Journal of Pharmaceutical and Scientific Innovation

www.jpsionline.com (ISSN : 2277 -4572)

Research Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE ESTIMATION OF DEFERIPRONE

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DOI: 10.7897/2277-4572.076114

Received on: 12/11/18 Revised on: 23/12/18 Accepted on: 27/12/18

ABSTRACT

Selective and precise RP-HPLC method was developed for estimation of Deferiprone in pure form. Acetonitrile: 0.1% formic acid (70: 30 v/v) was used as optimized mobile phase during the method development at 280 nm. Retention time of Deferiprone was 3.942 min. The developed method was validated according to ICH guidelines in the range of 10µg/mL to 60µg/mL. The linearity of Deferiprone shows a co-relation coefficient of 0.999. Precision was found to be 1.77 (%RSD). Percentage mean recovery of Deferiprone was found to be 100.34%. The developed method can be successfully employed for the quality control analysis of Deferiprone in its pure form.

Keywords: Deferiprone, RP-HPLC, Method Development, Validation, ICH guidelines

INTRODUCTION

Deferiprone is a chelating agent and used to treat thalassemia syndrome as a second line drug. Deferiprone is chemically 3-Hydroxy-1, 2-dimethyl-4(1H)-pyridone. Deferiprone is more selective for iron in which other metals such as zinc, copper, and aluminum have a lower affinity and route of elimination is through urine. Deferiprone is soluble in water, ethanol and methanol.[1, 2] some of the Manufacturers of Deferiprone pure drug in India is Apotex pharma chem India, Cadila Healthcare Ltd. Literature survey reveals that few methods were reported for estimation of Deferiprone.[3,4,5,6,7] The present research work aims to develop RP-HPLC method for the quantification of Deferiprone in pure form and validate according to ICH guidelines[8].

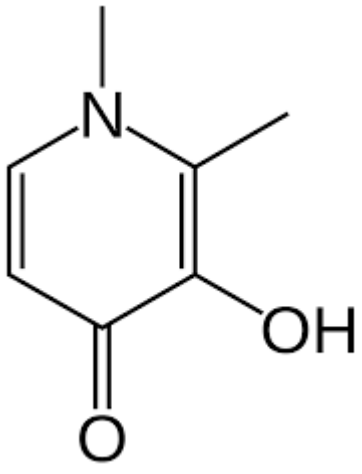

Figure 1: Deferiprone chemical structure

MATERIALS AND METHODS

Instruments

RP-HPLC instrument of Shimadzu make, LC-20 AD equipped with a PDA detector was used for the study.

Reagents and Chemicals

Deferiprone pure drug was procured as gift sample from Varun Herbals, Hyderabad, India. Acetonitrile and Water used are of HPLC grade. Formic Acid used is of AR Grade.

Liquid Chromatographic Conditions

Mobile phase containing Acetonitrile and 0.1% formic acid in the ration of 70: 30 v/v at a flow rate of 1mL/min through C18 Phenomenex Luna (250x4.6 mm; 5µ) at 280 nm was used. Injection volume was 20µl. Temperature was ambient.

Preparation of Standard Stock Solution

Accurately weighed 100 mg of standard Deferiprone was dissolved in 100 mL of mobile phase with proper sonication which gives strength of 1000 mcg/mL. This stock solution was filtered through 0.4 µ membrane filter paper.

Calibration Curve for Deferiprone

From the standard stock solution of Deferiprone respected aliquots are pipette out into 10mL volumetric flask and dilutions are made with mobile phase to obtain concentration range from 10-60µg/mL.

Sample Preparation

Drug equivalent to 10 mg of Deferiprone was weighed and transferred into 10 mL volumetric flask. The drug was dissolved in 10 mL of mobile phase and sonicated for 5mins.

RESULTS & DISCUSSION

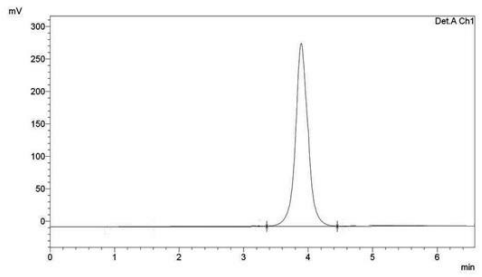

Figure 2: Optimized Chromatogram

In Fig. 2, chromatogram represents Deferiprone with an average retention time of 3.942 ± 0.127 min and with no interfering peaks which indicates the specificity of the HPLC method developed. The retention time was comparable with the shorter published data for Deferiprone.

System Suitability

System suitability test was an integral part of method development and has been used to ensure adequate performance of the chromatographic system.

Table 1: Results of System Suitability

Parameter	Result	Acceptance Limit
Retention time (Rt)	3.942 min	--
Resolution factor	NA	--
Number of theoretical plates (N)	2358	More than 2000
Tailing factor (T)	1.51	Less than 2

The system suitability was performed by 6 replicate analyses of Deferiprone at a concentration of 40 µg/mL. The tailing factor as determined for Deferiprone peak from standard solution was found to be 1.51. The theoretical plates were found to be 2358.The acceptance criterion for Number of theoretical plates (N) is more than 2000 and tailing factor is less than 2.

Specificity by Direct Comparison Method

Table 2: Specificity Data

S. No	Peak Name	Observation	
1	Blank	Nil	
2	Placebo	Nil	
3	Standard	Rt :3.942 min	Peak area :240541

No additional peak was detected close to the retention time of Deferiprone which was found to be 3.942 min. whereas in the blank and placebo chromatograms no peak was detected at retention time of Deferiprone from this it was concluded that this method is specific.

Results for Intraday and Interday Precision

Table 3: Precision Results of Deferiprone

S. No.	Intraday precision Area	Interday precision Area
1	244125	242145
2	242451	241245
3	244512	248596
4	242010	245487
5	241201	242403
6	254125	251593
Mean	244737.3	245244.8
Std Dev	4353.645	3769.877
%RSD	1.77	1.53

The optimized method was applied repetitively to analyze multiple replicates in three different occasions. Intraday precision was performed by analyzing of six replicates at a concentration of 40 µg/mL of standard Deferiprone within the same day while the inter-day precision was performed by analyzing of six replicates at a concentration of 40 µg/mL of standard of Deferiprone. The total precision of the method was expressed as the relative standard deviation (%RSD). The intraday and interday %RSD for Deferiprone was 1.77 and 1.53 respectively meets the acceptance criteria of less than 2.0. %RSD

Linearity and Range

Table 4: Results of Calibration Curve at 280 nm for Deferiprone

S. No	Concentration (µg/mL)	Peak Area
1	10	68451
2	20	121021
3	30	184241
4	40	241520
5	50	304512
6	60	365412

Linearity was determined by six different Deferiprone concentrations (10, 20, 30, 40, 50 and 60 µg/mL). The average peak areas were plotted against concentrations and it was observed that the peak area is directly proportional to the concention of Deferiprone. Then linearity was evaluated using the calibration curve to calculate coefficient of correlation,slope and intercept. In general, a value of correlation coefficient (r2) > 0.998 is considered as the evidence of an acceptable fit for the data to the regression line. The Correlation coefficient of Deferiprone was found to be 0.9994.

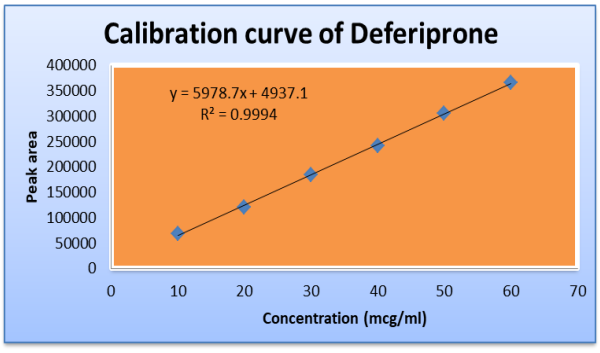

Figure 3: Calibration curve of Deferiprone

Accuracy

Accuracy of the method was determined by Recovery studies. To the formulation (pre-analyzed sample), the reference standards of the drugs were added at the level of 50%, 100%, 150%.

Table 5: Determination of Accuracy Results for Deferiprone

Spiked Concentration (µg/mL)	Peak area	Amount added (µg/mL)	Amount Found (µg/mL)	Recovery	% Mean Recovery
20	121021	20.01	20.1248	100.5737	101.51
	120041		19.96184	99.7593	
	125412		20.85499	104.2228	
40	241520	40.02	40.1628	100.3568	101.34
	248745		41.36426	103.359	
	241452		40.15149	100.3286	
60	365412	60.01	60.76503	101.2582	100.62
	364120		60.55018	100.9001	
	359874		59.8441	99.72355	

The recovery studies were carried out 6 times and the percentage recovery were calculated. From the data obtained recoveries of Deferiprone standard concentrations in the 20 µg/mL, 40 µg/mL, 60 µg/mL were found to be 101.51,101.34,100.62 respectively (Table 5). The method will be considered as accurate when the % recoveries were in between 98.0 and 102.0 according to ICH guidelines.

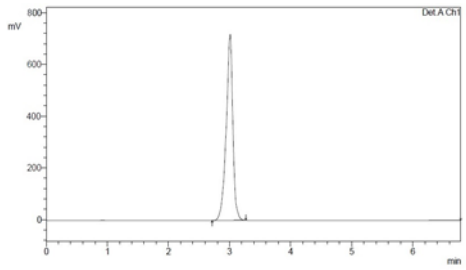

Figure 4: Chromatogram showing Accuracy 50% recovery

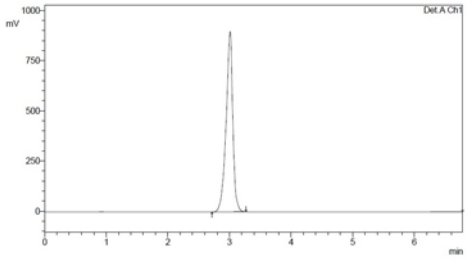

Figure 5: Chromatogram showing Accuracy 100% recovery

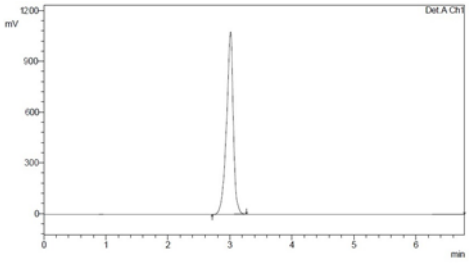

Figure 6: Chromatogram showing Accuracy 150% recovery

LOD & LOQ

Table 6: Results for Detection and Quantitation Limits

S.NO	Parameter	Slope	Standard Deviation	LOD & LOQ Value(µg/mL)
1	Limit of Detection	5978	4353	2.40
2	Limit of Quantification			7.28

LOD & LOQ are the least concentration of the drug that can be detected and quantified respectively. From LOD & LOQ the sensitivity of the method will be assessed. The LOD and LOQ were calculated as LOD = 3.3 σ/S and LOQ = 10 σ/S Where r is the standard deviation of the lowest standard concentration and S is the slope of the standard curve. The LOD & LOQ was found to be 2.40 and 7.28 respectively.

Robustness

Table 7: Change of Flow Rate (± 10%)

S. No	Flow Rate	0.9mL/min	1mL/min	1.1mL/min
1		249875	247851	241782
2		241578	246987	249514
3		239874	238745	237891
4	Mean	243775.6667	244527.7	243062.3
5	Std dev	4368.623302	4104.148	4830.664
6	% RSD	1.79	1.67	1.98

Table 8: Change in Temperature (± 5^{O}C)

S. No	Temperature	30 ºC	35 ºC	40 ºC
1		231457	234154	241598
2		241547	231451	246587
3		239874	241548	239852
4	Mean	237626	235717.7	242679
5	Std dev	4415.288062	4267.797	2853.825
6	% RSD	1.85	1.81	1.17

The robustness of the method was studied by deliberate changes in the method like alteration in flow rate and temperature. The robustness studies of Deferiprone samples were passed the acceptance criteria of less than 2% RSD which indicated that that there were no marked changes in the chromatograms and demonstrate that the HPLC method developed was robust.

Assay

The assay of Deferiprone pure drug was found to be 101.15 %

CONCLUSION

Selective and precise RP-HPLC method was developed for estimation of Deferiprone in pure form. Retention time of Deferiprone was 3.942 min which reduces the analysis time. The method underwent various validation parameters and the results were below the acceptance criteria. So the method can be used for the routine analysis of Deferiprone pure form without any interference of Excipients.

REFERENCES

1. Drugbank.ca [homepage on internet]. Canada Drug created on December 28, 2012 12:38 / Updated on December 23,

2018 18:24. Available from: [http://www.drugbank.ca/drugs/DB08826].
2. Wikipedia.org [homepage on internet]. Available from: https://en.wikipedia.org/wiki/Deferiprone.
3. Hinesha Barot, Darshil Shah, Dr. Dilip Maheshwari. Development of stability indicating UV Spectroscopy method development for the estimation of deferiprone in pharmaceutical formulation. Am. J. Pharm Tech Res 2015; 5(1):622-632.
4. 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 pharm and biosci 2015; 4(3):169-176.
5. 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 J of Pharma and Pharmaceu Sci 2015; 4(3): 831-838.
6. Mateen Abbas, Rakhshanda Nawaz, Tahira Iqbal, Muhammad Alim and 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 2012; 25(2): 343-348.
7. K. Sai Sireesha, B. Siva sai kiran, K.B. Chandra Sekhar and Shaik Muneer. A new RP-HPLC method development and validation of deferiprone in bulk and its pharmaceutical dosage form. Int. J. Adv. Res 2016; 4(8): 2174-2179.
8. Validation of analytical procedure: text and methodology, in. international conference on harmonization (ICH), Q2 (R1), IFPMA, Geneva, Switzerland, 2005.

How to cite this article:
Badithala Siva Sai Kiran and Sundararajan Raja. Development and validation of RP-HPLC method for the estimation of deferiprone. J Pharm Sci Innov. 2018;7(6):231-234.
http://dx.doi.org/10.7897/2277-4572.076114

Source of support: Nil, Conflict of interest: None Declared