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METHOD DEVELOPMENT AND VALIDATION FOR THE ESTIMATIN OF DEFRIPRONE USING RP HPLC IN BULK AND PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT
A simple and selective LC method is described for the determination of Deferiprone dosage forms. Chromatographic separation was achieved on a c_{18} column using mobile phase consisting of a mixture of Triethylamine (pH 3.5): ACN (50:40v/v), with detection of 280 nm. Linearity was observed in the range 125-375 µg /ml for Deferiprone (r^2 =0.994) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim. 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.
Key Words: Deferiprone, pharmaceutical dosage form, LC method

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INTRODUCTION
Chromatography equipment look rather intimidating to anyone who has not handled them before, but on a closer look and as you get familiar with the equipment you realize that behind the network of wires, complex plumbing and circuitry is a simple machine with only a few major parts. Different combinations of these parts namely pumps, detectors and injectors yield an infinite number of configurations based on the application. Just like an understanding of human anatomy makes you conscious of the vital role of each and every body organ towards your well being and vitality. Similarly you need to have a good understanding of the parts of Your HPLC system to generate data of highest reliability. A conceptual understanding of the function of each component will add to your comfort level with your HPLC system. You will ensure long time usage with high reliance on output data. The present module is intended to serve this very purpose and in simple terms you will appreciate the role of each part and its contribution to overall system efficiency. HPLC is a technique for separation, identification and quantification of components in a mixture. It is especially suitable for compounds which are not easily volatalised, thermally unstable and have high molecular weights. The liquid phase is pumped at a constant rate to the column packed with the stationary phase. Before entering the column the analysis sample is injected into the carrier stream. On reaching the column the sample components are selectively retained on the basis of physico-chemical interactions

between the analyte molecules and the stationary phase. The mobile phase moving at a steady rate elutes the components based on the operating conditions. Detection techniques are employed for detection and quantification of the eluted components. We now introduce you to the significance and role of each component part of the HPLC system (1-4).

Deferiprone is an oral iron chelator used as a second line agent in thalassemia syndromes when iron overload from blood transfusions occurs. Thalassemias are a type of hereditary anaemia due a defect in the production of hemoglobin. As a result, erythropoiesis, the production of new red blood cells, is impaired. Deferiprone is an iron chelator that binds to ferric ions (iron III) and forms a 3:1 (deferiprone:iron) stable complex and is then eliminated in the urine. Deferiprone is more selective for iron in which other metals such as zinc, copper, and aluminum have a lower affinity for deferiprone. Deferiprone is absorbed in the upper gastrointestinal tract. Absorption is rapid with maximum plasma concentrations occurring after 1 hour in the fasted state and after 2 hours in the fed state. Deferiprone is mainly metabolized by UGT1A6 to the 3-O-glucuronide metabolite. This metabolite cannot chelate iron. Within 5-6 hours of administration, more than 90% of deferiprone is eliminated from the plasma. 75 to 90% of deferiprone is excreted in the urine as the metabolite. Deferiprone is indicated in thalassemia syndromes when first line chelation agents are not adequate to treat transfusional iron overload (5).

Method development involves considerable trial and error procedures. The most difficult problem usually is where to start, what type of column is worth trying with what kind of mobile phase. Single dosage forms with combination of drugs are widely used today due to their advantages and their simultaneous estimation of individual component is a challenging task. Aim is to develop new RP HPLC method for the estimation of Deferiprone in pharmaceutical dosage form.

MATERIALS AND METHODS
Preparation of standard stock solution of Deferiprone (6-8)
10mg of Deferiprone was weighed and transferred in to 10ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol.

Preparation of standard solution
Weigh accurately 10 mg of Deferiprone in 10 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 250µg/ml of Deferiprone is prepared by diluting 2.5 ml of Deferiprone to 10ml with mobile phase. This solution is used for recording chromatogram.

Preparation of mixed standard solution
Weigh accurately 10 mg of Deferiprone in 10 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 250µg/ml of Deferiprone is prepared by diluting 2.5 ml of Deferiprone to 10ml with mobile phase. This solution is used for recording chromatogram (table-1).

Table-1 Optimized chromatographic conditions

Mobile phase	Triethylamine Buffer: ACN (50:50)
pH	3.5
Column	INERTSIL column,C18(150x4.6 ID) 5µm
Flow rate	1.0 ml/min
Column temperature	Room temperature(20-25ºC)
Sample temperature	Room temperature(20-25ºC)
Wavelength	280 nm
Injection volume	20 µl
Run time	6min
Retention time	About 4.9 min for Deferiprone

Preparation of samples for Assay
## Preparation of mixed standard solution

Weigh accurately 10 mg of Deferiprone in 10 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 250µg/ml of Deferiprone is prepared by diluting 2.5 ml of Deferiprone to 10ml with mobile phase. This solution is used for recording chromatogram.

Preparation of sample solution

5 Capsules (each Capsules contains 250 mg of Deferiprone) were weighed and taken into a mortar and make it fine powder and uniformly mixed. Capsules stock solutions of 250µg/ml were prepared by dissolving weight equivalent to 10 mg of Deferiprone dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 10 ml with mobile phase. Further dilutions are prepared in 5 replicates of 250 µg/ml of Deferiprone was made by adding 2.5 ml of stock solution to 10 ml of mobile phase.

RESULTS AND DISCUSSION

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 resulting spectra are shown in the fig-1 and the absorption curve shows characteristic absorption maxima at 280 nm for Deferiprone.
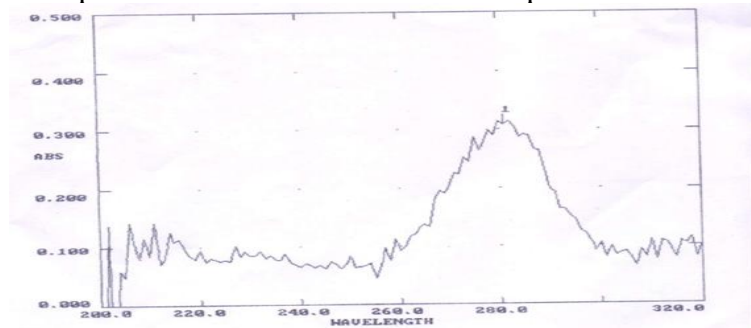
**Fig-1 UV-VIS spectrum of Deferiprone**

The amount of Defer prone present in the taken dosage form was found to be 99.08 %.

The correlation coefficient for linear curve obtained between concentrations vs. Area for standard preparations of Deferiprone is 0.994.

The relationship between the concentration of Deferiprone and area of Deferiprone is linear in the range examined since all points lie in a straight line and the correlation coefficient is well within limits (Table-2 and fig-2).

Table-2 linearity of Deferiprone

S.No.	Conc.(µg/ml)	Area
1	125	1904.438
2	187.5	2901.665
3	250	3680.717
4	312.5	4620.500
5	375	5220.440
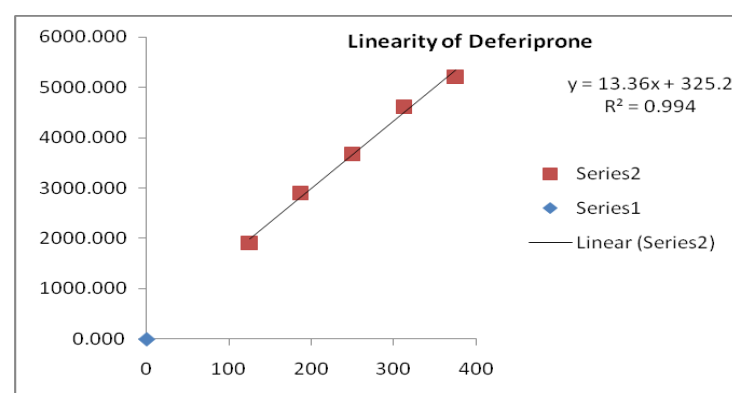
Fig-2 linearity graph of Deferiprone

The % recovery of Deferiprone should lie between 98% and 102%. Test results for Deferiprone are showing that the %RSD of Assay results are within limits. The results were shown in table-3.

Table-3 Results for Method precision of Deferiprone

Deferiprone		
S.No.	Rt	Area
1	4.987	3059.295
2	4.973	3091.558
3	4.950	3314.065
4	4.983	3293.678
5	4.987	3067.163
6	4.980	3315.153
avg	4.9767	3290.152
stdev	0.0141	23.203
%RSD	**0.28**	**0.70**

From the observation the %RSD between two analysts Assay values not greater than 2.0%, hence the method was rugged (Table-4).

Table-4 Results for Ruggedness

Deferiprone	%Assay
Analyst 01	98.98
Analyst 02	98.10
%RSD	0.63

CONCLUSION

The above experimental results and parameters it was concluded that, this newly developed method for the estimation of Deferiprone was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories, bio-pharmaceutical and bio-equivalence studies and in clinical pharmacokinetic studies in near future

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