CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

SJIF Impact Factor: 7.187
https://doi.org/10.5281/zenodo.7548262

Available online at: http://www.iajps.com *Research Article*

METHOD DEVELOPMENT AND VALIDATION FOR DEFERIPRONE BY RP-HPLC METHOD

A.Mounika, V.Kiran Kumar

Mother Teresa College of Pharmacy, Ghatkesar, NFC Nagar, Hyderabad, Telangana, India

Abstract:

Another investigative technique was set up for estimation of RP-HPLC strategy utilizing deferiprone cases. The chromatographic conditions were effectively created for the partition of Deferiprone by utilizing INERTSIL column, C18(150x4.6 ID) 5µm, stream rate 1.0ml/min, versatile stage proportion Triethylamine Buffer: ACN (50:50v/v), recognition frequency was 280nm. The maintenance time was seen as 4.9mins. The % virtue of deferiprone was seen as 99.08%. The framework appropriateness boundaries for deferiprone, for example, hypothetical plates and following elements were seen as 2567,1.512. The logical technique was approved according to ICH guidelines (ICH, Q2, (R1)). The linearity concentrate for Deferiprone was found in fixation scope of 125-375µg/ml and relationship coefficient were seen as 0.994. The % recuperation was seen as 98.40%. The %RSD was seen as 0.28. The accuracy study was precise, robust and repeatable. LOD esteem was 22.93µg/ml and LOQ esteem was 96.37µg/ml. As this strategy has shorter maintenance time and high goal, makes the technique more satisfactory and financially savvy and can be viably applied for routine examination in research establishments, quality control divisions in ventures and in clinical pharmacokinetic concentrates in not-so-distant future.

Keywords: *Deferiprone, RP-HPLC, Method development, Validation*

Corresponding author:

A.Mounika,
M. Pharmacy,
Department of pharmaceutical Analysis,
Mother Teresa College of Pharmacy, Ghatkesar, NFC Nagar
Hyderabad, Telangana, India
E-mail: mounikayadav249@gmail.com

QR code

Please cite this article in press A.Mounika et al, ***Method Development And Validation For Deferiprone By RP-HPLC Method..,*** *Indo Am. J. P. Sci, 2022; 09(12).*

INTRODUCTION:

Deferiprone is indicated in thalassemia syndromes when first line chelation agents are not adequate to treat transfusional iron overload. 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.[1-3] IUPAC name is 3-hydroxy-1,2-dimethyl-1,4-dihydropyridin-4-one. Molecular formula $C_7H_9NO_2$. Molecular Weight is 139.15. Deferiprone is highly soluble in water at pH 1-7.5 and has high permeability, thus is a BCS class 1 drug.

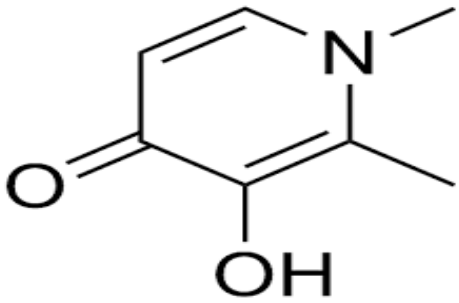

Figure 1: Structure of Deferiprone

The literature survey revealed that There are very few methods reported in the literature for analysis of Deferiprone alone or in combination with other drugs in the pure form and pharmaceuticals formulations by UV and RP-HPLC.[4-7] In view of the need for a suitable, cost-effective RP-HPLC method for routine analysis of Deferiprone estimation of in pharmaceutical dosage form. Attempts were made to develop simple, precise, accurate and cost-effective analytical method for the estimation of Deferiprone. The proposed method will be validated as per ICH guidelines. The objective of the proposed work is to develop a new, simple, sensitive, accurate and economical analytical method and validation for the estimation of Deferiprone in pharmaceutical dosage form by using RP-HPLC. To validate the developed method in accordance with ICH guidelines for the intended analytical application i.e., to apply the proposed method for analysis of the drug in its dosage form.

MATERIALS AND METHODS:

Chemicals and Reagents: Deferiprone were Purchased from market. NaH_2PO_4 was analytical grade supplied by Finerchem limited, Orthophosphoric acid (Merck), and Water and Methanol for HPLC (Lichrosolv (Merck).

Equipment and Chromatographic Conditions: The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, UV detector and Empower 2 software. Analysis was carried out at 280 nm with column INERTSIL column, C18(150x4.6 ID) 5µm, dimensions at Ambient temperature. The optimized mobile phase consists of Triethylamine Buffer: ACN (50:50v/v). Flow rate was maintained at 1 ml/min.

Preparation of solutions:
Preparation of Triethylamine buffer

5ml of triethylamine in 1000ml of water and its pH was maintained at by using orthophosphoric acid.

Mobile Phase

A mixture of 50 volumes of Triethylamine pH 3.5 & 50 volumes of Acetonitrile were prepared. The mobile phase was sonicated for 10min to remove gases.

Preparation of orthophosphoric acid

3ml orthophosphoric acid is diluted in 10ml water

Preparation of standard stock solution of Deferiprone

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 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.

METHOD:
The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.

System suitability parameters: To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 1.0 ml/min for 30 minutes to equilibrate the column at ambient temperature. Chromatographic separation was achieved by injecting a volume of 20 µL of standard into INERTSIL column, C18(150x4.6 ID) 5µm column, the mobile phase of composition Triethylamine Buffer: ACN (50:50v/v) was allowed to flow through the column at a flow rate of 1.0 ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in table 1.

Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine Deferiprone in tablet dosage form. The result obtained for was comparable with the corresponding labeled amounts and they were shown in Table-2.

Validation of Analytical method:

Linearity: The linearity study was performed for the concentration of 125 ppm to 375 ppm level. Each level was injected into chromatographic system. The area of each level was used for calculation of correlation coefficient. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The results are shown in table 3.

Accuracy studies: The accuracy was determined by help of recovery study. The recovery method carried out at three level 75%, 100%,125%. Inject the standard solutions into chromatographic system. Calculate the Amount found and Amount added for Deferiprone and calculate the individual recovery and mean recovery values. The results are shown in table 4.

Precision Studies: precision was calculated from Coefficient of variance for six replicate injections of the standard. The standard solution was injected for six times and measured the area for all six Injections in HPLC. The %RSD for the area of six replicate injections was found. The results are shown in table 5.

Ruggedness: To evaluate the intermediate precision of the method, Precision was performed on different day. The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found. The results are shown in table 6.

Robustness: As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition was made to evaluate the impact on the method. The results are shown in table 7.

LOD and LOQ: The sensitivity of RP-HPLC was determined from LOD and LOQ. Which were calculated from the calibration curve using the following equations as per ICH guidelines. The results are shown in table 8.

LOD = 3.3σ/S and
LOQ = 10 σ/S, where
σ= Standard deviation of y intercept of regression line,
S = Slope of the calibration curve

RESULTS AND DISCUSSION:

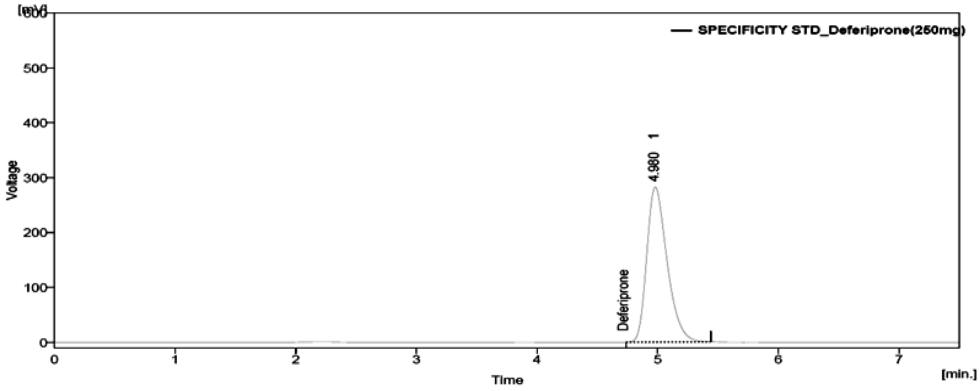

Figure 2: Standard chromatogram

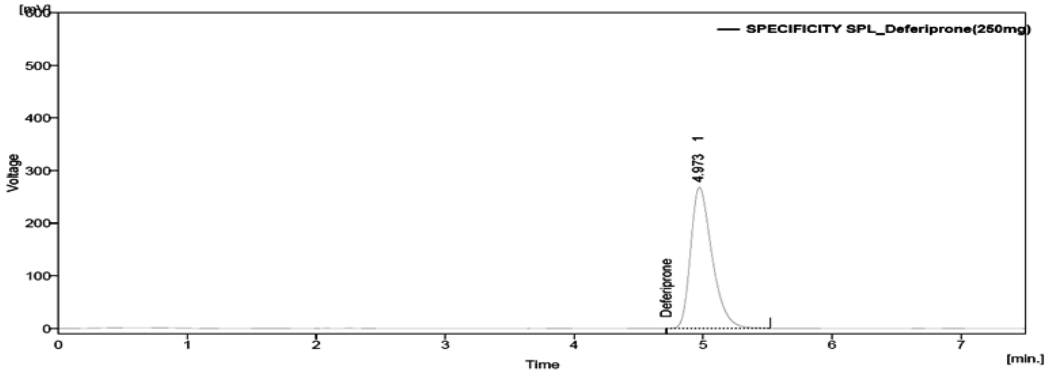

Figure 3: Sample chromatogram

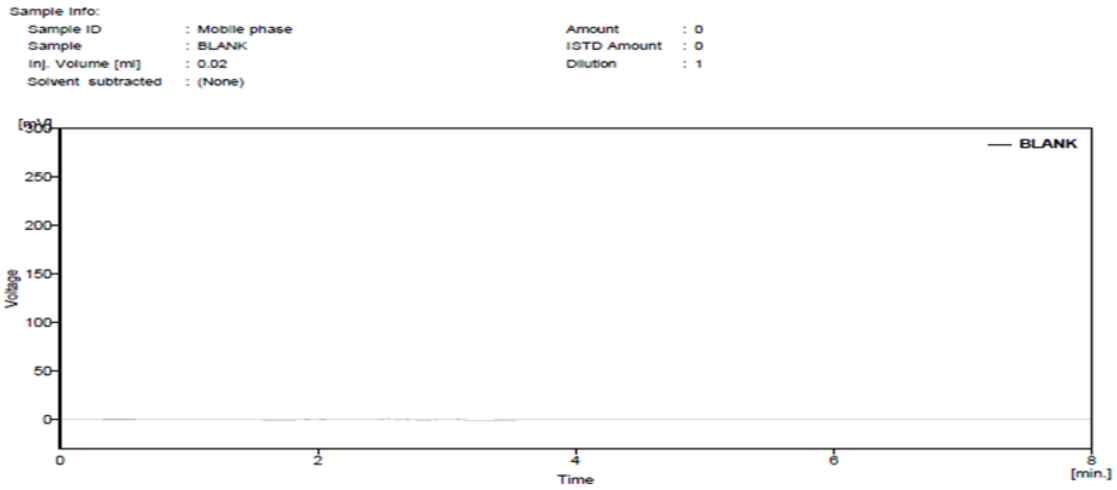

Figure 4: Blank chromatogram

Table 1: System suitability parameters

Injection	Retention time (min)	Peak area	Theoretical plates	Tailing factor
1	4.947	2576.974	2476	1.500
2	4.937	2535.582	2554	1.477
3	4.950	2549.337	2550	1.512
4	4.953	2538.795	2576	1.477
5	4.947	2544.742	2567	1.512
Mean	4.9767	2549.086	-	-
SD	0.01418	16.46919	-	-
%RSD	0.207261	0.646082	-	-

Table 2: Assay results for Deferiprone

	Label Claim (mg)	% Assay
Deferiprone	10	99.08

Table 3: Linearity results 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

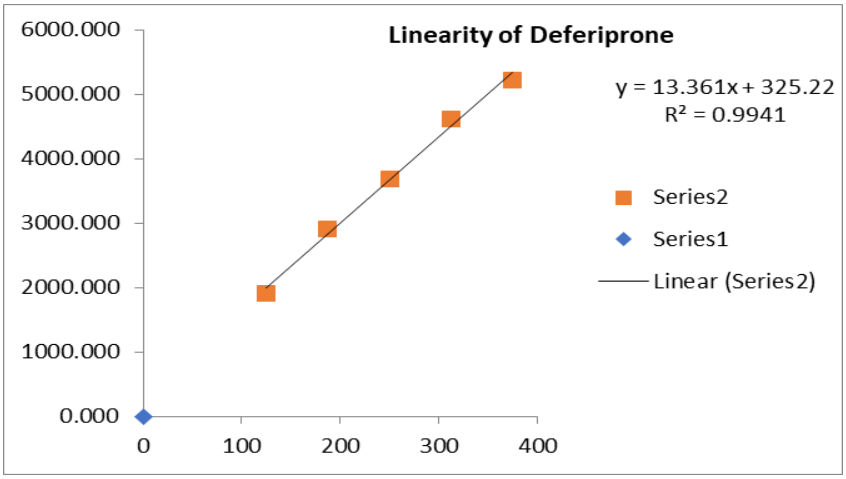

Figure 5: Linearity graph for Deferiprone

Table 4: Showing accuracy results for Deferiprone

Recovery level	Accuracy Deferiprone					Average % Recovery
	Amount taken(mcg/ml)	Area	Average area	Amount recovered(mcg/ml)	%Recovery	
75%	75	3404.393	3256.777	73.60	98.14	**98.40**
	75	3069.834				
	75	3296.104				
100%	100	3838.430	3483.758	98.98	98.98	
	100	3285.170				
	100	3327.673				
125%	125	4838.317	4760.862	122.63	98.0	
	125	4781.051				
	125	4663.219				

Table 5: Precision results for 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
St dev	0.0141	23.203
%RSD	**0.28**	**0.70**

Table 6. Ruggedness results of Deferiprone

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

Robustness results

Table 7: Flow variation results for Deferiprone

Parameter	Deferiprone	
	Retention time(min)	Tailing factor
Flow		
0.8ml/min	6.080	1.783
1.2ml/min	4.233	1.588
Wavelength		
278nm	4.973	1.641
282nm	4.963	1.641

Table 8: LOD, LOQ of Deferiprone

Drug	LOD	LOQ
Deferiprone	22.93	96.37

CONCLUSION:

The Developed HPLC method was validated and it was found to be simple, precise, accurate and sensitive for the estimation of Deferiprone in its pure form and in its pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of Deferiprone in pure and its pharmaceutical dosage forms.

REFERENCES:

1. Roberts DJ, Brunskill SJ, Doree C, Williams S, Howard J, Hyde CJ: Oral deferiprone for iron chelation in people with thalassaemia. Cochrane Database Syst Rev. 2007 Jul 18;(3):CD004839. [Article]

2. Victor Hoffbrand A: Deferiprone therapy for transfusional iron overload. Best Pract Res Clin Haematol. 2005 Jun;18(2):299-317. [Article]

3. FDA Approved Drug Products: Ferriprox (deferiprone) oral tablets [Link]

4. Hinesha Barot, Darshil Shah, Dr. DilipMaheshwari. Development of stability indicating UV Spectroscopy method development for the estimation of deferiprone in pharmaceutical formulation. Am. J. PharmTech Res, 5(1), 2015, 622-632.

5. Manzoor Ahmed, Vinod Kumar Jangade, Satishkumar Shetty A, Vijaya Krishna C. Aradhya, Anil Kumar S.M. Development and validation of first order derivative spectrophotometric method for the estimation of deferiprone in bulk and capsule dosage form.Int.J.of universal pharmacy and biosciences. 4(3), 2015, 169-176.

6. Manzoor Ahmed, Vinod Kumar Jangade*, Satishkumar Shetty A, Vijayakrishna C. Aradhya, Kuppast I.J and Anil kumar S.M. RP-HPLC method development and validation for estimation of deferiprone in capsule dosage form. World Journal of Pharmacy and Pharmaceutical Sciences. 4(3), 2015, 831-838.

7. Mateen Abbas, Rakhshanda Nawaz, Tahira Iqbal2, Muhammad Alim, Muhammad Rafique Asi. Quantitative determination of deferiprone in human plasma by reverse phase high performance liquid chromatography and its application to pharmacokinetic study. Pak. J. Pharm. Sci.25(2), 2012, 343-348.