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# A VALIDATED UV SPECTROSCOPIC ASSAY METHOD DEVELOPMENT FOR THE ESTIMATION OF DEFERIPRONE IN BULK AND ITS FORMULATION

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## ABSTRACT

The objective of work was to develop and validate a UV spectrophotometric method for deferiprone in bulk and its dosage form. The solvent and wavelength of detection were optimized in order to maximize sensitivity of proposed method. The method was validated for different parameters like linearity, precision, specificity, accuracy, limit of detection (LOD), limit of quantitation (LOQ) and robustness as per ICH guidelines (Q2). A wavelength maximum absorption of Deferiprone in 50% v/v ethanol was monitored at 278nm. The method was found to be linear in the range of 2 to 12µg/ml with a correlation coefficient ($R^2$) of 0.999. The accuracy of the method was studied by recovery study and % recovery was found to be 101.07%. The LOD and LOQ were found to be 0.1808µg/ml and 0.547µg/ml respectively. The method is simple, accurate and requires relatively inexpensive instrument. The method was used successfully for determination of Deferiprone in bulk and its pharmaceutical dosage form.

**KEYWORDS:** Deferiprone, Spectrophotometric method, ICH Guidelines, validation.

## INTRODUCTION

Deferiprone is an oral iron chelator used as a second line agent in the treatment of Thalassemia [1] when iron overload from blood transfusionoccurs.[2-6] Deferiprone has higher binding affinity for iron than other metals such as copper, aluminium, and zinc.[7-9] Thalassemia is a type of hereditary anaemia due a defect in the production of haemoglobin. As a result, erythropoiesis, the production of new red blood cells, is impaired. It is chemically 3-hydroxy-1, 2-dimethyl-1, 4-dihydropyridin4-one.[10] The chemical structure of deferiprone was shown in Figure 1. From the thorough literature survey very few analytical methods have been reported for the determination of Deferiprone in pure drug and pharmaceutical dosage forms using UV Spectroscopy.[8-10] Hence an attempt was made to develop and validate simple, rapid and reliable analytical method to quantify Deferiprone in bulk and its formulation. The developed method to be validated in accordance to ICH Q2 (R1) guidelines.[11]

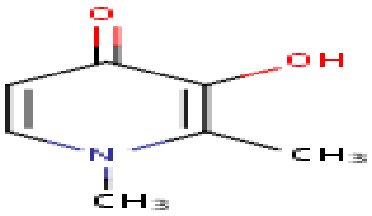


**Fig.1 Chemical structure of Deferiprone**

## MATERIALS AND METHOD

**Chemicals and Reagents:** An analytically pure sample of Deferiprone was procured as gift sample from MSN laboratories (Hyderabad, India). Capsule formulation [Kelfer], Cipla Formulation Pvt. Ltd. India] was purchased from a local pharmacy with labelled amount250mg. Analytical reagent grade ethanol was purchased from SD Fine Chem. Pvt. Ltd and distilled water was used as diluent for further preparations of the drug.

**Instruments Used:** For the current study UV/VIS double beam spectrophotometer, Shimadzu 1800 incorporated with UV probe as chemstation was used for the sample data analysis and was scanned using 1 cm matched quartz cell.

### Method Development

**Selection of solvent:** The solvent was selected by determining thesolubility of Deferiprone in various solventsnamely distilled water, 0.05M Hydrochloric Acid, 0.05M Sodium Hydroxide

Solution, Methanol, Ethanol. Finally, Ethanol 50%v/v was chosen as the solventfor Deferiprone depending on absorption at itsanalytical wavelength.

**Preparation of stock solutions:** Deferiprone pure drug 10 mg was weighed and transferred to a 10 ml volumetric flask and was dissolved in 50% v/v ethanol. It was dissolved properly and diluted up to the mark with diluent to obtain final concentration of 1000µg/ml. From the stock solutionvarious aliquots were prepared using distilled water.

**Analysis of Marketed formulation (Sample Preparation):** Weight equivalent of 10 mg of Deferiprone capsule formulation was takenand transferred into 10 ml volumetric flask. The contents were dissolved with 50% v/v ethanol and sonicated for 5 min to enhance solubility of the drug and then finally made up to the volume. From thisaliquot of 6µg/ml-1 was prepared and used.

**Validation of developed method**

**Linearity and Range:** From the standard stock solution six aliquots of drug solutions were prepared to obtaindynamic linearity range between 2-12µg/ml, obeys Beer's law. Standard calibration curve was plotted from the absorbance values obtained for the six aliquots of drug solutions by taking concentration (µg/ml) on x-axis and absorbance values on y-axis.Calibration curve was shown in Figure 4. The linear regression equation was found to be Y= 0.0745x+0.0815 and $R^2$ as0.999. The results were tabulated intable 1.

**Accuracy:** Accuracy is determined as the closeness of the true value of analyte concentration. To determine the accuracy of the proposed method the recovery studies were carried out at different levels (50%, 100% and 150%). The procedure was repeated for three times. The mean percent recovery was calculated and shown in table 2.

**Precision:** It is an analytical procedure expressed as repeatability of set of results under the prescribed conditions. The precision studies were carried for both repeatability and intermediate precision (interday) and %RSD was calculated. Intraday studies were carried for all the samples and absorbance (n=6) was recorded. Inter-day studies were carried for repeated days and absorbance (n=6) was recorded. The %RSD for both interday and intraday was found to be lessthan 2.

**Limit of Detection and Quantification (LOD& LOQ):** The LOD and LOQ were calculated from the slope of regression equation obtained from calibration curve and standard deviation was taken from precision studies. The result obtained was 0.18 and 0.547µg/ml respectively.

**Robustness:** The evaluation of robustness is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.By using working standard solutions of Deferiprone the Robustness was performed at altered wave length of ± 2 nm. The %RSD was calculated and found to be less than 2 and hence method was robust.

## RESULTS AND DISCUSSION

The results obtained shows the method was novel, simple, economic and accurate spectrophotometric method for the effective quantitative determination of Deferiprone as an active pharmaceutical ingredient as well as in pharmaceutical preparations without the interferences of other constituent in the formulations. The maximum absorption for the drug was shown in figure 2 and overlay spectra for respective concentrations is shown in figure 3. The developed method was validated in accordance with ICH Q2(R1) guidelines and overall summary of validation parameters were tabulatedin Table 3.

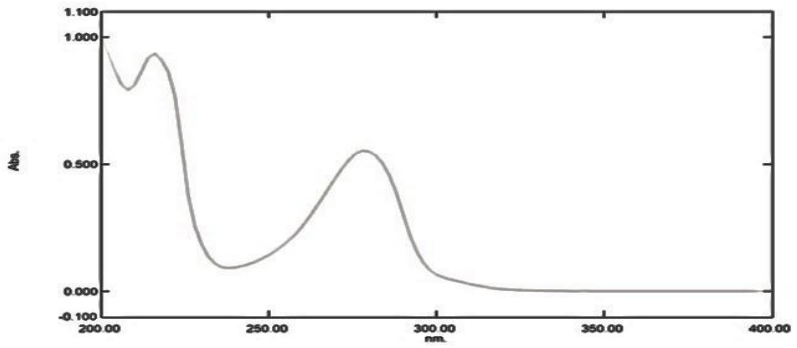


**Fig. 2: UV Spectra of Deferiprone.**

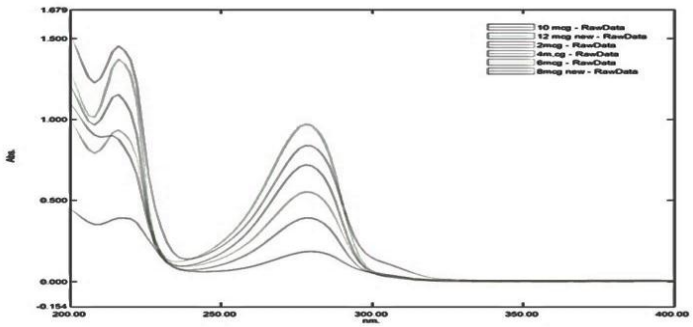


**Fig. 3: Overlay UV Spectra of Deferiprone.**

**Table. 1: Results of calibration curve for Deferiprone.**

| S. No | Concentration (µg/ml) | Absorbance ± Std Dev |
|---|---|---|
| 1 | 2 | 0.217±0.002 |
| 2 | 4 | 0.393±0.012 |
| 3 | 6 | 0.534±0.015 |
| 4 | 8 | 0.678±0.010 |
| 5 | 10 | 0.824±0.009 |
| 6 | 12 | 0.973±0.004 |

Regression value must be not more than 0.999. Llinearity of Deferiprone within 2-12µg/ml with regression value of 0.999.

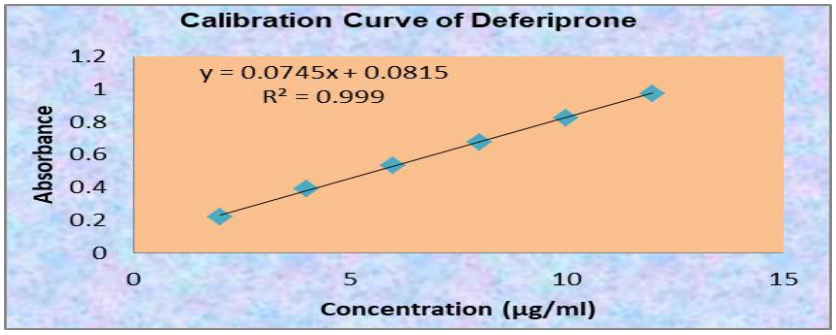


**Fig. 4 Linearity curve for Deferiprone.**

**Table. 2: Determination of Accuracy results for Deferiprone.**

| S. No | Spike Level | Absorbance (n=3) | Amount Added µg/ml | Amount Found µg/ml | % Recovery |
|---|---|---|---|---|---|
| **1** | 50 % | 0.279 | 1.48105 | 1.5 | 101.35 |
| **2** | 100 % | 0.554 | 5.92417 | 5.98 | 101.035 |
| **3** | 150 % | 0.805 | 13.3294 | 13.11 | 100.846 |

The mean % recovery was found to be 101.07%.

**Table 3: The total Summary of Optical characteristics and Other Parameters.**

| S No. | Parameters | Results |
|---|---|---|
| 1. | Beer's-Lambert's range (µg/ml) | 2-12 |
| 2. | Regression equation (y) | Y = 0.0745x - 0.0815 |
| 3. | Slope (b) | 0.0745 |
| 4. | Intercept (a) | 0.0815 |
| 5. | Correlation coefficient ($r^2$) | 0.999 |
| 6. | Intraday precision (% RSD) | 0.24 |
| 7. | Interday precision (% RSD) | 0.56 |
| 8. | Accuracy (% mean recovery) | 101.07 |
| 9. | Limit of detection (µg /ml) | 0.18083 |
| 10. | Limit of quantification (µg / ml) | 0.547 |
| 11. | Assay of tablets (%Purity) | 100.083 |

Y = bx + a where x is the concentration of Deferiprone in mcg / ml and Y is the absorbance at the respective $\lambda_{max}$.

## CONCLUSION

UV spectrophotometric method has been developed and validated according to ICH guidelines. The proposed study describes a novel UV spectrophotometric method for the estimation of Deferiprone in bulk and pharmaceutical dosage form using suitable diluent. The present UV spectrophotometric method was found to be simple, sensitive, accurate, precise, reproducible and robust relatively inexpensive. So, the developed method can be easily applied for the routine Quality Control analysis of Deferiprone in pharmaceutical preparations.

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