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Development of Stability Indicating UV Spectroscopy Method for the Estimation of Deferiprone in Pharmaceutical Formulation

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ABSTRACT

The study describes the simple, sensitive, accurate, rapid and reliable ultra violet spectroscopic method has been developed for determination of Deferiprone in bulk drug and pharmaceutical formulation. Deferiprone is use as second line agent for thalassemia when iron overload from blood transfusion occurs.in order to investigate the stability of drug, a stress testing of drug sample by exposing it to variety of force degradation conditions has been recommended. Deferiprone was subjected to stress degradation under different condition recommended by international conference on harmonization (ICH). Deferiprone shows maximum absorbance at 279nm & calibration graph linear in the concentration range 5-25 Mcg/ml with correlation co-efficient 0.9997.The higher percentage of recovery study indicates that there is no interference of excipients in the presence of formulation. The stability study indicates appreciable changes were observed by treating the drug with acidic hydrolysis, basic hydrolysis and oxidation. However, there is no appreciable changes were observed for thermal stress and photolytic degradation.

Keywords: Deferiprone, UV spectroscopy, Force degradation, Stability indicating method, Validation.

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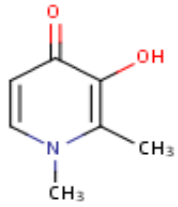
INTRODUCTION

Deferiprone is an oral iron chelator used as a second line agent in Thalassemia when iron overload from blood transfusion occurs. Deferiprone molecular formula$C_7H_9NO_2$and molecular weight is 139.15 g/mol.Deferiprone designated chemically as 3-hydroxy-1, 2-dimethyl-1,4-dihydropyridin4-one Literature review reveals that, Estimation of Deferiprone by UV & HPLC method is available .but there is no stability indicating method reported for assay determination of Deferiprone. Literature review reveals that estimation of Deferiprone by UV spectroscopy & HPLC method is available. But there is no stability indicating method reported for assay determination of Deferiprone. HPLC coupled with MS/MS detection, LC, terbium sensitized fluorescence method for determinations of Deferiprone in combination with Deferasirox have been reported. In view of these points an attempt was made to develop a simple, accurate & validated stability indicating UV method for estimation of Deferiprone in pharmaceutical dosage form.

Structure



MATERIALS AND METHOD

Chemicals and reagents

Deferiprone API was procured from the Symbolic pharma, suratand Deferiprone capsules are procured from the Cipla pharmaceutical ltd., India as a gift sample.

Instrumentation

- Double beam UV-visible spectrophotometer (Shimadzu UV-1800)
- Analytical weighing balance (wenstar)

Preparattion of Solution

Preparation of stock solution:

Accurately weighed quantity of Deferiprone 100mg was transferred to 100 ml volumetric flask and volume made up to the mark with distilled water to give a stock solution having strength of 1000 µg/ml.

Preparation of standard solution

From the stock solution of 1000 µg/ml pipette out 10 ml in 100 ml volumetric flask and volume made up to the mark with a distilled water to give a standard solution of 100 µg/ml.

Preparation of calibration curve

Calibration curve for Deferiprone consist of different concentration of standard solution ranging from 5-25 µg/ml. the solution were prepared by pipetting out 0.5,1,1.5,2,and 2.5 standard solution of Deferiprone in 10 ml volumetric flask and the volume was adjusted to mark with distilled water. Absorbance of each solution was measured at 279nm against distilled water as a blank and calibration curve was plotted.

Force Degradation Study

Solution stability

The assay result during 0, 3, 6 and 12 hour time period were within 96.15-99.30% of the initial value & no peak for degradation product was observed in any of the chromatogram. The standard solution is therefore stable for at least 1 day under normal laboratory conditions. The results of analysis of solution stability are as shown in table.

Acid hydrolysis

Take 10 ml from the standard stock solution of 1000 µg/ml, and transferred it into 100 ml volumetric flask. Than made up the volume with the 0.1N/1 N HCl up to the mark. And it refluxed for 8hr at 80 ^{O}c on water bath. The acidic degradation performed under dark condition to exclude the possible photolytic degradation. The degradation sample was cooled at room temperature and neutralized the sample with same strength of NaOH. Suitable aliquots of resultant degradation samples were pipette out and made up the volume with the distilled water.

Basic hydrolysis

Take 10 ml from the standard stock solution of 1000 µg/ml, and transferred it into 100 ml volumetric flask. Than made up the volume with the 0.1/1N NaOH up to the mark. And it refluxed for 8hr at 80 ^{O}c on water bath. The acidic degradation performed under dark condition to exclude the possible photolytic degradation. The degradation sample was cooled at room temperature and neutralized the sample with same strength of HCl. Suitable aliquots of resultant degradation samples were pipette out and made up the volume with the distilled water.

Oxidative condition

Take 10 ml from the standard stock solution of 1000 µg/ml, and transferred it into 100 ml volumetric flask. Than made up the volume with the 3/6 % H2O2 up to the mark. And it refluxed for 8hr at 80 ^{O}c on water bath. The acidic degradation performed under dark condition to exclude the possible photolytic degradation. The degradation sample was cooled at room temperature.

Suitable aliquots of resultant degradation samples were pipette out and made up the volume with the distilled water.

Thermal condition

For dry heat degradation the sample were placed in the oven at 80^{O}c for 24hrs under the dark condition and then cooled at room temperature. Degradation sample were subjected to analysis after its dilution with the distilled water.

Photolytic condition

For photolytic degradation the sample were placed in the sunlight for 24hrs. Degradation sample were subjected to analysis after its dilution with distilled water.

Method for Validation

Parameters to be considered for the validation of method are,

Linearity and Range

The linearity response was determine by analysing 5 independent concentration levels of calibration curve in range of 5-25µg/ml for Deferiprone .the calibration curve of absorbance vs. respective concentration was plotted and correlation coefficient and regression line equation for Deferiprone were calculated.

Accuracy

Accuracy may often be expressed as % recovery by the assay of known, added amount of analyte. It measure of the exactness of the analytical method. The recovery experiments were carried out in triplicate by adding previously analysed samples of the Deferiprone with three different concentration of standard at 80%, 100%, and 120% respectively. Absorbance of solution was measured at 279 nm. The amount of Deferiprone was calculated at each level and % recoveries were computed.

Precision

I. Repeatability: aliquots of 1.8, 2, and 2.2 ml of working standard solution of DEF (100µg/ml) were transferred to a series of 10ml volumetric flask. The volume was adjusted up to mark with distilled water. The absorbance of above solution was measured three times and % RSD was calculated.

II. Intraday precision: solutions containing 10, 15, and 20 µg/ml of Deferiprone was prepared & analysed 3 times on the same day and % RSD was calculated.

III. Interday precision: solution containing 10,15, and 20 µg/ml of Deferiprone was prepared analysed 3 times on 3 different days and %RSD was calculated.

Robustness

Solution containing 10, 15 and 20 µg/ml of Deferiprone were prepared & analysed at 3 different wavelengths (277,279& 281) and %RSD was calculated.

Limit of detection and limit of quantitation

LOD and the LOQ of the drug were calculated using the following equations as per ICH guidelines.

LOD=3.3 x (SD/slope)

LOQ= 10 x (SD/slope)

Where, SD= the standard deviation of intercept of calibration curves.

Slope= the mean slope of the calibration curve

Assay

Powder of twenty capsules was weighed. The quantity of the powder equivalent to 500mg of Deferiprone was dissolved in 50ml distilled water in volumetric flask. 1ml from above solution was taken in to a 100ml volumetric flask and the volume was adjusted up to mark with distilled water. Further 5ml from this solution was taken and again diluted up to 100 ml with distilled water in volumetric flask the resulting solution was filtered through 0.45nm membrane filter. The absorbance of resulting solution was measured at 279 nm.

RESULTS AND DISCUSSION

Deferiprone is give maximum absorbance at 279nm.

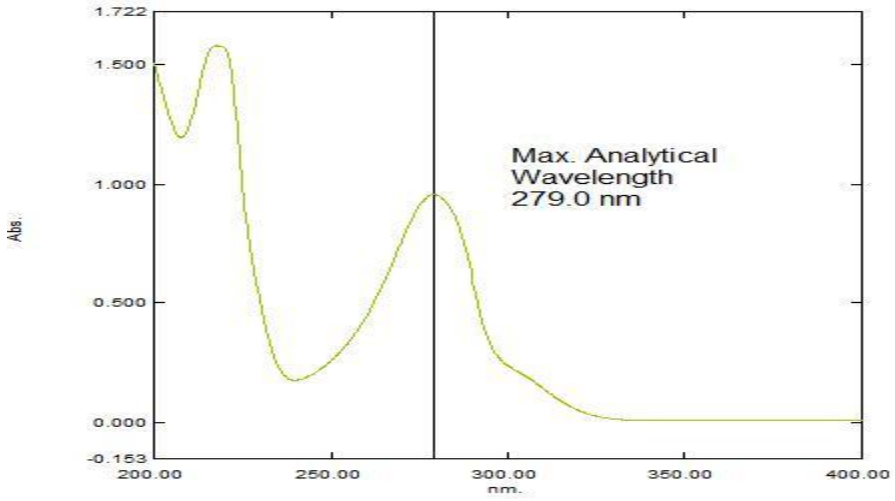


Figure 1: Spectra of Deferiprone Showing λmax

Force Degradation Study

Solution stability

The results of analysis of solution stability are as shown in table.

Time (hr.)	% Assay
0	99.87
3	98.53
6	97.76
12	96.66

Acid hydrolysis

UV spectra of Deferiprone for acidic degradation with 0.1 N HCl is shown in the figure.

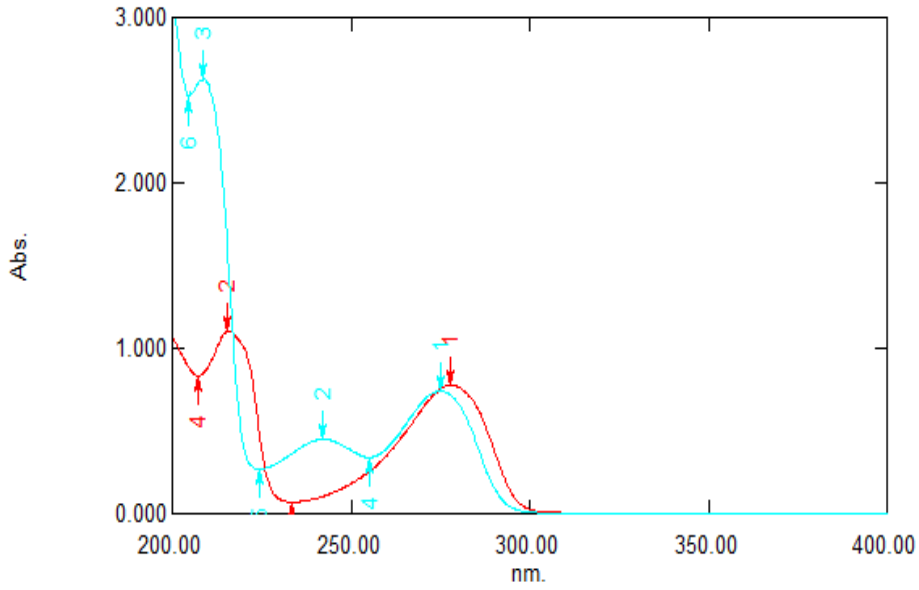


Figure 2: UV Spectra of Acid Hydrolysis by 0.1 N HCl

UV spectra of Deferiprone for acidic degradation with 1 N HCl is shown in the figure.

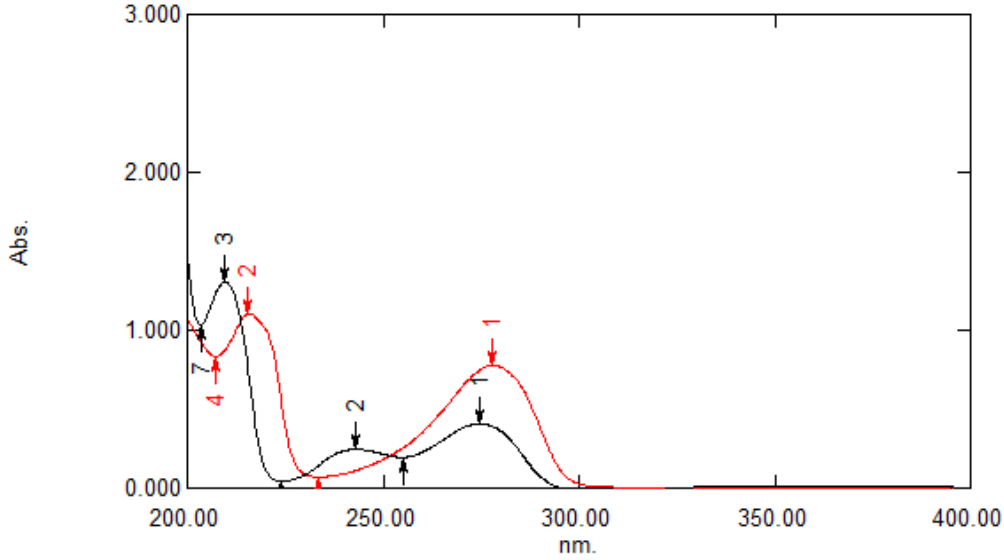


Figure 3: UV Spectra of Acid Hydrolysis by 1N HCl

Basic hydrolysis

UV spectra of Deferiprone for basic degradation with 0.1 N NaOH is shown in the figure.

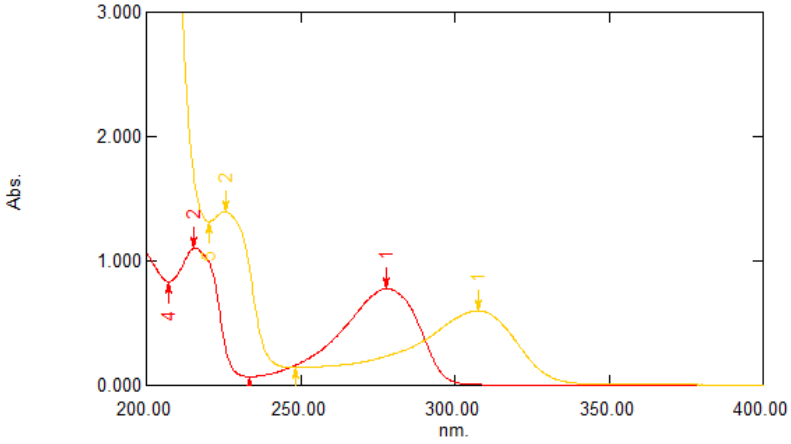


Figure 4: UV Spectra of Base Hydrolysis by 0.1N NaOH

UV spectra of Deferiprone for basic degradation with 1 N NaOH is shown in the figure.

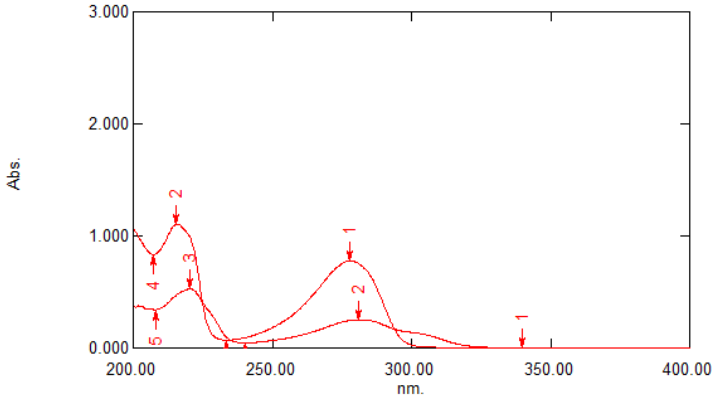


Figure 5: UV Spectra of Base Hydrolysis by 1N NaOH

Oxidative degradation

UV spectra of Deferiprone for oxidative degradation with 3% H2O2 is shown in the figure.

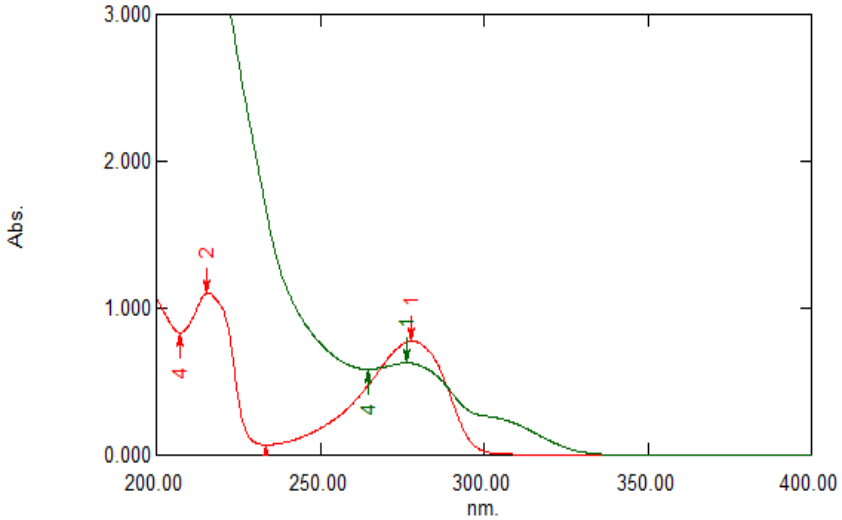


Figure 6: UV Spectra of Oxidative Degradation by 3% H2O2

UV spectra of Deferiprone for oxidative degradation with 6% H2O2 is shown in the figure.

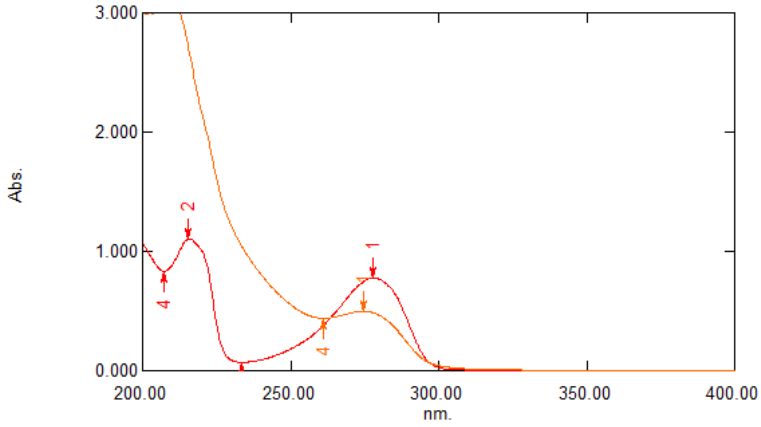


Figure 7: UV Specra of Oxidative Degrdation by 6% H2O2

Thermal degradation

A UV spectrum of Deferiprone for Thermal degradation is shown in the figure.

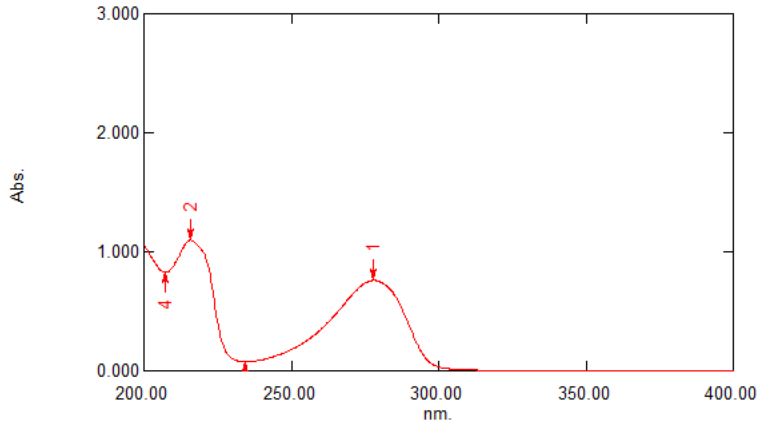


Figure 8: UV Spectra of Thermal Degradation

Photolytic degradation

A UV spectrum of Deferiprone for photolytic degradation is shown in the figure.

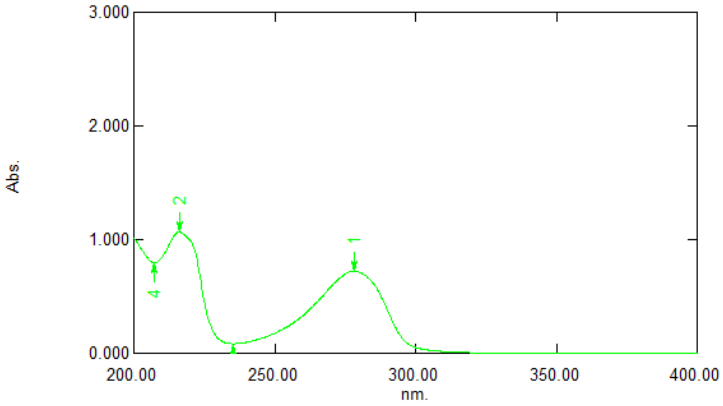


Figure 9: UV Spectra of Photolytic Degradation

Stability of the Drug Under Stress Condition

Table 1: Stress Study of Deferiprone

Sr no.	Parameter	Absorbance at 279nm	Percentage drug estimated
1	Normal	0.778	100
2	0.1N HCl	0.740	95.11
3	1N HCl	0.373	31.34
4	0.1N NaOH	0.600	76.14
5	1N NaOH	0.252	32.39
6	3% H2O2	0.627	80.59
7	6% H2O2	0.498	64.01
8	Thermal	0.760	97.68
9	Photolytic	0.722	91.62

Method Validation

Linearity and range

The linearity range for Deferiprone was found to be in the range of 5-25µg/ml. linearity data for Deferiprone at 279 nm are depicted in table 1.

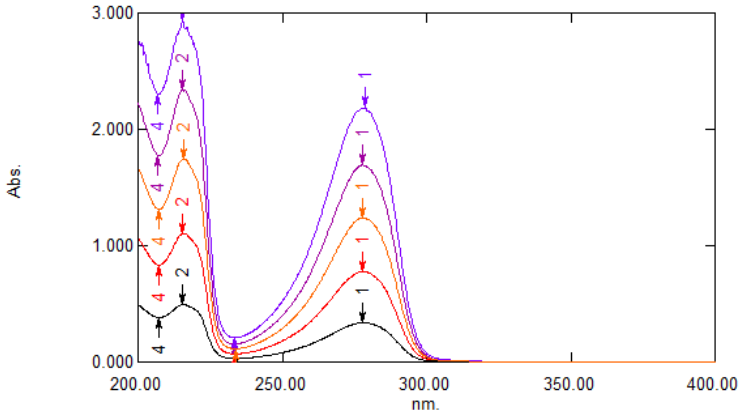


Figure 10: Overlay Spectra of Deferiprone

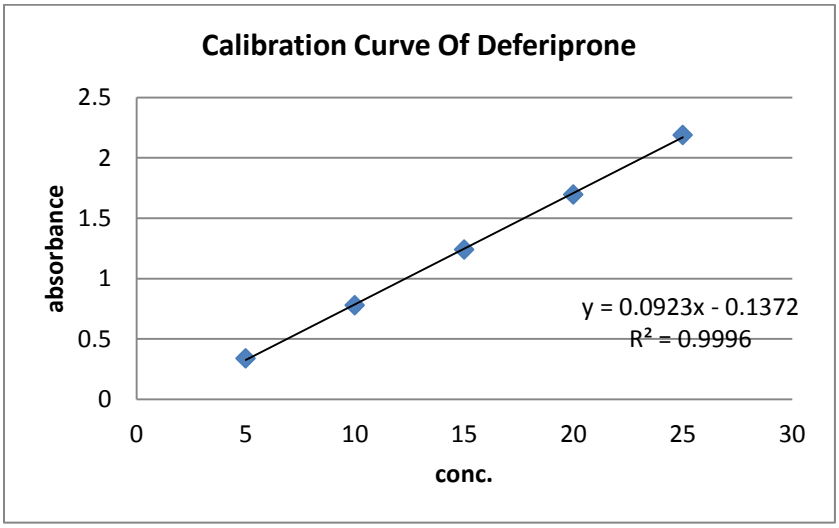


Figure 12: Calibration Curve of Deferiprone

Table 2: Linearity of Deferiprone

CONC. (µG/ml)	Absorbance at 279nm Mean ± Std. Deviation	%RSD
5	0.338 ± 0.000471	0.139331
10	0.783 ± 0.005715	0.729946
15	1.242 ± 0.002055	0.165399
20	1.694 ± 0.000816	0.048199
25	2.187 ± 0.000816	0.003733

Accuracy

Percentage recovery for Deferiprone was 99.77-100.37%.

Table 3: Recovery Data for Deferiprone

% Level	Amount of Deferiprone in sample (µg/ml)	Amount of standard Deferiprone added(µg/ml)	Absorbance	Amount of DEF found (µg/ml)	% recovery	Mean± S.D.
80	1.0	0.8	1.526	18.00	100	99.77± 0.8
	1.0	0.8	1.537	18.12	100.66	
	1.0	0.8	1.504	18.00	98.66	
100	1.0	1.0	1.711	20.00	100	100.2± 0.5
	1.0	1.0	1.706	19.93	99.65	
	1.0	1.0	1.728	20.19	100.95	
120	1.0	1.2	1.924	22.07	100.35	100.37± 0.4
	1.0	1.2	1.909	22.15	100.68	
	1.0	1.2	1.891	21.95	99.77	

Precision

Repeatability

The data for repeatability for Deferiprone 10µg/ml at 279nm is shown in the table 4.

Table 4: Repeatability of Deferiprone

Sr no.	Absorbance
1	0.778
2	0.779
3	0.767
4	0.759
5	0.772
6	0.769
Mean	0.770667
SD	0.006799
RSD	0.88222

Intraday precision

The data for Intraday precision for Deferiprone at 279nm is shown in the table.

Table 5: Intraday Precision Data for Deferiprone

Concentration (µG/ML)	Absorbance at 279nm Mean ± S.D. (n=3)	%RSD
10	0.768 ± 0.007789	1.0141
15	1.243 ± 0.020548	1.6526
20	1.556 ± 0.028674	1.8420

Interday precision

The data for Intraday precision for Deferiprone at 279nm is shown in the table.

Table 6: Interday Precision Data for Deferiprone

Concentration (µG/ML)	Absorbance at 279nm Mean ± S.D. (n=3)	%RSD
10	0.7676 ± 0.0066	0.8597
15	1.2366 ± 0.0205	1.6615
20	1.5500 ± 0.0216	1.3937

Assay

Table 7: Assay of Dosage Form

Brand	Label claim (mg)	Recovered conc.	%Mean recovery ± SD (n=3)
KELFER	Deferiprone 500	5.012	100.40 ± 0.49

Summary of Validation Parameter

Table 8: Summary of Validation

Parameter	Deferiprone
Linearity	5-25 µg/ml
Correlation co-efficient	0.997
Slope	0.0922
Intercept	0.1337
Limit of detection	0.1123
Limit of quantitation	0.3404
Repeatability	0.8822
Interday	0.85-1.66
Intraday	1.01-1.84
Accuracy	99.77-100.37

The validation parameters were studied at 279 nm wavelengths for the method. Accuracy and reproducibility was determined by calculating the recovery that was close to 100%.

CONCLUSION

The proposed method is simple, precise, accurate and reproducible. Due to high sensitivity and simple sample preparation, the method can be used for routine analysis. Results of analysis were validated as per the ICH guidelines. Stability study include effect of temperature, oxidation, photolysis and susceptible to hydrolysis across wide range of pH.

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