International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 6 Nov-Dec 2022, pp: 1724-1730 www.ijprajournal.com ISSN: 2456-4494

Deferiprone: A review of Analytical methods

Diptee D. Marchande[1*], Ashish Jain[2]

[1]Department of Pharmaceutical Quality Assurance, D,D,Vispute College of Pharmacy, New Panvel,410206.
[2]Department of Pharmacognosy & Phytochemistry, D,D,Vispute College of Pharmacy, New Panvel,410206

Submitted: 15-12-2022 Accepted: 26-12-2022

ABSTRACT

Deferiprone is an iron chelating agent, used as second line agent during thalassemia disorder. Thalassemias are form of genetic anemia which occurs due to deficiency in hemoglobin production.It is generally selective to iron than some othermetals like zinc, aluminum and copper. Deferipronedrug is absorbed in the upper gastro intestinal tract where absorption is fast with high plasma concentrations arising after two hours in fed state and onehour in fasted state.More than half of deferiprone drug is detached through plasma in 5-6 hours of administration. Determinations are categorized into different analytical methods that are used. Efforts are taken to collect all related articles published till May 2021.This article covers analytical methods that are reported so far for estimation of deferiprone in pharmaceutical preparations and biological samples.They include various techniques like spectrophotometry, High performance liquid chromatography,Liquid chromatography-mass spectroscopy and electrochemical methods. The techniques discussed in this review follow the ICH guidelines for method validation.

I. INTRODUCTION

Deferiprone is chemically 3-hydroxy-1, 2-dimethylpyridin-4(1H)-one. It is sparingly soluble in water and methanol and slightly soluble in ethanol and chloroform. The molecular formula is $C_7H_9NO_2$ andmolecular weight is 139.152 g/mol. Deferiprone drug is an oral iron chelator, mostly used as second line agent in thalassemia disorder during iron overload that occurs after blood transfusions. Basically thalassemias are form of genetic anemia which is generally due to deficiency in hemoglobin production. As a result, erythropoiesis, the production of new red blood cells, is impaired. Deferiprone normally binds to the ferric ions and also forms 3:1 (deferiprone: iron) stable complex, later then eliminated through urine. It is generally selective to iron than some metals like zinc, aluminium and copper having lesser attraction for deferiprone. [1]

Deferiprone belongs to alpha-ketohydroxpyridines familywhich is a comparatively new class of chelating agents. Deferiprone can eliminateextra iron from different parts of the body of iron-loaded patients, including the liver and the heart. The drug is usedworldwide to treat leukemia, hemodialysis, cancer and some other diseases.

Deferiprone is absorbed in the upper gastro intestinal tract (GIT). Basically absorption is fast with extreme plasma concentrations arising after 2 hrs in fed state and 1hour in fasted state. It is usually metabolized by using UGT1A6 to 3-O-glucuronide which cannot chelate the iron. More than 90% of deferiprone is removed through plasma in 5-6 hours of administration. 75 to 90% deferiprone drug is eliminate in urine as metabolite.[2]

UV SPECTROSCOPIC METHOD

It is the cheapest and easiest working analytical tool available used in the pharmaceutical laboratories and research. The analytical applications of the UV spectroscopy are qualitative and quantitative estimation. There are various spectrophotometric techniques which are used in the pharmaceutical world for the analysis of the pharmaceutical ingredients. [3]

In the literature 5 methods were reported for the estimation of deferiprone using spectrophotometry.Table1 shows the summary of reported spectroscopic methods indicating basic principle,wavelength, solvent and results.

Reliable UV spectroscopic technique has been established for quantitative determination of Deferiprone. Deferiprone was exposed to stress degradation recommended by the standard guidelines. Deferiprone displays maximum absorbance at wavelength of 279nm and also calibration graph shows linearity in range 5-25 µg/ml using 0.9997correlation co-efficient.The higher proportion of recovery indicates that no

interference of excipients. Stability study specifies appreciable variations were seen by treating the drug with acidic as well as basic hydrolysis and with oxidation. [4]

To develop First Order derivative spectrophotometric way for determination of deferiprone and validatethe method by ICH guidelines is reported. Method is passed using Distilled water as solvent with absorption wavelength set at 270nm. Linearity was proven over the range of 2-10 μg/ml where correlation coefficient was about 0.9998. The results been validated statistically plus recovery studies confirmed the accuracy of method. [1]

Spectrophotometric approaches for estimation of three drugs along with deferiprone were developed based on oxidation by $KMnO_4$. Initial rate and also fixed time process are used forconstruction of calibration curves in the range 4-24 μg/mlfor deferiprone. Recovery studies by pure samples and formulations have been done. Excellent recoveries specify thatapproaches are accurate as well as precise. Methods been validated by ICH guidelines. [5]

Spectrophotometric method was developfor deferiprone in its dosage form. The solvent and wavelength were optimized to maximize sensitivity method. The method was validated for different factors like linearity, precision, accuracy, limit of detection and limit of quantitation as per ICH guidelines. Maximum absorption of Deferiprone was monitored at 278nm. The method was linear in the range of 2 to 12μg/ml with a correlation coefficient of 0.999. The accuracy was studied and % recovery was found to be 101.07%. The method is modest, accurate and needs inexpensive instrument. [6]

A precise spectrometric method including Area under curve has been developed in bulk and capsule form for estimation of deferiprone. Four UV spectrometric methods were carrired out for deferiproneby using double beam UV spectrophotometer.Deferiprone with distillled water shows maximum absorbance at 278 nm. Deferiprone obeys beer lamberts law in range of 2-10 μg/ml. By proposed method % recovery found to be 98-101%. %RSD indicates precise nature of method. [7]

Table No.1 UV SPECTROSCOPIC METHOD

Sr.no	Method	Matrix	Detection	Solvent	Linearity, LOD, LOQ (μg/ml)	Reference
1	Stability indicating UV Spectoscopy	Bulk and formulation	279 nm	water	Linearity: 5-25 LOD: 0.1123 LOQ: 0.3404	[4]
2	First Order derivative spectrophotometric method	Bulk and capsule form	270 nm	water	Linearity: 2-10 LOD: 0.150 LOQ: 0.455	[1]
3	Kinetic spectrophotometry	Bulk drug and capsule	610 nm	Water, $KMnO_4$, NaOH	Linearity: 4-24 LOD: 1.324 LOQ: 4.01	[5]
4	UV Spectroscopic Assay Method	Bulk and formulation	278 nm	Water and ethanol	Linearity: 2-12 LOD: 0.18083 LOQ: 0.547	[6]
5	Area under curve spectrophotometric method	Bulk and capsule	278 nm	water	Linearity: 2-10	[7]

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 6 Nov-Dec 2022, pp: 1724-1730 www.ijprajournal.com ISSN: 2456-4494

High Performance Liquid Chromatography

Liquid Chromatography is now one of the most powerful tool in analytical chemistry. It has the ability to separate, identify and quantify the compounds that are present in any sample that can be dissolved in a liquid. HPLC is the most accurate analytical method widely used for the quantitative as well as qualitative analysis of drug product and used for determining drug product stability. [8]

Analytical methods for the determination of deferiprone in pharmaceutical dosage form using HPLC are shown in table 2

HPLC method was used for quantification of deferiprone in human plasma using UV/VIS detector. Chromatographic separation was carried out on C_{18} column, with a mobile phase of methanol-buffer (18:82, v/v), pH 3.5 and caffeine was used as an internal standard. The calibration curve was linear over the range 0.25-10 µg/mL in human plasma. The deferiprone plasma concentration showed a rapid absorption and average area under the plasma concentration-time curve (AUC) of deferiprone was 17.0 ± 1.23 h.µg/mL. Average absorption and elimination half-life values of deferiprone of 24 volunteers were 0.62 ± 0.12 and 2.65 ± 0.43 hours. This study confirms the rapid absorption of deferiprone in humans. [9]

The objective of the study was to develop a simple, accurate, precise and rapid RP-HPLC method and subsequently validate as per ICH guidelines for the determination of Deferiprone using mobile phase [mixture of Phosphate buffer pH-3.6 and methanol in the ratio of 20:80 v/v] as the solvent. The retention time of Deferiprone was found to be 5.404 at 280 nm. The linearity of the proposed method was investigated in the range of 10-50 µg/ml and regression was found to be (R^2= 0.9998). The method was statistically validated for its linearity, accuracy and precision. [10]

A method was developedfordeferiprone where separation was done using column $C_{18}$with mobile phase entails water and acetonitrile in ratio of 55:45v/v ratio. The detection wavelength was 280 nm with flow rate of 1 ml/min and temperature of 30^0C. Themethod beenvalidated as per standard guidelines. In range of 10- 50µg/ml, linearity of Deferiprone shows R^2= 0.999 and precision was found in % RSD to be 0.70. The mean recovery were98.40 %.[11]

A method was develop for the estimation of deferiprone in formulation by using RP-HPLC. The separation was carried on Inertsil ODS C18, 250x 4.6mm, 5µm i.d. column using mobile phase as 60 volumes of Mixed Phosphate buffer (KH_2PO_4+K_2HPO_4) and 40 volumes of methanol. Detection was 280nm using PDA detector. Theprocess found to be validated for accuracy, precision, specificity, linearity and sensitivity. Stability studies reported absence of impurities at the peak retention time. The drug was steady to different situations like alkali, thermal,acidic and photolytic condition. [12]

RP-HPLC method was developed for Deferiprone in pure form. Acetonitrile and 0.1% formic acid (70: 30 v/v) as mobile phase during the method development at 280 nm.Retention time was 3.942 min. Method was validated by using ICH guidelines. In the range of 10µg/mL to 60µg/mL, linearity of Deferiprone shows a R^2=0.999. Precision study found of 1.77 (%RSD). Percentage mean recovery of Deferiprone was found to be 100.34%.[13]

LC method is described for the determination of Deferiprone. Chromatographic separation was achieved on a c18 column using mobile phase consisting of a mixture of Triethylamine: ACN (50:50v/v), with detection of 280 nm. Linearity was detected in the range 125-375 µg/ml (R^2 =0.994). The accuracy of the methods was assessed at three different levels by recovery studies. By the repeatability analysis and showing %RSD less than 2, this indicates the method to be precise. [2]

Table No. High Performance Liquid Chromatography Method

Sr. no	Method	Matrix	Stationary phase	Mobile phase	Detection	Result (µg/ml)	Ref
1	Bio Analytical RP-HPLC	Plasma	C_{18}Discovery Supelco (250 x 4.6; 5µm)	Methanol-buffer(pH 3.5) 18:82 (v/v)	280 nm	Linearity: 0.25-10 LOD: 0.1 LOQ: 0.25	[9]

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 6 Nov-Dec 2022, pp: 1724-1730 www.ijprajournal.com ISSN: 2456-4494

2	RP-HPLC	Capsule	C_{18}(250 x 4.6; 5µm)	Phosphate buffer(pH3.6)-methanol 20:80 (v/v)	280 nm	Linearity: 10-50 LOD: 132.17 LOQ: 400.53	[10]
3	RP-HPLC	Bulk and capsule	C_{18} Phenomenxluna column	Water – ACN 55:45 (v/v)	280 nm	Linearity: 10-50 LOD:0.0659 LOQ:0.199	[11]
4	Stability indicating RP-HPLC	Capsule	C_{18} Inertsil ODS (250 x 4.6; 5µm)	Mixed phosphate buffer (KH2PO4 + K2HPO4) pH 3.0, methanol 60:40 (v/v)	280 nm	Linearity: 75–125 LOD: 3.9 LOQ: 11.8	[12]
5	RP-HPLC	Pure drug	C_{18} Phenomenex Luna (250x4.6 mm; 5µ)	Acetonitrile and 0.1% formic acid 70: 30 (v/v)	280 nm	Linearity:10-60 LOD: 2.40 LOQ: 7.28	[13]
6	RP-HPLC	Bulk and capsule	Inertsil column,C_{18}(150x4.6 ID; 5µm)	Triethylamine buffer (pH 3.5): ACN 50:50(v/v),	280 nm	Linearity: 125-375	[2]

LC-MS

It is one of the hyphenated technique uses for the determination of chemical entity.It separates chemicals on basis of mass to charge ratio.There are only two method reported for determination of deferiprone given in table 3

LC-MS/MS assay for thedeferiprone estimation in the human plasma. To attain protein precipitation,analytes were extracted using acetonitrile. Separation was done using a Synergi Fusion-RP 80A column. Mobile phasecomposed of methanol plus0.2% formic acid holding 0.2 mM EDTA (60:40, v/v) with flow rate set for 0.8 mL/min. Validation was estimated for linearity, recovery, precision, stability and accuracy.[14]

For affinity studies,sensitive techniques wereestablished for estimation of metalbound deferiprone. The method being carried on monolithic column with mobile phase having ammonium formate solution, water and methanol. Identification was attained on single quadrupole mass spectrometer. [15]

Table No 3. LC-MS Method

Sr.no	Matrix	Stationary phaase	Mobile phase	Result	Reference
1	Plasma	Synergi Fusion-RP 80A column (4 mm, 150 × 4.6 mm i.d.; Phenomenex, USA).	methanol and 0.2% formic acid containing 0.2mM EDTA (60:40 v/v),	Linearity: 0.1-20 µg/ml LOD: 0.05 µg/ml	[14]
2	Bulk drug	monolithic column Chromolith	ammonium formate so lution (pH 7.4; 10 mM), H2O,	Linearity: 0.5–17.5 mM LOD:17.7 µM LOQ:53.8 µM	[15]

International Journal of Pharmaceutical Research and Applications
Volume 7, Issue 6 Nov-Dec 2022, pp: 1724-1730 www.ijprajournal.com ISSN: 2456-4494

			and methanol		

ELECTROCHEMICAL METHOD

In electrochemical method, voltametery,spectroflurometery and potentiometery methods has been developed for determination of deferiprone shown in table 4

The deferiprone was examined on modified carbon nanotube glassy carbon electrode inwith pH7.4 in phosphate buffer. Voltammetric study specified that oxidation method is diffusion controlled and irreversible. In electro-oxidation procedure,amount of the exchanged electrons was gained and data specified that deferiprone was oxidized through two-electron stages. The results exposed that the carbon nanotube helps the oxidation rate by increase in peakcurrent.Thus deferiprone will be oxidized at lesser potentials which are mostly thermodynamically favorable. This result has been confirmed using impedance measurements. A sensitive differential-pulse voltammetricmethodwas established forstudy of deferiprone. [16]

A very rapid fluorometric technique have been reported for estimation of deferiprone drug in serum samples and urine. This technique is built on development of luminescent compound by Tb3+ ion and assessed in terms of validation parameters. Relative intensities are linear at 545nm, with range of 0.072–13 mmol/L. The result values are all less than 5% for precision whereas accuracy is in range 97.1–103.8%. The method can be effectively applied to deferiprone determination in serum samples and urine. [17]

Fluorometric technique for deferiprone determination was develop. Method was built on development of luminescent compound with Tb3+. Determined emission and excitation wavelengths were 545 and 295 nm individually. The validation outcomes specify that at 545 nm this relative intensity has linear connection with concentration of drug. Precision results were lesser than 5% and also recovery results were within the standard limit. [18]

Selective potentiometric method was developed for deferiprone. Method is built on fabricating a PVC membrane sensor that helps to estimate the studied drug. The method was linear over range of 10-2-10-5 M with a nernstian slope of 58.4mV. The accuracy and precision of the technique was found to be within the standard limits. Besides, the methods were statistically associated to a reported method.[19]

Table No.4.Electrochemical Method

Sr.no	Method	Matrix	Solvent	Linearity and LOD	Reference
1	Electrochemical (voltametric method)	Bulk form	phosphate buffer solution pH 7.40	LOD: 5.25×10^{-7} M. LOQ: 1.75×10^{-6} M	[16]
2	fluorometric method	urine and serum	water	Linearity 0.072–13 mmol/L , The LOD and LOQ: 0.014 and 0.045 mmol/L for urine and 0.022 and 0.072 mmol/L for serum samples.	[17]
3	fluorometric method	Tablet form	water	Linearity: 7.2 × 10-9 to 1.4 × 10-5 M LOD and LOQ: 6.3 × 10-9 and 2.1 × 10-8 M	[18]
4	potentiometric method w	Bulk and capsule	potassium chloride buffer pH (2.0)	Linearity:10^{-5}-10^{-2} M, LOD: 3.3x10-6	[19]

II. CONCLUSION

There are varieties of techniques available for deferiprone analysis in biological samples andformulations. Deferiprone an iron chelator, used for treatment of thalassemia.In present review,different analytical approaches areused for analysis study of deferiprone.Numerous practices like HPLC,UV-Spectroscopy,LC-MS and electrochemical has been performed for assessment of deferiprone drug in plasma, bulk and formulation.Review study reveals that most common method for identification plus quantification for deferiprone is HPLC.UV-Spectroscopy methods mostly used for analysis purpose of bulk and formulation whereas LC-MS only practiced for biological fluids used for detection plusquantification of drug content in plasma. The present review article reveals that there is not a single article performed for HPTLC analysis. This review give information which is useful for future study for researcher involved in formulation development and quality control of deferiprone.

REFERENCES

[1]. Manzoor A, Jangade VK, Satishkumar SA, Vijaya Krishna CA, Anil Kumar SM. Development and validation of first order derivative spectrophotometric method for the estimation of deferiprone in bulk and capsule dosage form. Int J Univers Pharm Bio Sci. 2015; 4(3):169-76.

[2]. Adapa S, Jyothi CH N, Hemalatha R, Deepthi B, Gopikrishna U, Shabareesh P. Method development and validation for the estimatin of deferiprone using RP-HPLC in bulk and pharmaceutical dosage forms. Int J Pharma Res Novel Sci.2019; 4(2):600-03.

[3]. Badyal PN, Sharma C, Kaur N, Shankar R, Pandey A, Rawal RK. Analytical Techniques in Simultaneous Estimation: An Overview. Austin J Anal Pharm Chem. 2015; 2(2):1-14.

[4]. Barot H, Shah D, Dr.Maheshwari D. Development of stability indicating UV Spectroscopy method development for the estimation of deferiprone in pharmaceutical formulation. Am J Pharm Tech Res. 2015; 5(1): 622-32.

[5]. Prashanthi M, Venkateshwarlu G. Kinetic spectrophotometric determination of drugs based on oxidation by alkaline kmno4. World J Pharm Res.2016; 5(6): 1429-43

[6]. Padma A, Dr. K. Thejomoorthy, Pallavi A, Snehith B, Kumar MP. A Validated UV spectroscopic assay method development for the estimation of deferiprone in bulk and its formulation. World J Pharm Res.2019; 8(5):1163-69.

[7]. Malik A, Firke S, Patil R, Shirkhedkar A, KalaskarM.Development and validation for zero and first order derivative area under curve spectrophotometric methods foor the determination of deferiprone in bulk material and capsules. Asian J Pharm Anal.2019;9(2):49-54.

[8]. Bhardwaj SK, Dwivedi K, Agarwal DD. A Review: HPLC Method Development and Validation. Int J Anal Bioanal Chem. 2015; 5(4): 76-81.

[9]. Abbas M, Nawaz R, Iqbal T, Alim M, Muhammad R A. 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; Vol.25, No.2 :343-348

[10]. Manzoor A, Jangade VK, Satishkumar SA, Vijayakrishna CA, Kuppast IJ, Anil kumar SM. RP-HPLC method development and validation for estimation of deferiprone in capsule dosage form. World J Pharm Pharm Sci. 2015; 4(3): 831-38.

[11]. Sireesha KS, Badithala Siva SK, Chandra Sekhar KB, Shaik M. A new RP-HPLC method development and validation of deferiprone in bulk and its pharmaceutical dosage form. Int J Adv Res. 2016; 4(8): 2174-79.

[12]. Reddy PV, Ranjani VA, Sekhar RC, Sundar MS. Stability indicating method development and validation of deferiprone in pharmaceutical dosage form by RP-HPLC. J Innov Pharm Biol Sci. 2017; 4(2):53-7.

[13]. Badithala Siva SK, Sundararajan R. Development and validation of RP- HPLC method for the estimation of deferiprone. J Pharm SciInnov. 2018; 7(6):231-34.

[14]. Song TS, Hsieh YW, Peng CT, Liu CH, Chen TL, Hour MJ. Development of a fast LC-MS/MS assay for the determination of deferiprone in human plasma and application to pharmacokinetics. BiomedChromatogr. 2012;26(12):1575-81.

[15]. Mufarreh A, Ahmed M. A-M, Michalcová L,Glatz Z, El Deeb S. Analytical approaches for the determination of deferiprone and its iron (III)complex: Investigation of binding affinity based on liquid chromatographymass spectrometry (LC-ESI/MS) and capillary electrophoresis-frontal analysis (CE/FA).Microchemical Journal.2020;154.
[16]. Yadegari H, Jabbari A, Heli H, Moosavi-Movahedi A A, Karimian K, Khodadadi A. Electrocatalytic oxidation of deferiprone and its determination on a carbon nanotube-modified glassy carbon electrode.Electrochimica Acta.2008; 53: 2907–2916.
[17]. Manzoori JL, Amjadi M, Soleymani J, ElnazTamizi , AzimRezamand ,AbolghasemJouyban. Determination of deferiprone in urine and serum using a terbium-sensitized luminescence method.2012;27(4):258-73
[18]. Manzoori JL, Mo Amjadi M, Soleymani J,Tamizi E,Panahi-Azar V ,Jouyban A. Development and Validation of a Terbium-Sensitized Luminescence Analytical Method for Deferiprone. Iranian Journal of Pharmaceutical Research .2012; 11 (3): 771-780
[19]. Attia KAM, El-Abasawi NM, El-Olemy A and Serag A. Electrochemical Determination of Deferiprone Using PVC Membrane Sensors. Austin J Anal Pharm Chem. 2018; 5(1): 1098.