Content available at: https://www.ijcrips.com/current-issue

International Journal of Current Research and Innovation in Pharma Sciences

Journal homepage: https://www.ijcrips.com/

Research Article

Development and Validation of RP HPLC Method for Estimation of Deferiprone and Its Related Impurity in Pharmaceutical Dosage Form.

Shweta Ubale[*1], Mayur Bhosale[1], Dr. S. K. Parajne[1]

[1]*Department of pharmaceutical chemistry, Pravara rural college of pharmacy, Loni, Ahmednagar, Maharashtra, India.*

ARTICLE INFO

Article history:
Received 08-06--2022
Accepted 13-06-2022
Available online 16-06-2022

Keywords:
Deferiprone,
Maltol impurity,
Method validation,
RP HPLC.

ABSTRACT

The aim of this study is to develop a new, precise, sensitive, simple, efficient, selective and accurate high-performance liquid chromatographic method for the separation and determination of Deferiprone and its impurity in capsule dosage form. An extensive literature survey revealed no method for estimation of the above said. The chromatographic separation was achieved on Agilent Zorbax Bonus-RP (250 x 4.6 mm, 5 µ) with a mobile phase composed of Methanol: 0.1% O-Phosphoric acid (10:90, % v/v) in 1000 ml of Methanol: Water (50: 50, % v/v) using a diluent. gradient program at a flow rate of 1 mL/min with UV detectation at 280 nm. The developed method was validated as reported by ICH guidelines. The linearity of the calibration curve for Deferiprone and its process-related impurity in the concentration range of 4.0-6.0µg/ml was good. There exists a qualitative correlation between peak area and analyte concentration. The retention time for Deferiprone was found to be 2.29 min and its impurity was 8.65 min. Relative standard deviation values for Deferiprone is 0.45 and its process-related impurity is 0.17. All the results reveal that the proposed method was found to be highly sensitive, simple, precise, accurate, and fast. A large number of samples can be analyzed in a shorter time due to shorter retention times, so it can be successfully applied for routine analysis of Deferiprone and related maltol impurity in bulk and pharmaceutical dosage forms.

INTRODUCTION:

Deferiprone is in a class of medicines called iron chelators. Deferiprone is used for the management of Thalassemia major. Thalassemia most important takes place when a child inherits mutated two genes, one from every parent. Most importantly, children born with thalassemia expand the signs of intense anaemia within the first year of life. They cannot produce normal, personal hemoglobin and experience continual fatigue. They may also fail to thrive [2]. Deferiprone binds to iron in the blood. It treats and help to prevent too much iron in the blood. It helps to prevent and treat too much iron in the blood caused by blood transfusions [8]. Deferiprone is an associate iron chelator that binds to metal ions (iron III) and forms a 3:1 (deferiprone: iron) stable complex and is then eliminated within the urine.

*** Corresponding author.**
E-mail address: shwetarubale97@gmail.com
Shweta Ubale

[Doi: 10.1000/ijcrips/2022/04]

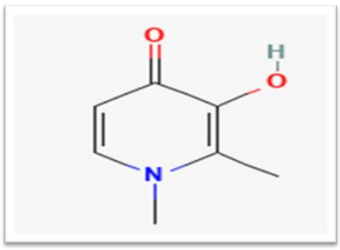
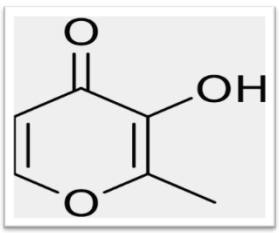
Deferiprone is a lot of selective for iron during which alternative metals like zinc, copper, and aluminum have a lower affinity for deferiprone. [3,5] Deferiprone is chemically 3-hydroxy-1,2-dimethyl pyridine-4-one with molecular formula C7H9NO2 [1,5]. Unwanted chemicals present within the formulation and active pharmaceutical ingredient which affects the quality, safety, and efficacy of the medicinal products are called impurity. A significant aspect of ensuring the safety of medicinal products is the qualification of impurities. [4] Literature survey revealed that few analytical methods have been reported for the estimation of deferiprone alone or in combination with other drugs by ultraviolet (UV) spectrometry high-performance liquid chromatography (HPLC) and LC-mass spectrometry. However, there is no reported method about the separation and determination of deferiprone impurity. Hence, an attempt was made to develop simple, accurate, precise, and sensitive HPLC method for estimation of deferiprone in the presence of its above-mentioned impurity.

METHODS:

Chemicals and reagents:

Deferiprone and its impurity were obtained as a gift sample from Adhar life science Pvt.Ltd Solapur. Kelfer capsules were purchased from local pharmacy, Solapur, Maharashtra. Analytical grade Methanol, O-Phosphoric acid and water were also available from Adhar life science Pvt.Ltd Solapur.

Table 1: Chemical name and structure of deferiprone and its related impurity [6,7]

Sr.no.	Name of compound	Chemical Structure	Molecular formula	IUPAC name
1	Deferiprone		[C7H9NO2]	3-hydroxy-1,2-dimethyl pyridine-4-one
2	Impurity [Maltol]		[C6H6O3]	3-Hydroxy-2-methyl-4*H*-pyran-4-one

Instrumentation and conditions:

The chromatographic column used for separation was Agilent Zorbax Bonus-RP (250 x 4.6 mm, 5 µ). The mobile phase used for the separation of both API and impurity was 0.1%ml of ortho-phosphoric acid and make the volume with 1000 ml of Water: methanol (50: 50v/v). Ambient temperature was maintained. Detection was made at a wavelength of 280.0 nm. The validation study was carried out using same optimized condition with suitable preparation of standard and sample solutions.

Standard Preparation:

Deferiprone Standard Stock Solution-I (DSSS-I):

Initially Prepare a Standard Stock Solution (SSS-I) of by adding 5 mg of Deferiprone in 10 ml volumetric flask & add 5 ml diluent, mix for 2 minutes and make the volume to 10 ml with diluent. (Conc. of Deferiprone = 500 µg/ml).

Impurity Standard Stock Solution-I (ISSS-I):

Then prepare a Standard Stock Solution (SSS-II) of impurity [Maltol] by adding 5 mg in 10 ml volumetric flask & add 5 ml diluent, mix for 2 minutes and make the volume to 10 ml with diluent. (Conc. of Maltol = 500 µg/ml).

Preparation of resolution solution:

Then add 1.0 ml of DSSS-I & 1.0 ml ISSS-I in 10 ml volumetric flask and add 5 ml diluent and vortex and make up the volume with diluent. (Conc. of Deferiprone =50 µg/ml & Maltol = 50 µg/ml).

Further, pipette out 1 ml of above solution in 10 ml volumetric flask and add 5 ml diluent and vortex and make up the volume with diluent. (Conc. of Deferiprone =5 µg/ml & Maltol = 5 µg/ml).

Preparation of sample solution:

10 Capsule contents were weighed and average weight was calculated and power/granules was crushed & mixed in mortar and pestle. Powder weight equivalent to 5 mg Deferiprone was weighed into 10 ml volumetric flask & add 5 ml diluent, sonicate for 10 minutes and make the volume to 10 ml with diluent. (Conc. of Deferiprone =50 µg/ml). Further, pipette out 0.1 ml of above solution in 10 ml volumetric flask and add 5 ml diluent and vortex and make up the volume with diluent. (Conc. of Deferiprone =5 µg/ml).

Method Validation:

The method validation was performed as per the international conference on harmonization (ICH) guidelines [8]. The parameters such as specificity, linearity and range, precision, accuracy, limit of detection (LOD), limit of quantitation (LOQ), were evaluated.

Specificity:

The specificity was demonstrated by injecting deferiprone standard solution, blank solution and sample spiked with impurity solution, and the chromatograms were checked for

interferences.[9]

Accuracy:

The accuracy of an analytical procedure is the closeness of the test results. Accuracy is expressed as % recovery of the standard spiked to already analyzed test sample of capsule. (10) It was measured in drug products by spiking known quantities (80%, 100%, and 120%) of the analyte into the analyzed tablet powder and each concentration was injected into the column for 3 times and percent recovered was calculated.

System suitability:

System suitability studies form an integral part of method development and ensures good performance of chromatographic system. The standard solutions of deferiprone [5 µg/ml]and maltol impurity [5 µg/ml] of about 10 µl were injected under optimized chromatographic conditions to evaluate the suitability of the system.

Precision:

The precision was studied by repeatability or reproducibility and intermediate precision (11). The precision was checked by injecting the sample solution spiked with impurities in six replicates, and the intermediate precision was evaluated by different analyst using different columns on different days. The %RSD of % total impurity was calculated.

Linearity and range:

Linearity was checked for standard solutions of drug and impurity at concentrations of 80%, 90%,100%, 110%and 120%. Aliquot solutions of deferiprone and Maltol impurity were prepared in the range of 4.0-6.0 µg/ml respectively. The chromatographic system was set to equalize and samples of study were injected, keeping the injection volume constant, i.e., 10 µl.

Limit of detection and limit of quantitation:

LOD is defined as the lowest concentration of an analyte in the sample that an analytical method differentiates from background levels (12). The LOQ is defined as the lowest concentration of an analyte in the sample that can be measured with acceptable accuracy, precision, and variability. The LOD and LOQ were calculated from the linearity curve by applying the formulae:

LOD= 3.3 S / σ

LOQ = 10 S / σ

Where, σ is the standard deviation of the y-intercept and

S is the slope of the calibration plot**.**

Table 2: Optimized chromatographic conditions

Sr.no.	Parameters	Results
1	Dilution	Methanol: Water (50: 50, % v/v)
2	Mobile phase	Methanol: 0.1% O-Phosphoric acid (10:90, % v/v)
3	Column	Agilent Zorbax Bonus-RP (250 x 4.6 mm, 5 µ)
4	Flow rate	1 ml/min
5	Detection	280 nm
6	Injection volume	10µl
7	Temperature	30℃
8	Retention time	2.29 min for deferiprone and 8.65 min for maltol impurity
9	Run time	11 minutes

RESULT:

Method development:

Analytical detection wavelength of 280 nm was selected for the proposed HPLC method based on the UV absorption of deferiprone and its impurities. the separation of the impurity from the drug was achieved with Agilent Zorbax Bonus-RP (250 x 4.6 mm, 5 µ) column and with a mobile phase Methanol: 0.1% O-Phosphoric acid (10:90, % v/v), and the peak shape of the drug and the impurity was good. The optimized parameters were listed in Table 2. Chromatogram for standard solutions of deferiprone and maltol impurity was presented in Figs. 1.

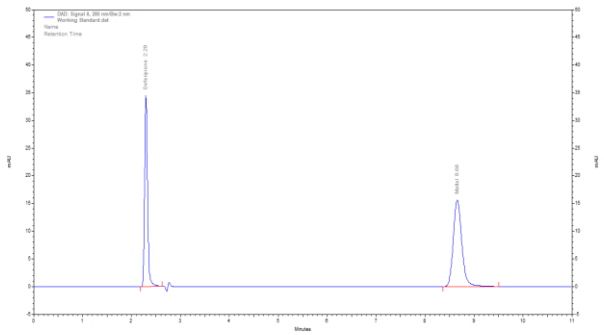


Fig.1: Chromatogram of resolution for deferiprone and impurity.

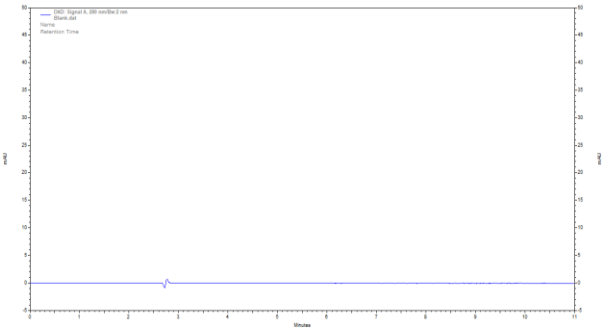


Fig.2: Chromatogram of the blank solution.

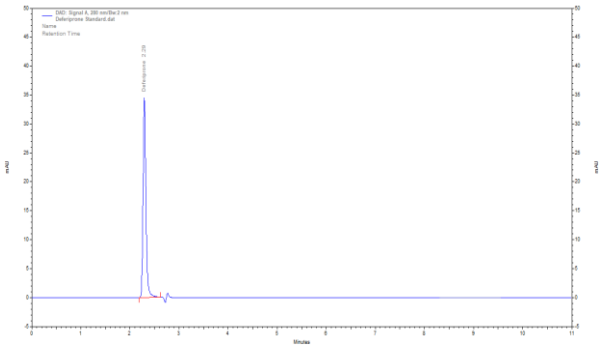


Fig.3: Chromatogram for standard solution of deferiprone.

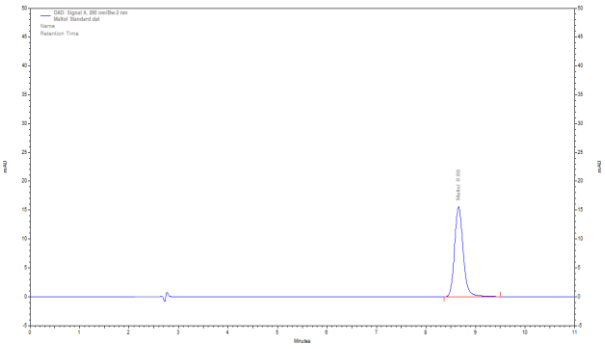


Fig.4: Chromatogram for standard solutions of impurity.

Specificity:

The specificity of the method was tested by comparing the chromatograms of blank (Fig. 2), deferiprone standard solution (Fig. 3), and impurity solution (Fig. 4). No interference peaks were observed at the deferiprone RT of due to the blank, impurities, and placebo.

Accuracy:

The accuracy of the proposed method was determined by analyzing the deferiprone sample solution spiked with impurities at three concentration levels of 80%, 100%, and 120% of each impurity in triplicate. The mean percentage recovery was calculated and reported in Tables 3 and 4.

Table 3: Accuracy study of deferiprone.

Sr.no	% Level	Area	% Recovery	AVG
1	80%	238021	100.47	100.52
		238154	100.53	
		238177	100.54	
2	100%	298091	100.67	99.96
		294473	99.44	
		295471	99.78	
3	120%	359095	101.06	100.85
		358045	100.76	
		357953	100.73	

Table 4: Accuracy study of Impurity.

Sr.no	% Level	Area	% Recovery	AVG
1	80%	316121	99.82	99.85
		316275	99.87	
		316298	99.87	
2	100%	395689	99.95	99.93
		395712	99.96	
		395354	99.87	
3	120%	473682	99.71	99.66
		473183	99.61	
		473384	99.65	

Table 5: System suitability parameters.

Sr.no.	Parameters	Deferiprone	Impurity maltol
1	Retention time	2.29	8.65
2	Theoretical plate	7737	11765
3	Tailing factor	1.24	1.17
4	Resolution	0.00	30.03

System suitability studies:

system suitability parameters such as the number of theoretical plates, tailing factor, and the resolution were calculated. The results are listed in Table 5.

Precision:

Precision of the method by repeatability and intermediate precision was assessed by injecting sample solution spiked with impurities six times, and the results were expressed in terms of %RSD of the percentage of total impurities. The results are presented in Tables 6,7 and chromatogram were presented in Figs. 5 and 6.

Linearity:

The linearity graph was plotted by taking the concentration in the x-axis and peak area in y-axis over the calibration ranges tested for deferiprone and impurities. Results are shown in Table 8, and the linearity curves are shown in Figs. 7 and 8.

Limit of detection (LOD) and limit of quantitation (LOQ):

LOD and LOQ for deferiprone were found to be 0.27 ug/ml and 0.82 ug/ml. LOD and LOQ for maltol impurity were found to be 0.24 ug/ml and 0.74 ug/ml respectively.

Table 6: Precision studies for standard solutions of Deferiprone.

Sample ID	Area	RT
100%Rep 1	298091	2.29
100%Rep 2	294473	2.29

100%Rep 3	295471	2.29
100%Rep 4	297344	2.29
100%Rep 5	295478	2.29
100%Rep 6	295867	2.29
AVG	296121	2.29
STDEV	1341.119	4.86E-16
%RSD	0.45	0.00

Table 7: Precision studies for standard solution of Impurity Maltol.

Sample ID	Area	RT
100%Rep 1	395689	8.66
100%Rep 2	395712	8.66
100%Rep 3	395354	8.66
100%Rep 4	395121	8.66
100%Rep 5	396671	8.66
100%Rep 6	396713	8.66
AVG	395877	8.66
STDEV	668.875	1.95E-15
%RSD	0.17	0.00

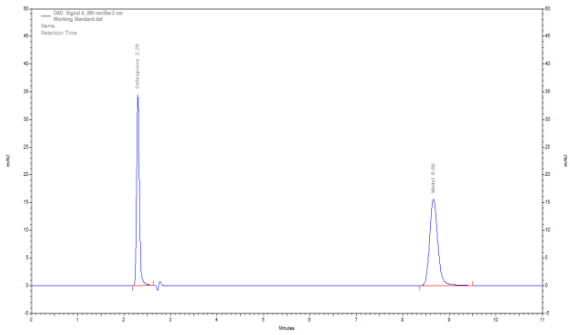


Fig.5: Precision chromatogram for standard solution of Deferiprone and Impurity Maltol.

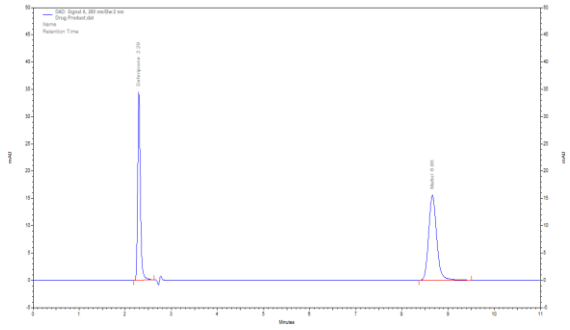


Fig.6: precision Chromatogram for sample solution.(Kelfer)*

Table 8 Linearity studies for deferiprone and maltol impurity.

Sr.no.	Area of deferiprone	Area of maltol
1	238021	316121
2	265596	355454
3	298091	395689
4	330319	438212
5	359095	473682

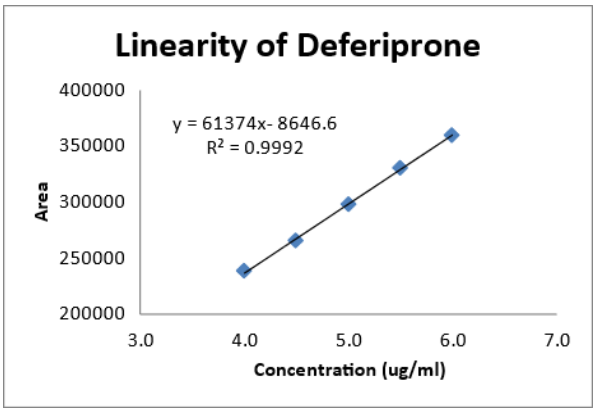


Fig.7: Calibration curve for deferiprone.

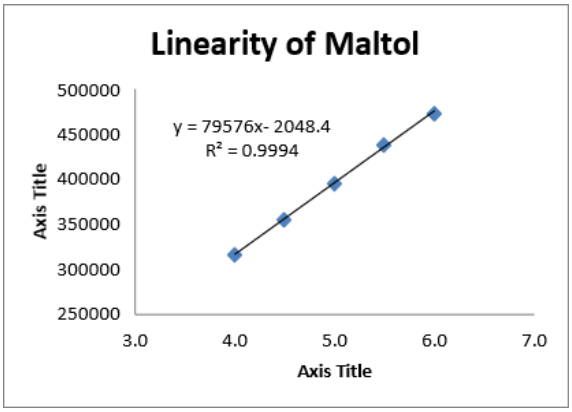


Fig.8: Calibration curve for maltol.

DISCUSSION:

Various solvent system combinations for the determination of deferiprone and its related impurity (maltol) in bulk and pharmaceutical dosage forms were studied and finally a mixture 0.1% O-Phosphoric acid (10:90, % v/v) in 1000ml of water and methanol was selected as mobile phase as it gave better resolution. The effect of inflow rate was studied in 1 ml/min was preferred. The retention time (RT) was found to be 2.29 min for Deferiprone and 8.65 min for maltol impurity. The chromatographic system is considered suitable when it obtains the following criteria. The resolution between the peaks must be >2, the number of theoretical plates should be more than 2000, and tailing factor must be lower than 2. as per ICH guidelines [11]. All the parameters like retention time (RT), number of theoretical plates (TP), tailing

factor (T), and resolution were within the acceptable limits, so the optimized method is suitable for analysis of both compounds. specificity study meets the acceptance criteria [8], as that no interference was observed from the blank at the RT of known impurities. It is evident that from the got data that all the peaks were well resolved, and the method is said to be specific. Percent recovery was found to be 100.52, 99.96%, and 100.85% for drug and 99.85%, 99.93%, and 99.66% for related substance at 80%, 100%, and 120% respectively. All experimental results are in the acceptable criteria, i.e., 97-102% for drug and 50-150% for related substance and the method was found to be accurate. The % RSD values for the peaks were found within the limits, RSD ≤ 2 [13] as shown in results, so the method was found to be precise. A calibration curve was met by plotting a graph between peak area and concentration. Excellent correlation was obtained between peak area and concentration with R2 = 0.9992 for deferiprone and 0.9993 for maltol impurity as per the limit R2 > 0.999 [12]. The LOD and LOQ were calculated from the slope of regression equation gained from calibration curve and standard deviation was taken from precision studies. The result obtained was 0.27 ug/ml and 0.82 ug/ml for deferiprone and 0.24 ug/ml and 0.74 ug/ml for maltol impurity respectively.

CONCLUSION:

The method proposed for the analysis of deferiprone and related impurity in bulk and pharmaceutical dosage forms was found to be specific, precise, accurate, quickly, and economical. The developed method was validated in terms of accuracy, linearity, LOD, LOQ, and precision in accordance with ICH guidelines. Short retention time enabled analysis of deferiprone and maltol impurity with minimal amount of mobile phase. The method was found to be precise and accurate. Due to quantitation limits and low detection, the method was said to be sensitive. This method can be applied successfully for the determination of deferiprone and its related maltol impurity in bulk and pharmaceutical dosage forms.

Authors' contributions:

The research work, manuscript preparation, and grammar check using the software Grammarly were done by Miss. SHWETA UBALE, the research work was guided by Dr. S.K. PARJANE, and critical revision and final proofreading of the manuscript were done by Mrs. M. S. BHOSALE.

Abbreviations:

LOD: Limit of detection; LOQ: Limit of quantitation; RSD: Relative standard deviation; DSSS-I: Deferiprone Standard Stock Solution-I; ISSS-I: Impurity Standard Stock Solution-I.

Acknowledgements:

I thank Adhar life science Pvt.Ltd Solapur laboratories, for providing a gift sample of deferiprone and Maltol. I thanks to my guide and co-guide for support me and help me and I also thanks to my family.

Competing interests:

The authors declare that there are no conflicts of interest.

REFERENCES:

1. P. Vamsi Reddy*, et.al., Stability indicating method development and validation of Deferiprone in pharmaceutical dosage form by RP-HPLC, Journal of Innovations in Pharmaceutical and Biological Sciences, Vol 4 (2), 53-57, 2017.
2. Thalassemia foundation of Canada. Thalassemia Major « Thalassemia;2022.
3. FDA Approved Drug Products: Ferriprox (deferiprone) oral tablets;1-16;2011. https://www.accessdata.fda.gov/drugsatfda_docs/label/2021/021825s010lbl.pdf.
4. Federal Register International Conferences on Harmonization. Draft Revised guidance on Impurities in New Drug Substances, Q3A(R); 2000. p. 45085-90
5. Manzoor Ahmed, Vinod Kumar Jangade*, et.al., RP-HPLC method development and validation for estimation of deferiprone in capsule dosage form, world journal of pharmacy and pharmaceutical science, 2015;831-838.
6. K. Sai Sireesha, et.al., A new RP-HPLC method development and validation of deferiprone in bulk and its pharmaceutical dosage form, international journal of advance research,2016;2174-2179.
7. Introduction to Food Toxicology A volume in Food Science and Technology Book; Chapter 7 - Food Contaminants from Industrial Wastes;1Pages 117-140.
8. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use. Validation of Analytical Procedures: Text and Methodology ICH Q2 (R1); 2005.
9. Daksh S, Goyal A, Pandiya CK. Validation of analytical methods strategies and significance. Int J Res Dev Pharm Life Sci 2015; 4:1489-97
10. Guidance Document, Validation of Analytical Methods, Document ID IPC/GD/04 Version 1.0 Issue Date 16th September 2021;1-13.
11. ICH guidelines validation of analytical procedures; Text & methodology Q2 R1;2005.

12. David A Armbruster1*Limit of Blank, Limit of Detection and Limit of Quantitation; PubMed; National Library of Medicine; 2008 Aug; 29(Suppl 1): S49–S52.
13. United States Pharmacopeia (USP) 34 (NF 29), Chapter 621, Edition 2011.
14. European medical agency withdrawal assessment report of deferiprone;2020;13-18; "UPKANZ, INN-deferiprone" (europa.eu).

Author biography

Shweta Ubale, Student.

Mayur Bhosale, Professor.

Dr. S. K. Parajne, Professor.

Cite this article: Shweta Ubale , Mayur Bhosale1, Dr. S. K. Parajne1, Development and Validation of RP HPLC Method for Estimation of Deferiprone and Its Related Impurity in Pharmaceutical Dosage Form. *International Journal of Current Research and Innovation in Pharma Sciences, 2022;1(1):22-28*