IJBPAS, January, 2022, 11(1): 104-110

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

A QUANTITATIVE ESTIMATION OF DEFERIPRONE IN BULK DRUG AND PHARMACEUTICAL DOSAGE FORM BY RP-HPLC

SIVA PRASAD M*, KAMAKSHI DEVI N AND RAMA RAO N

Department of Pharmaceutical Analysis, Chalapathi Institute of Pharmaceutical Sciences, Lam, Guntur-522034

***Corresponding Author: Dr. Siva Prasad M; E Mail: kamakshidevinalam1@gmail.com; Ph.: 9701512825**

Received 8th March 2021; Revised 6th April 2021; Accepted 6th May 2021; Available online 1st Jan. 2022

https://doi.org/10.31032/IJBPAS/2022/11.1.5811

ABSTRACT

A reverse phase liquid chromatography (RP- HPLC) method has been developed and subsequently validated as per ICH guidelines for the determination of Deferiprone in bulk and its pharmaceutical dosage form. Separation was achieved with Symmetry C8 column (4.6 x 150mm, 5m, Make: XTerra) with ambient temperature. A mixture of phosphate buffer and acetonitrile (40:60 v/v) was used as mobile phase, p^H was adjusted to 3.5 with 0.1% orthophosphoric acid at a flow rate of 0.6 ml/min with a run time of 10 min by using UV detector at 285 nm. The retention time was found to be 2.976 minutes. The method was rapid, simple and sensitive. The described method of Deferiprone was linear in the range of 20-60µg/ml with correlation coefficient of 0.999. The method enables accurate, precise and rapid analysis and it can be conveniently adopted for routine quality control analysis of bulk and pharmaceutical dosage form.

Keywords: Deferiprone, Thalassaemia, Kefler, Validation

INTRODUCTION

Deferiprone **Figure 1** chemically known as 3-hydroxy-1,2-dimethylpyridin-4(1H)-one **[1]**. Its molecular formula is $C_7H_9NO_2$and molecular weight is 139.152 g/mol **[2]**. Deferiprone has a melting point of 272-278 ^{0}C, it was soluble in water with pKa of 3.5 **[3, 4]**. Deferiprone, sold under the brand name Ferriprox among others, is a medication that chelates iron and is used to treat iron overload in thalassaemia major **[5]**. It was first approved and indicated for

use in treating thalassaemia **[6, 7]**. The most common side effects include red-brown urine, nausea, abdominal pain and vomiting. Less common but more serious side effects are agranulocytosis and neutropenia **[8, 9]**. Aim of the present work is to develop a new simple precise, accurate, reliable and rapid analytical method to estimate deferiprone in capsules by RP-HPLC method. Objective of the work is to validate the developed method in accordance with ICH guidelines to test its suitability for use in quality control laboratories.

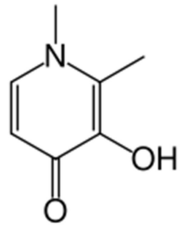

Figure 1: Structure of Deferiprone

MATERIALS AND METHODS

Materials:

Deferiprone was obtained from Sigma Aldrich. Kefler capsules containing 500 mg manufactured by Cipla was procured from local market. Other chemicals and reagents such as Potassium dihydrogen phosphate, ortho phosphoric acid, water, methanol, acetonitrile of HPLC grade was obtained from Merk.

Instrumentation and chromatographic conditions:

The chromatographic separation was carried out by Shimadzu 2010C with UV detector, Symmetry C8 column (4.6 x 150mm, 5m, Make: XTerra) with ambient temperature. Mobile phase consisting of phosphate buffer: acetonitrile (40:60) at pH 3.5 was used. pH was adjusted to 3.5 with OPA and filtered through 0.45µ Millipore Nylon filter. The mobile phase was degassed and pumped from the solvent reservoir in the ratio 40:60 v/v into the column at a flow rate of 0.6 ml/min. The injection volume was set to be 10 µl with detectionat 285 nm.

Preparation of standard stock solution:

Accurately weighed and transferred 50 mg of Deferiprone into a 50 mL volumetric flask.

30mL of diluent was added, sonicated for 10 min to dissolve, diluted up to the mark with diluent.

Preparation of standard solution

4mL of the above solution was pipetted into a 100mL volumetric flask and diluted up to the mark with diluent (40µg/ml).

Preparation of sample solution

20 capsules were accurately weighed and average weight of capsules was determined. Accurately weighed and transferred 50mg powder equivalent to drug into a 50 mL volumetric flask. 30mL of diluent was added, sonicated for 10 min to dissolve, diluted up to the mark with diluent and filtered through 0.45µ Millipore Nylon filter. Further 4 mL of the

above solution was diluted to 100mL with diluent.

ASSAY:

40 µg/ml of the standard solution was injected five times into the chromatographic system, chromatograms were recorded and peak areas were measured.40 µg/ml of the sample solution was injected in duplicate into the chromatographic system, chromatograms were recorded and peak areas were measured. The assay values are calculated by using formula:

$$\text{Amount present} = \frac{AT1}{A_{S1}} \times \frac{DS1}{D_{T1}} \times \frac{P1}{100} \times AW$$

Where, A_{T1} = Average area of Deferiprone peak in chromatogram of sample solution
A_{S1} = Average area of Deferiprone peak in chromatogram of standard solution
D_{S1} = Dilution factor for the standard solution
D_{T1} = Dilution factor for the sample solution
P_1 = Percentage potency of Deferiprone standard used (As is basis)
AW = Average weight of tablet

METHOD VALIDATION

The proposed method was validated according to the ICH guidelines which include system suitability, specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ) and robustness. Under the validation study, the following parameters were studied **[10]**.

RESULTS & DISCUSSION

System Suitability:

HPLC system was optimized as per the chromatographic conditions. 10 µlof standard solutions of drugs were injected in triplicate into the chromatographic system. To ascertain the system suitability for the proposed method, the parameters such as retention time, number of theoretical plates, resolution, tailing factor and % RSD were calculated and compared with standard specification of system. These values are represented in **Table 1**.

From the system suitability studies it was observed that all the parameters are within limit, hence it is concluded that the Instrument, Reagents and Column are suitable to perform Assay.

Specificity:

The specificity of method was determined by comparing the chromatograms of blank, standard and sample and in blank chromatogram analyte was not detected, the retention times of standard and sample was found to be same i.e., 3 min. **Figure 2** represents standard chromatogram.

Linearity:

Linearity of the method was analysed by preparing calibration curves using different concentrations of the standard solutions. Linearity was established from 20 to 60

µg/ml represented in **Table 2**, **Figure 3** represents the calibration curve.

The correlation coefficient was within the acceptance limit.

Accuracy:

The accuracy of the method was determined by analyzing three solutions containing deferiprone approximately 50%, 100% and 150% **(Table 3)**.

The overall % recovery was found to be 99.46%, these values are within the acceptancelimits which indicates the method was accurate.

Precision:

System and method precision was carried out for Deferiprone by giving six injections **(Table 4)**.

For system precision % RSD was found to be 0.620 and for method precision % RSD was 0.642. These values are within the acceptance limit NMT 2.

Limit of detection [LOD] & Limit of quantification [LOQ]:

LOD and LOQ of Deferiprone was found to be 0.4 and 1.2µg/ml which indicates the sensitivity of the method.

Robustness:

Various parameters such as flow rate, ratio of mobile phase and detection wavelength was changed and all the parameters were within the acceptance limit with a tailing factor more than 2 and plate count more than 2000.

Assay:

The % assay of Deferiprone was found to be 99.28% which was in the acceptance limit.

Table 1: System suitability parameters of Deferiprone

Parameters	Deferiprone	Acceptance criteria
Retention time (min)	2.974 min	NA
Number of theoretical plates	5800	NLT 2000
Tailing factor	1.17	NMT 2
% RSD	0.438	NMT 2

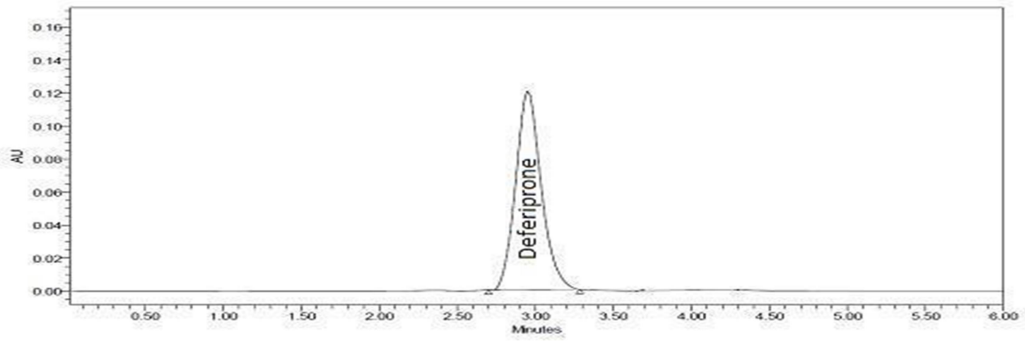

Figure 2: Standard chromatogram of Deferiprone

Table 2: Linearity of Deferiprone

Linearity levels	Concentration (µg/ml)	Peak Area
I	20	1012432
II	30	1538397
III	40	2051196
IV	50	2563995
V	60	3152132

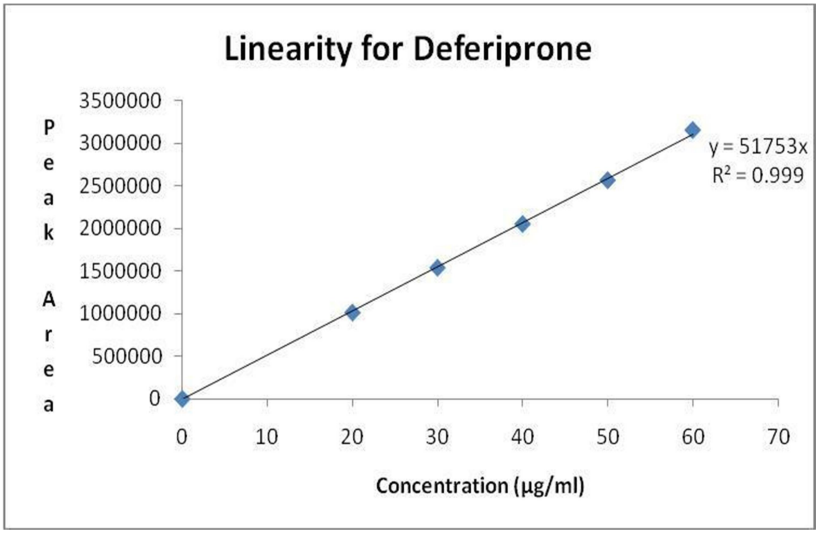

Figure 3: Calibration curve of Deferiprone

Table 3: Accuracy of Deferiprone

Accuracy	Peak area	%Recovery	Mean % Recovery	Overall % Recovery
50	1001121	99.88	99.37	99.46
50	1005721	99.34		
50	1011213	99.88		
100	2034521	99.19	99.28	
100	2031421	99.04		
100	2043541	99.63		
150	3142814	99.71	99.74	
150	3137541	99.54		
150	3151321	99.97		

Table 4: Precision of Deferiprone

Injection number	System Precision		Method Precision	
	Retention time (min)	Peak area	Retention time (min)	Peak area
1.	2.976	2021168	2.975	2063801
2.	2.972	2038961	2.970	2065987
3.	2.975	2014516	2.976	2030457
4.	2.976	2042143	2.975	2054897
5.	2.977	2011967	2.976	2044985
6.	2.975	2022468	2.976	2055854
Average		2025204	Average	2052664
SD		12565.2	SD	1381.92
%RSD		0.620	%RSD	0.642

CONCLUSION

In the present work, a newer, sensitive, simple, accurate and economic RP-HPLC method was developed. It is successfully applied for the determination of Deferiprone in pharmaceutical preparations without the interferences of other constituent in the formulations and is validated as per ICH guidelines. All the parameters such as %RSD, % Recovery, theoretical plates, tailing factor were within the acceptance criteria as per ICH Q2 guidelines. Hence this method was used for routine analysis.

REFERENCES

[1] Siva Sai Kiran B., Sundararajan Raja., (2018). "Development and validation of RP-HPLC method for the estimation of Deferiprone", J. Pharm. Sci. Innov, 7(6): Pp. 231-234.

[2] Manzoor Ahmedz., Vinod kumar G *et al*., "RP-HPLC method development and validation for estimation of Deferiprone in capsule dosage form", World. J. Phar. Phar. Sci, 4(3):Pp. 831-838.

[3] Aamir Malik., Sandip Firke *et al*.,(2019). "Development and Validation of Zero and First-Order Derivative Area under Curve Spectrophotometric Methods for the Determination of Deferiprone in Bulk Material and Capsules". Asian J. Pharm. Ana, 9(2): Pp. 49-54.

[4] Hajjizadeh M., Jabbari A., Heli H., Moosavi MA., (2008), "Electrocatalytic oxidation and determination of Deferasirox and Deferiprone on a nickel oxyhydroxidemodified electrode", Analytical Biochemistry, 373(2), Pp.337–48.

[5] Antonis K., Vassilios L., Helen B., Nikolaos L K., Efthymia A., (2007), "Iron chelation treatment with combined therapy with Deferiprone and Deferioxamine: A 12-month trial, Blood cells", Molecules, and Diseases, 36(1): Pp. 21–5.

[6] FDA Approves (2011), "Ferripox (deferiprone) to Treat Patients with Excess Iron in the Body". *U.S. Food and Drug Administration (FDA)*.

[7] "Drug Approval Package: Ferriprox (deferiprone) Tablet NDA #021825". *U.S.* Food and Drug Administration *(FDA). 30 November 2011*. Retrieved 20 May 2020.

[8] *Ferriprox EPAR". European Medicines Agency (EMA). Retrieved 20 May 2020.* Text was copied from this source which is European Medicines Agency. Reproduction is authorized provided the source is acknowledged.

[9] Viens AM., Savulescu J., (2019), "Introduction to The Olivieri

symposium". Journal of Medical Ethics, 30 (1):Pp. 1–7.

[10] Validation of analytical methods (1996): methodology, in processing of the 3rd International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH '96). Guideline on Validation of Analytical Procedure-Methodology, Geneva, Switzerland.