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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR DEFERIPRONE IN BULK AND TABLET DOSAGE FORM BY USING RP-HPLC METHOD

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ABSTRACT

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Deferiprone was done by RP-HPLC. The chromatographic conditions were effectively created for the partition of Deferiprone by utilizing INERTSIL column, C18(150×4.6 ID). The detection was carried out using UV detector at 280nm.The solutions were chromatographic at a constant flow rate of 1.0ml/min. Linear regression coefficient was not more than 0.994. The accuracy study was precise, robust and repeatable. LOD esteem was 22.93 µg/ml. and LOQ esteem was 96.37 µg/ml. The results obtained on the validation parameters met ICH and USP requirements it inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

KEYWORDS: INERTSIL C18 column, Deferiprone, RP-HPLC.

INTRODUCTION

THALASSEMIA: Thalassemia are acquired blood issues described by anomalous hemoglobin creation. Indications rely upon the sort and can fluctuate from none to serious. Regularly there is gentle to extreme iron deficiency (low red blood cells). Anemia can bring about inclination drained and fair skin. There may likewise be bone issues, an amplified spleen, yellowish skin, dim pee, and among youngsters moderate growth. Thalassemia's are hereditary issues acquired from an individual's folks.

There are two principle types

- alpha thalassemia
- beta thalassemia.

The seriousness of alpha and beta thalassemia relies upon what number of the four qualities for alpha globin or two qualities for beta globin are absent. Determination is ordinarily by blood tests including a total blood check, exceptional haemoglobin tests, and hereditary tests. Determination may happen before birth through pre-birth testing.

DEFERIPRONE

Deferiprone is an oral iron chelator utilized as a second line operator in thalassemia conditions when iron over-burden from blood bondings happens. Thalassemia's are a kind of genetic sickliness due an imperfection in the creation of hemoglobin.

Accordingly, erythropoiesis, the creation of new red platelets, is debilitated. FDA endorsed on October 14, 2011.

Category: Chelating Agents, Iron Chelating Agents, Pyridines, Sequestering Agents.

Structure

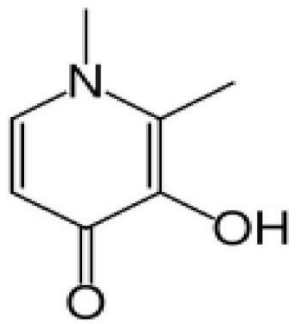

Fig. 1: Structure of Deferiprone.

IUPAC Name: 3-hydroxy-1,2-dimethyl-1,4-dihydropyridin-4-one

Chemical formula: $C_7H_9NO_2$ **Molecular weight :** 139.1519

Mechanism of action: Deferiprone is an iron chelator that ties to ferric particles (iron III) and structures a 3:1 (deferiprone:iron) stable complex and is then killed in the pee. Deferiprone is more specific for iron in which different metals, for example, zinc, copper, and aluminum have a lower fondness for deferiprone.

PLAN OF WORK

- Solubility assurance of DEFERIPRONE different solvents and cradles.
- Determine the assimilation maxima of both the medications in UV– Visible locale in various solvents/supports and choosing the solvents for HPLC strategy advancement.
- Optimize the versatile stage and stream rates for appropriate goal and maintenance times.
- Validate the created strategy according to ICH rules.

MATERIALS AND METHODS

- **Chemicals and Reagents:** Triethylamine buffer pH 3.5 : ACN

Instruments: UV: Nicolet evolution 100, HPLC: Shimadzu (LC 20 AT VP), Electronic Balance **(Shimadzu),** Ultra Sonicator **(Citizen),** and P^H Analyzer **(Global digital),** Distillation unit **(BOROSIL),** Vaccum filtration unit **(BOROSIL).**

Preparation of Triethylamine buffer: 5ml of tri ethylamine in 1000ml of water and its pH was maintained at by using ortho phosphoric acid.

Preparation of orthophosphoric acid: 3ml ortho phosphoric acid is diluted in 10ml water.

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.

Assay

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 DISCUSSIONS

The current examination detailed in the theory was intended to build up another technique improvement and approval for estimation of deferiprone by RP-HPLC strategy. Writing uncovers that there are no logical strategies announced for the estimation deferiprone by RP-HPLC strategy. 4.1

METHOD DEVELOPMENT

The detection wavelength was selected by dissolving the drug in mobile phase. The resulting solution was scanned in U.V range from 200-400nm.

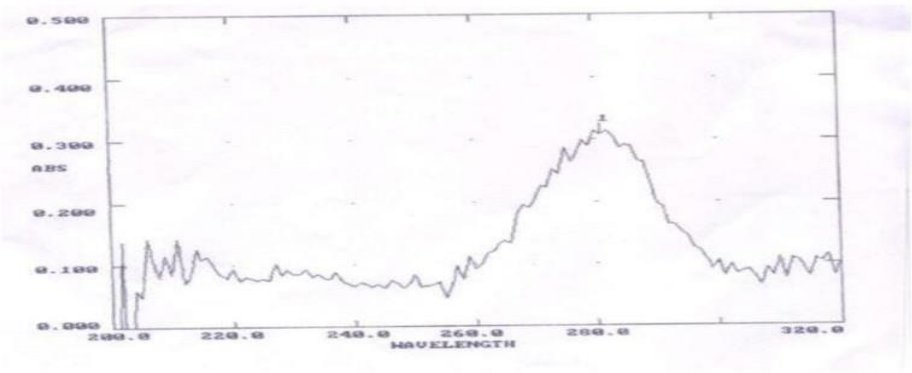

Fig. No. 2: Spectrum showing λmax of the deferiprone.

Observation: λmax was found to be 280nm for deferiprone shown in the above figure.

Table 1: Optimized chromatographic conditions.

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

Assay-1

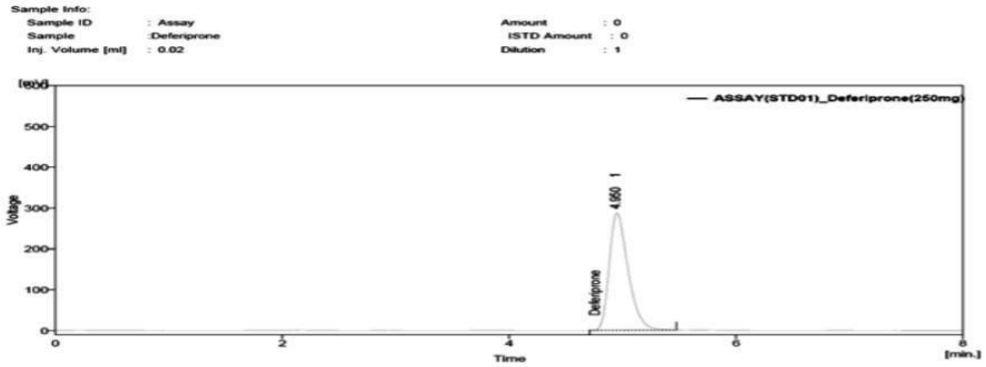

Fig.No.4.7 Chromatogram of Assay standard preparation-1

Fig. 3: Assay std preparation.

DEFERIPRONE

Table No. 2: Assay Results.

	STANDARD AREA	SAMPLE AREA
INJECTION POINT-1	3323.905	3315.153
INJECTION POINT-2	3320.771	2958.634
INJECTION POINT-3	3293.678	3099.478
INJECTION POINT-4	3274.549	3304.543
INJECTIION POINT-5	3193.689	3067.163
AVERAGE AREA	3281.318	3148.994
ASSAY(%PURITY)	**99.8**	**99.8**

OBSERVATION

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

VALIDATION REPORT

SPECIFICITY

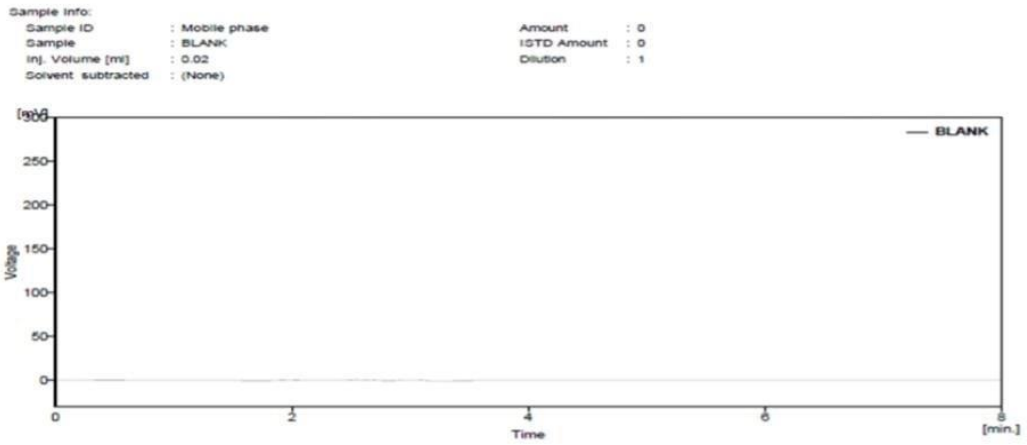

Fig. No. 4: Blank chromatogram for specificity.

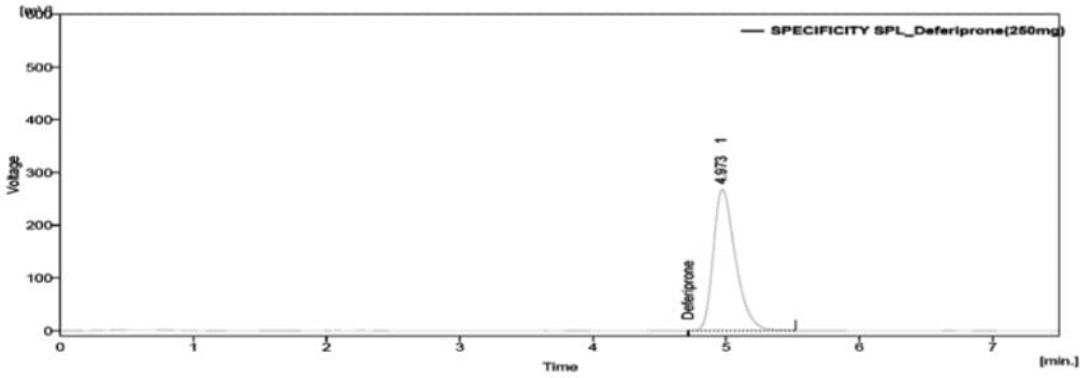

Fig. No. 5: Chromatogram for specificity of Deferiprone sample.

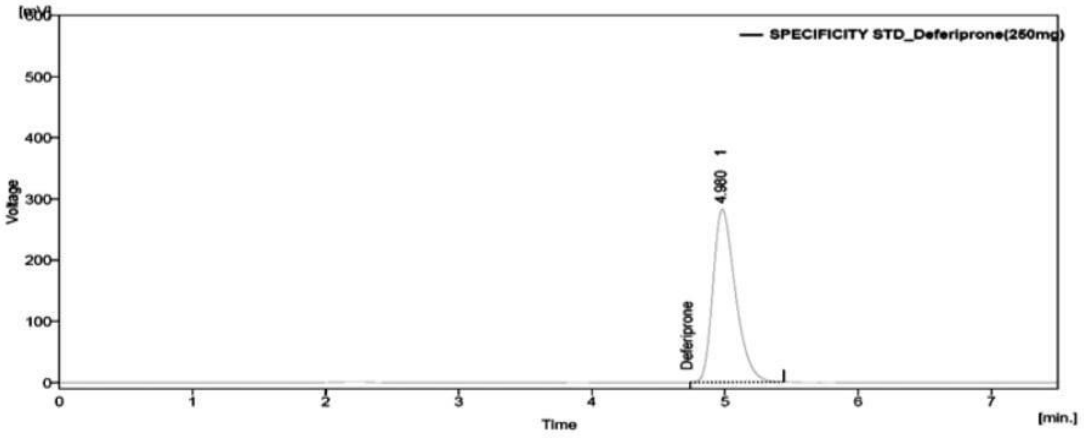

Fig. No. 6: Deferiprone chromatogram for specificity of standard.

Observation

- It is observed from the above data, diluents or excipient peaks are not interfering with the Deferiprone peaks.

LINEARITY

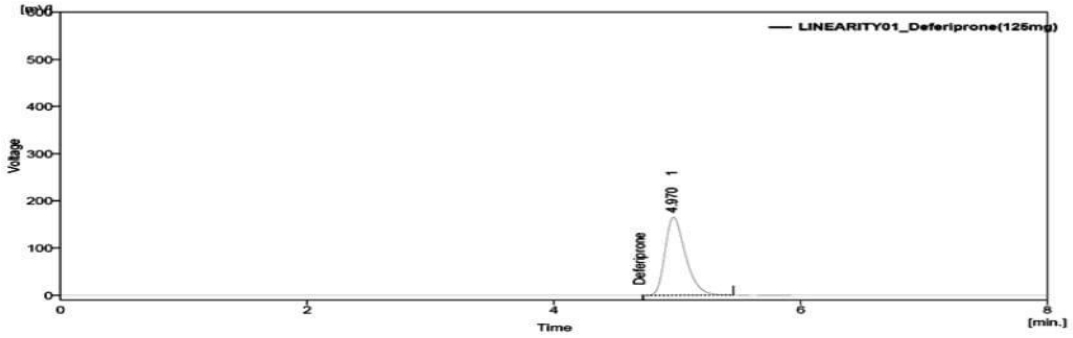

Fig. No. 7: chromatogram of Deferiprone preparation-1.

Table 3: 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

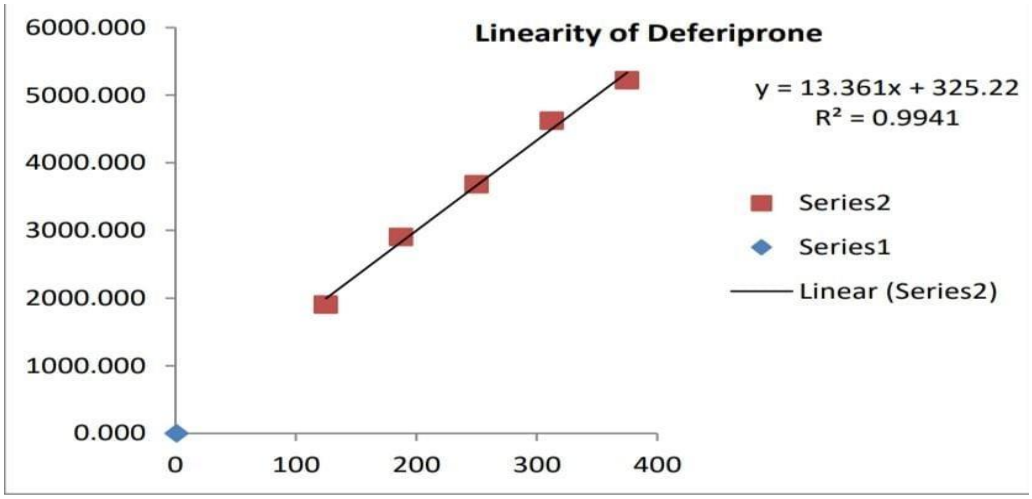

Fig. No. 8: Linearity of deferiprone.

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

Accuracy

Accuracy of the strategy was dictated by Recovery contemplates. To the detailing (pre broke down example), the reference norms of the medications were included at the degree of 75%, 100%, 125%. The recuperation considers were completed multiple times and the rate recuperation and rate mean recuperation were determined for tranquilize is appeared in table.

To check the exactness of the technique, recuperation examines were done by expansion of standard medication answer for pre-broke down example arrangement at three distinct levels 75%, 100% and 125%.Accuracy-75%

Table 4: Recovery 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				

Observation: The percentage mean recovery of Deferiprone is 98.40%.

Precision

Method precision

Table 5: 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	3067.163
Avg	4.9767 3	3290.152
stdev	0.0141	23.203
%RSD	**0.28**	**0.70**

Observation: Test results for Deferiprone are showing that the %RSD of Assay results are within limits.

Robustness

Chromatographic conditions variation

To demonstrate the robustness of the method, prepared solution as per test method and injected at different variable conditions like using different conditions like Temperature and wavelength. System suitability parameters were compared with that of method precision.

Table 6: Result of Robustness study.

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

Observation: From the observation it was found that the system suitability parameters were within limit at all variable conditions.

Ruggedness: The ruggedness of the method was studied by the determining the analyst to analyst variation by performing the Assay by two different analysts.

Table 7: Results for Ruggedness.

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

OBSERVATION

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

System suitability: Standard solutions were prepared as per the test method and injected into the chromatographic system. The system suitability parameters like theoretical plates, resolution and asymmetric factor were evaluated.

Table 8: Results for system suitability of Deferiprone.

Injection	Retention	Peak area	Theoretical	Tailing
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	-	-

Observation: The % RSD for the retention times and peak area of Deferiprone were found to be less than 2%. The plate count and tailing factor results were found to be satisfactory and are found to be within the limit.

Limit of Detection: Standard deviation valve of Deferiprone = 839.10

Observation: The LOD for this method was found to be 22.93 µg/ml of Deferiprone.

Limit of Quantification: Standard deviation valve of Deferiprone = 839.106

Observation: The LOQ for this method was found to be 96.37 µg/ml for Deferiprone

CONCLUSION

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 was 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.

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