	4620.5
5	15	10	150	5220.44

Table 5. Recovery of deferiprone

Recovery level	Accuracy			Average % Recovery
	Amount taken (µg/ml)	Amount recovered (µg/ml)	% Recovery	
75%	100	73.60	97.3	
	100			
	100			
100%	120	98.9	98.9	
	120			98.0%
	120			
125%	140	122.6	98.0	

Robustness

Robustness of the method was evaluated by assaying test solution under slight but deliberate changes in analytical conditions such as change in flow rate, change in wave length, change in proportions of mobile phase [5].

a)Flow rate change: In this experiment the test samples were analyzed at the flow rate of 0.8 ml/min, 1.0 ml and 1.2 ml/min each and the results were observed in terms of assay value and chromatographic compatibility. Blank, standard and sample solutions were prepared as per the assay procedure .The result of robustness of the developed assay method was established in table 6 .The result shown that during all variance conditions assay value of the test preparation solution was not affected and it was the accordance with that of actual system suitability parameters were also found satisfactory. Hence the analytical method would be concluded as robust.

b)Wave length change: In this experiment the test samples were analyzed at the wave length of 278, 280 and 282 nm each and the results were observed in terms of assay value and chromatographic compatibility. Blank, standard and sample solutions were prepared as per the assay procedure [6].

The result shown that during all variance conditions assay value of the test preparation solution was not affected and it was the accordance with that of actual. System suitability parameters were also found satisfactory. Hence the analytical method would be concluded as robust.

Table 6. Robustness of deferiprone

Parameter	deferiprone Retention time (min)	Tailing factor	%RSD
Flow			
0.8ml/min	4.881	1.632	0.44
1.0ml/min	4.980	1.647	
1.2 ml/min	4.233	1.622	
Wavelength			
278nm	4.973	1.641	0.41
280nm	4.963	1.611	
282nm	4.978	1.621	

Table 7. Ruggedness study of deferiprone

	%Assay
system 01	99.8
system 02	100.1
%RSD	0.14

Ruggedness study

The ruggedness of the method was studied by the determining the system to system variation and the analyst to analyst variation. The present ruggedness study was performed by two different HPLC systems.

The % Relative standard deviation of assay values between two systems should be not more than 2.0%. From the observation the %RSD between two systems assay values not greater than 2.0%, hence the method was rugged [7].

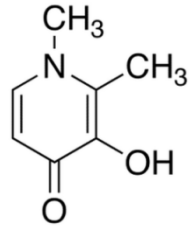


Figure 1. Chemical structure of deferiprone

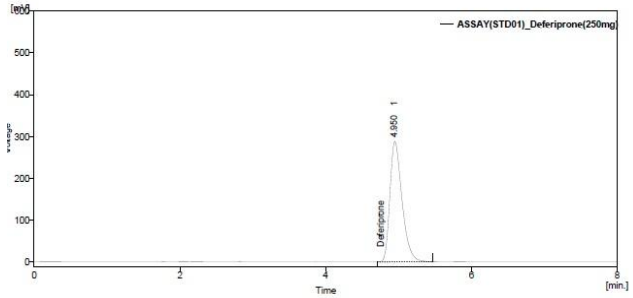


Figure 2. Standard chromatogram of deferiprone

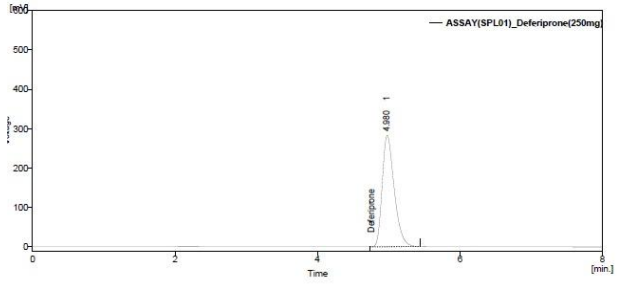


Figure 3. Sample chromatogram of deferiprone

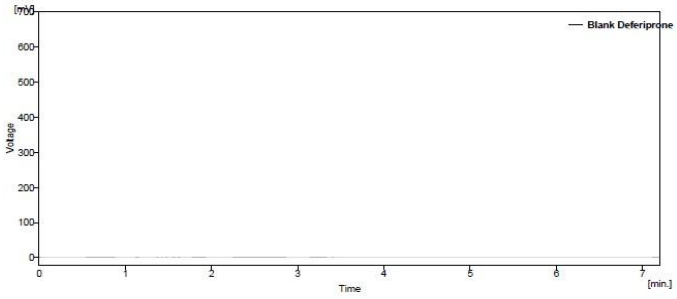


Figure 4. Chromatogram of specificity of deferiprone

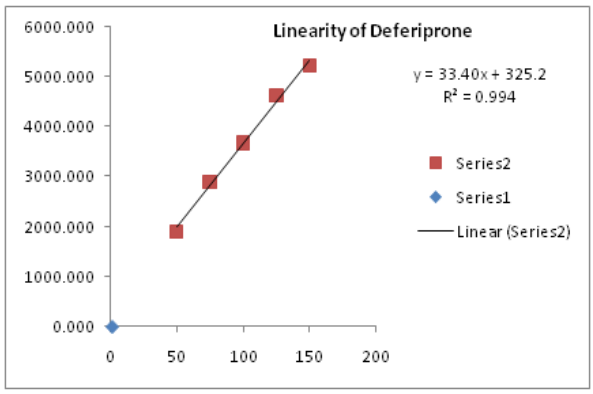


Figure 5. Linearity of deferiprone

Conclusion

In conclusion a validated stability indicating RP-HPLC method has been developed for determination of deferiprone in the bulk and capsules. The results show that the method was found to be specific, simple, accurate, precise and sensitive. The method was successfully applied for the determination of capsules in formulation. Forced degradation studies of the bulk drug carried out under conditions of acid, alkali, thermal, oxidation and UV. For heat and light study period was 10 days where as for acid, base and oxidative degradation it was about 48 hrs. There is no significant degradation of the sample was obtained in the acid, alkali, thermal, oxidation and UV. The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

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