Method Development and Validation of Stability Indicating RP- HPLC Method Using Iron Chelating Agent

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Abstract

The ideal of this present is to develop a simple, precise, accurate stability indicating system for the estimation of deferiprone in expression by using RP- HPLC.

The system was validated for delicacy, perfection, particularity, linearity and perceptivity. The total chromatographic analysis time per sample was about 5 min with deferiprone eluting at retention time of about 4.980 min.

The system was validated as per ICH companion lines. Stability studies reported absence of contaminations at the peak retention time. The medicine was stable to different conditions like acidic, alkali, thermal, oxidative conditions.

Confirmation studies demonstrated that the proposed HPLC system is simple, specific, rapid-fire, dependable and reproducible. The standard angles were direct over the attention range of 75- 125µg/ ml. The LOD and LOQ values for deferiprone were 0.14 and 0.45 µg/ mL, independently. The chance recovery was set up to be 0.77, 0.407, and 0.7023 at 80, 100, and 120 independently and the RSD for perfection was set up to be 0.78, 0.408, and 0.7028 independently.

The high recovery and low relative standard divagation confirm the felicity of the proposed system for the determination of deferiprone in bulk and capsule lozenge forms.

Keywords: RSD: Relative standard deviation, LOD: Limit of detection, SD: Standard deviation ICH: International Conference on Harmonization, UV: Ultraviolet, Rt: Retention Time, API: Active Pharmaceutical Ingredient, LOQ: Limit of Quantitation, RP: HPLC: Reversed Phase High Performance Liquid Chromatography, SIAM: Stability indicating assay method

Introduction

Analytical system development and confirmation are the crucial rudiments of pharmaceutical development program. Effective logical system ensures the optimization of coffers, system develops, insure the identity, chastity, energy, of the medicine that we use. The system may support the safety and characterization studies or evaluation of medicine performances.

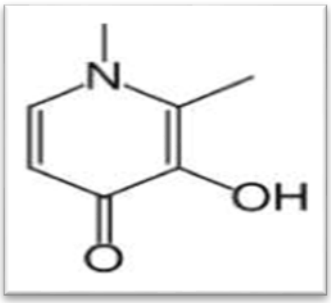
Pharmaceutical analysis plays a veritably important part in examination of pharmaceutical expression and bulk medicine related to the quality control and assurance. The rapid-fire increase in the pharmaceutical field and product of medicine in around the world brings forward a rise in ineluctable demand to seek new and methodical logical fashion in the pharmaceutical assiduity. The development in scientific and logical styles has been redounded from the advancement of logical instruments. The enhancement in logical system development and logical instruments should develop a cost effective and lower time consuming system. The ways for analysis are developed and validated for API, affiliated substances, medicine products, declination products and, residual detergents, etc.

Analytical system development eventually published in sanctioned test styles. also the quality control laboratories use these styles to estimate the identity, chastity, safety as well as performance of expression of the medicine. Analytical chemistry more simply Analysis is understood as an examination of a chemical substance with the thing of inspiring information regarding its ingredients their character form, quality, or chastity and volume also known as attention or content. The Analysis is a crucial element of the advanced technologies in determining and optimizing the attention of substances by opting a suitable logical system. By this, we can gain both qualitative as well as quantitative analysis. The logical system may be spectral, chromatographic, electrochemical, hyphenated or eclectic. The logical instrument plays a significant part in the process to achieve high quality and dependable logical data.

Analytical system development is the process of developing an accurate assay procedure to determine the composition of an expression or in a bulk of the specified substance. It's the process of proving that an logical system is respectable for use in a laboratory to measure the attention of posterior samples. Analytical styles should be used within criteria given as per nonsupervisory authorities and must be developed using the protocols and acceptance criteria as per the nonsupervisory guidelines.

❖ EXPERIMENTAL WORK

➢ Drug Profile



Chemical Structure of Deferiprone

DEFERIPRONE

IUPAC name	3-hydroxy-1,2-dimethylpyridin-4(1H)-one
Description	Solid
Molecular Weight	139.152 g·mol−1
Molecular Formula	C7H9NO2
Melting Point	272^0C-278^0C
Category	Iron chelating agent
Mode of Action	A chelating agent with an affinity for ferric ion (iron lll). Binds with ferric ion to form neutral 3:1 (deferiprone: iron) complex that are stable over a wide range of pH values.
Solubility	Soluble in methanol and maximum solubility in aqueous buffers.

Use	Used to remove excess iron in the body in people who have received a large number of blood transfusions to treat thalassemia and who have not benefitted enough from other treatments for excess iron.
Adverse Effects	Nausea, vomiting, abdominal pain or joint pain occurs. This effect is harmless and will disappear when the medication is stopped.

MATERIALS AND MATERIALS

Reference Standard

The following reference standard was used during the project work and is enlisted in Table

Table 6.1: Details of Reference Standard Used

Sr. No.	Name of Standard	Gift sample supplier	Purity
1	Deferiprone USP	Althos Chemical Pvt. Ltd. Morabhagal, Surat, Gujarat.	99.98%

Marketed Formulation

The following marketed formulation was used during the project work and is enlisted in Table No.6.2.

Table 6.2: Details of Marketed Formulation Used

Sr. No	Particulars	Details
1	Brand Name	Ferriprox 10 mg Tab
2	Manufactured by	Althos Chemical Pvt. Ltd. Morabhagal, Surat, Gujarat.
3	Content	Each tablet contains: Deferiprone USP…. 10mg Excipients…….q.s.
4	Colour	White
5	Average Weight	139.152g/mol

- **HPLC Instrumentation & Condition**

6.1 Instruments

The following instruments were used and are enlisted

Table 6.3: Details of Instrument Used

Sr. No	Name of Instrument	Make	Model

1	UV-Visible Spectrophotometer	Shimadzu Lab Solutions	UV-2600i (UV-2700i)
2	HPLC System	Waters Alliance and Agilent HPLC System with Empower & Chromeleon Software	Waters Alliance HPLC e2695 Series and Agilent HPLC 1260 Infinity
3	Analytical Balance	Mettler Toledo Balance and Sartorius Balance	Mettler Toledo Balance XSR603S & Sartorius Balance BSA224S-CW.
4	Sonicator	PCI Analytics Ultra Sonic Bath	WUC-4L

6.1 Chemicals and Reagents

The following chemicals and reagents were used and are enlisted in Table No 6.4.

Table 6.4: Details of Chemicals and Reagents used

Sr. No	Reagent	Grade	Manufacturer/Supplier
1	Methanol	HPLC Grade	JT Baker
2	Water	Milli-Q	Milli-Q
3	Hydrochloric acid	Laboratory Grade	Rankeme
4	Sodium hydroxide	Laboratory Grade	Merck Pvt. Ltd.
5	Hydrogen peroxide	Laboratory Grade	Merck Pvt. Ltd.

Analysis of Marketed Formulation Preparation of standard solution

400 µL of stock solution was pipette out and transferred to 10 mL of volumetric flask and then 5 mL of a mobile phase (solvent system) was added into it. The final volume was made up to the mark with selected mobile phase and the volumetric flask labeled as a standard solution for assay.

Sample preparation

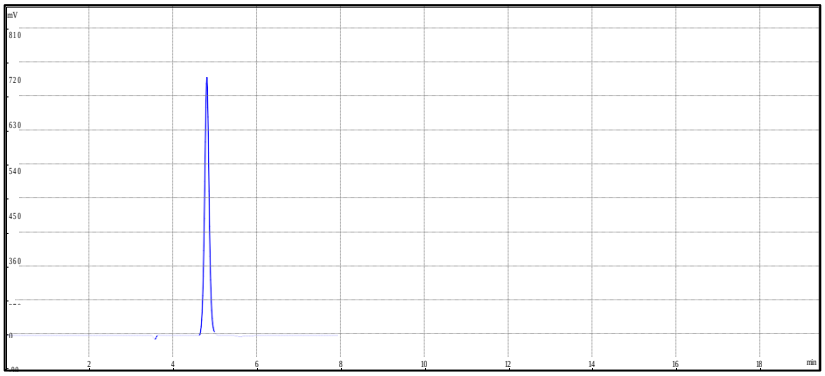
a. Twenty tablets of Deferiprone were weighed individually and the average weight of Deferiprone tablet was calculated accordingly (avg. wt. 0.2013 gm). Afterward the tablets were crushed and ground to fine powder using mortar and pestle.

b. An accurately weighed quantity of 0.2013g (equivalent to 10 mg of Deferiprone) tablet powder was transferred into 10 mL of volumetric flask containing about 8 mL of the mobile phase and the resultant solution was sonicated for 20 min with intermittent shaking, the final volume was made upto the mark with selected mobile phase; then final solution was centrifuged at 5000 RPM to settle the undissolved powder and pipette out 0.4 mL of the resultant solution from the above supernatant into a 10 mL of

volumetric flask and diluted it with selected mobile phase upto the mark, then the solution was filtered through 0.45µ membrane syringe filter-media to get concentration of 40 ppm solution of Deferiprone tablet sample.

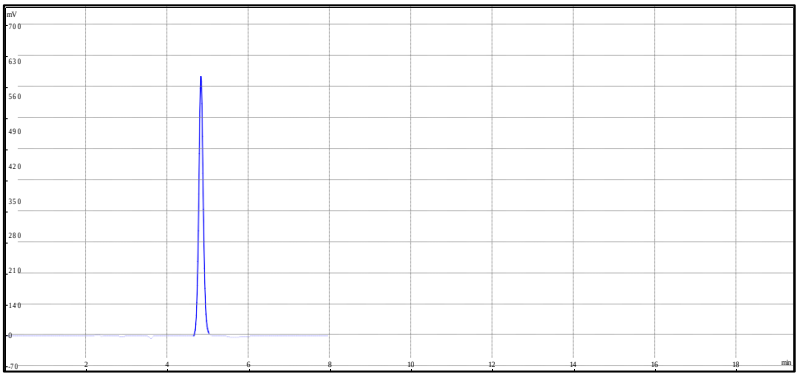
Procedure

Equal volumes (20 µL) of standard and sample solution were injected separately after equilibration of stationary phase, the sample solution was injected three times and the chromatograms were recorded. Finally, the percentage of drug in a tablet was calculated. The standard and sample solutions chromatograms are shown



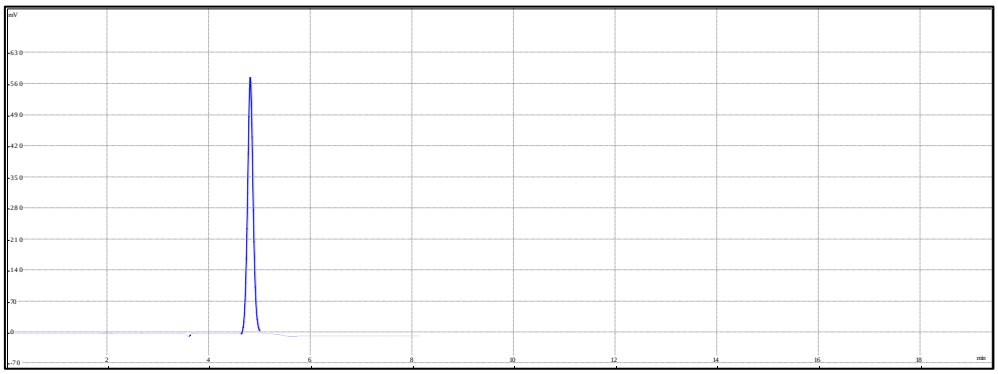
Rt	Peak area	Plate number	Asymmetric factor
4.767	5659586	8923	1.04

Fig 1.Chromatogram of standard solution of Deferipron



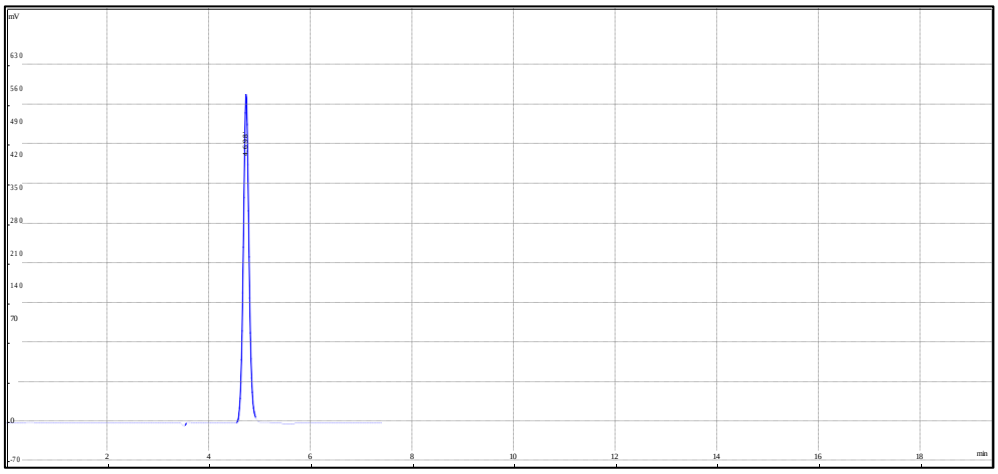
Rt	Peak area	Plate number	Asymmetric factor
4.797	5654265	7919	1.06

Fig2. Chromatogram of sample solution of Deferiprone



Rt	Peak area	Plate number	Asymmetric factor
4.783	5663804	8177	1.06

Fig 3.Chromatogram of sample solution of Deferiprone



Rt	Peak area	Plate number	Asymmetric factor
4.698	5668845	7769	1.06

Fig 4. Chromatogram of sample solution of Deferiprone (3)

Table : Result of assay

Parameters	**Deferiprone**
Sample area	5654265
	5663804
	5668845
Mean sample area	5662304
Standard area	5659586
% Assay	99.88%
Label claim found	9.98

6.5 Accuracy study

The recovery study was perform to evaluate the developed method was accurate for the analysis of Deferiprone. The 80%, 100% and 120% levels of recovery study were selected to perform the recovery study.

Preparation of test samples

The test samples for recovery study were prepared according to Table 6.15.

Table 6.14: Preparation of % recovery samples

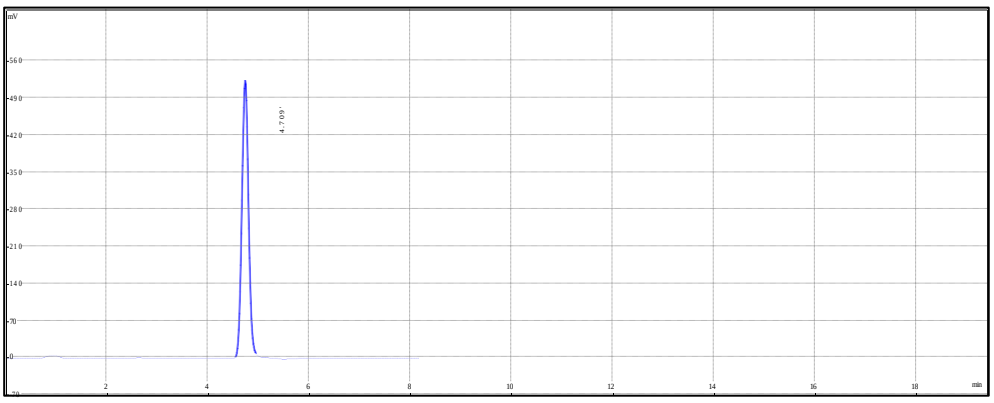
Sr. No	Level of % recovery	Amount of tab taken(µg/mL)	Amount of Std added(µg/mL)	Total Amount (µg/mL)
1	80	20	16	36
2	100	20	20	40
3	120	20	24	44

Preparation of standard solution

The solution of standard Deferiprone in concentration of 36, 40 and 44 ppm were prepared by dilution of stock solution for the evaluation of % recovery.

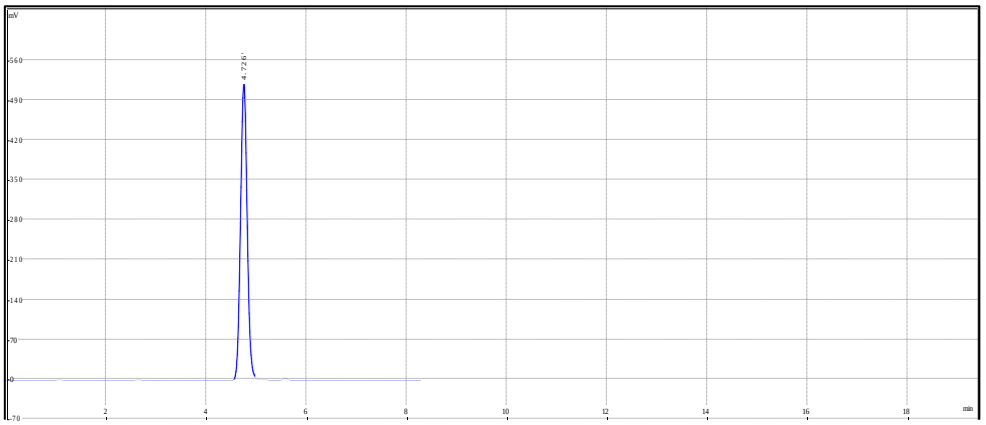
Procedure

Equal volume (20 µL) of test sample and standard solution of each level of % recovery were injected into three replicates and then chromatograms were recorded. The % recovery of each level was calculated and the standard deviations followed by % RSD of recovery study were reported



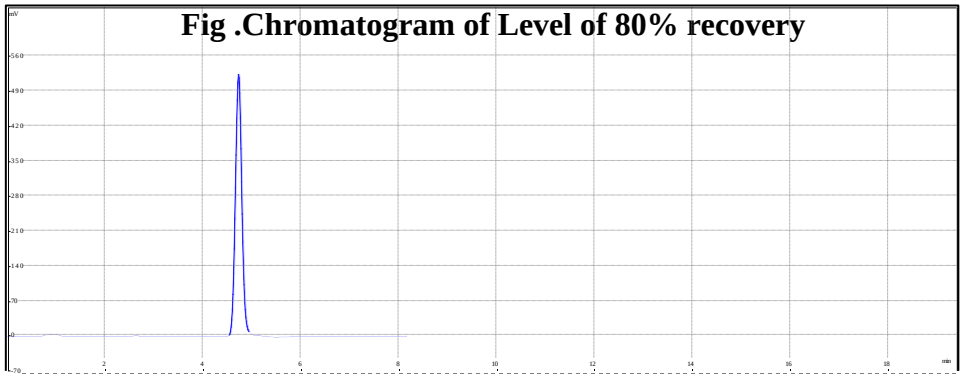
Rt	Peak area	Plate number	Asymmetric factor
4.709	5054364	7857	1.04

Fig.Chromatogram of Level of 80% recovery



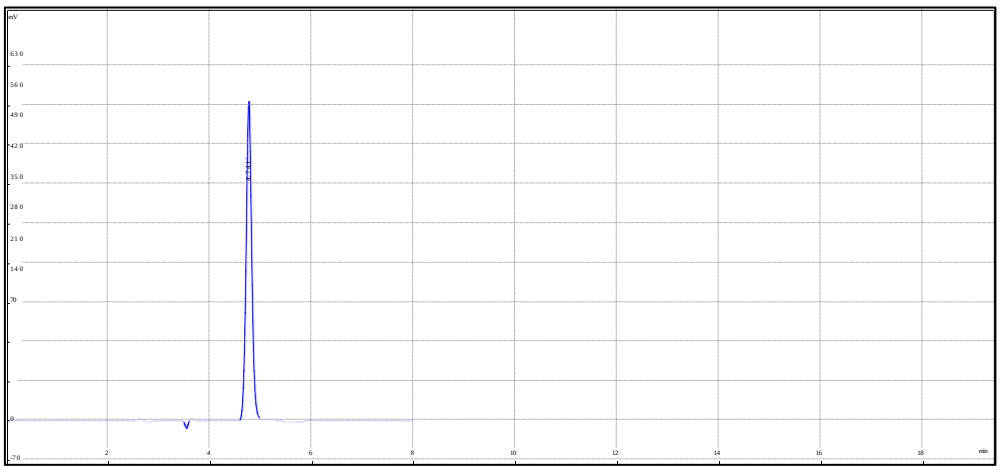
Rt	Peak area	Plate number	Asymmetric factor
4.726	5128132	7961	1.06

Fig .Chromatogram of Level of 80% recovery



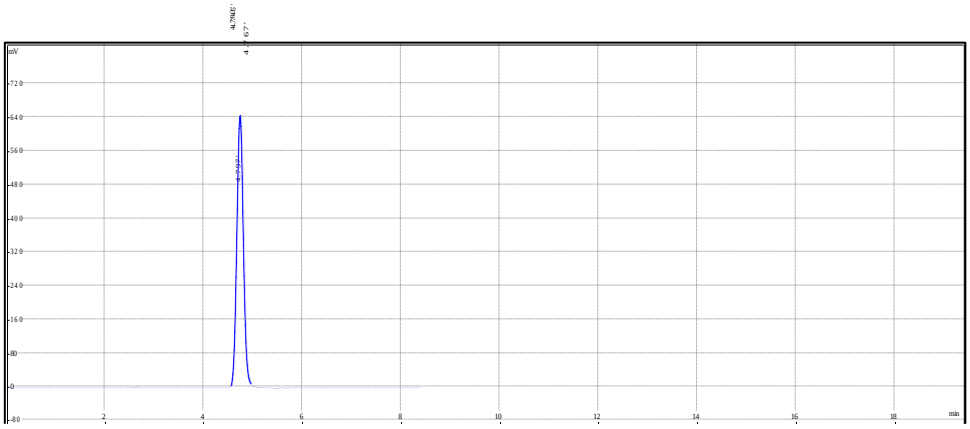
Rt	Peak area	Plate number	Asymmetric factor
4.709	5130064	7835	1.06

Fig: Chromatogram of Level of 80% recovery (3)



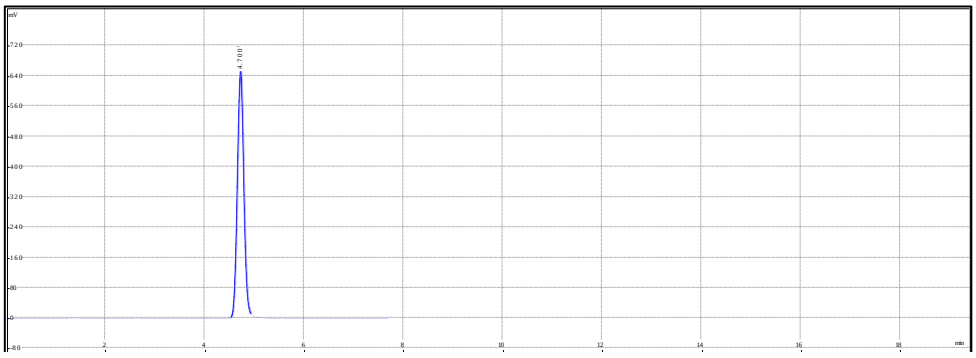
Rt	Peak area	Plate number	Asymmetric factor
4.741	5610694	7922	1.06

Fig : Chromatogram of Level of 100% recovery (1)



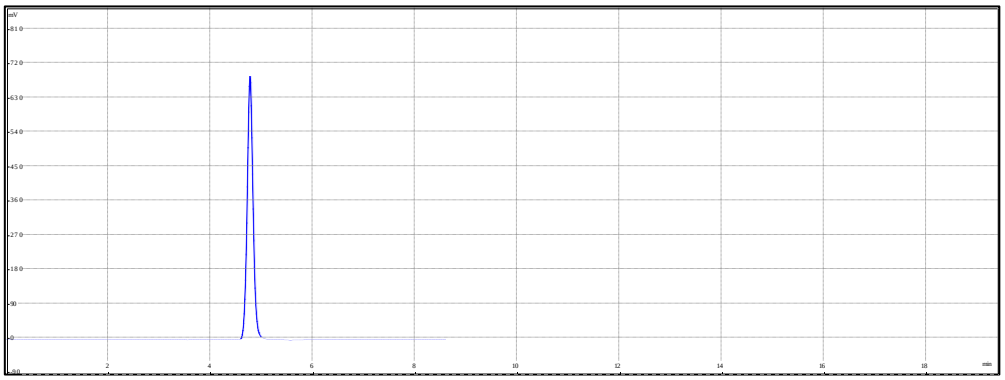
Rt	Peak area	Plate number	Asymmetric factor
4.713	5654733	7799	1.06

Fig: Chromatogram of Level of 100% recovery (2)



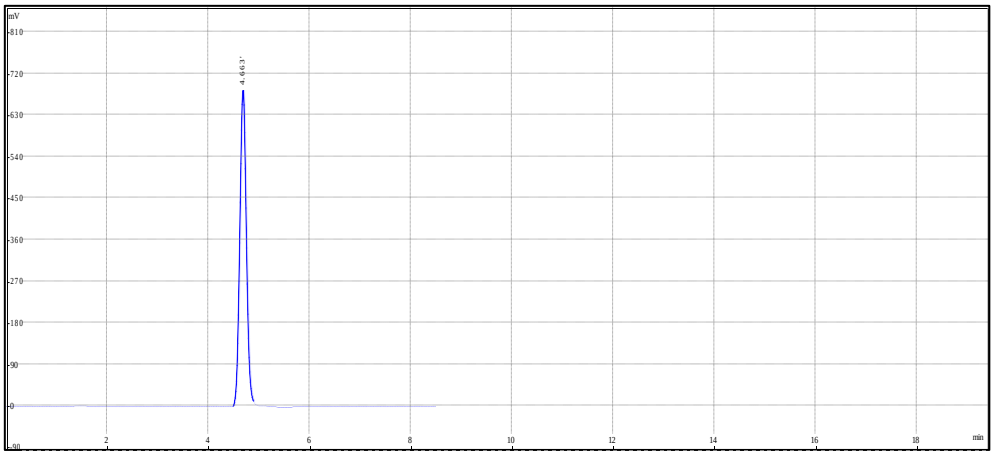
Rt	Peak area	Plate number	Asymmetric factor
4.700	5650492	7938	1.06

Fig : Chromatogram of Level of 100% recovery (3)



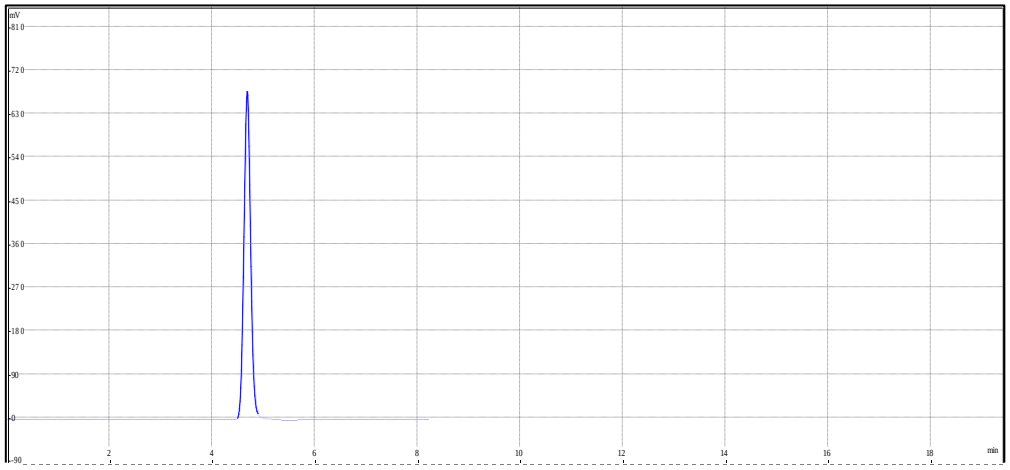
Rt	Peak area	Plate number	Asymmetric factor
4.745	6263775	7917	1.07

Fig: Chromatogram of Level of 120% recovery (1)



Rt	Peak area	Plate number	Asymmetric factor
4.663	6271198	7928	1.07

Fig: Chromatogram of Level of 120% recovery (2)



Rt	Peak area	Plate number	Asymmetric factor
4.659	6270865	7820	1.07

Fig 6.28: Chromatogram of Level of 120% recovery (3)

Level of % recovery	**Amt of sample taken (µg/mL)**	**Amt of STD added (µg/mL)**	**Total amount (µg/mL)**	**% recovery**	**Total Amount Found (µg/mL)**
80%	20	16	36	98.61	35.49
	20	16	36	99.97	35.98
	20	16	36	99.95	35.98
100%	20	20	40	99.29	39.71
	20	20	40	100.04	40.01
	20	20	40	99.94	39.97
120%	20	24	44	99.87	43.94
	20	24	44	99.93	43.96
	20	24	44	100.01	44.00

Table 6.15: Result of % recovery test

Table: Statistical result of accuracy

Level of % Recovery	Mean % recovery	SD	%RSD
80%	99.51	0.770	0.780
100%	99.75	0.407	0.408
120%	99.93	0.702	0.702

6.5 Precision

The precision of a method was carried out in a two parts such as Interday precision and Intraday precision. The 30 ppm standard Deferiprone solution was suitably selected for method repeatability.

Interday precision

The Interday precision was carried in two different consecutive days. In day 1, three replicates of standard solution were injected and the chromatograms were recorded, In day 2, injected the same standard solution in three replicates which was carried out in day 1, the % RSD of peak areas of the six chromatograms were reported for the fulfilment the accepted criteria. The results were shown as follows:

Table 6.17: Result of Interday precision

	ncentration (µg/mL)	Peak area
Day 1	30	4278925
	30	4272000
	30	4269134
Day 2	30	4279126
	30	4286916
	30	4281753
	Mean	4281753
	%RSD	0.15

Interday precision

The Intraday precision was carried in two different time period. In Morning, three replicates of standard solution were injected and the chromatograms were recorded, In Evening, injected the same standard solution in three replicates which was carried out in Morning, the % RSD of peak areas of the six chromatograms were reported for the fulfilment the accepted criteria.
The results were shown as follows:

Table Result of Intraday

	ıcentration (µg/ml)	Peak area
Morning	30	4271753
	30	4275822
	30	4269410
Evening	30	4281119
	30	4274775
	30	4272163
	Mean	4274174
	%RSD	0.10

Specificity

For the specificity study, solutions of blank, sample and standard solution were used, the standard and sample solutions of 40 ppm were used and each solution were injected into the system and chromatograms were recorded. It was found that the no interference from impurity and excipients.

Table: Result of robustness (change in flow rate)

Changed condition	Peak area
0.6 ml/min	2891426
0.8 ml/min	2896228
1.0 ml/min	2897348
Mean	2895001
%RSD	0.10

Ruggedness

The ruggedness or Intermediate precision of a method was assessed to check the effect of change in analyst on analysis of Deferiprone, the linearity as a method validation parameter was repeated by second/other analyst to verify the ruggedness, and % RSD was calculatedThe % RSD was found to be 0.070 which is under accepted criteria developed method was rugged.

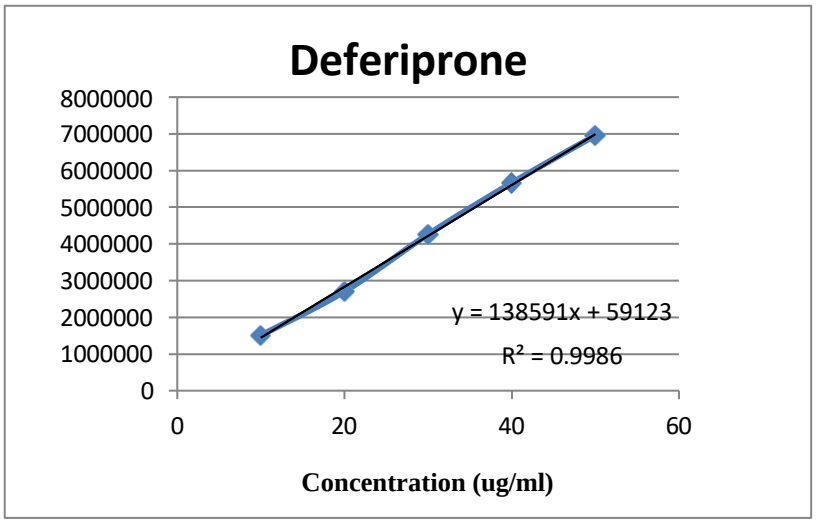


Table : Result of ruggedness

Analyst-1		**Analyst-2**	
Concentration (µg/mL)	**Peak area**	**Concentration (µg/mL)**	**Peak area**
10	1509903	10	1505313
20	2898763	20	2703320
30	4275906	30	4258983
40	5659586	40	5660353
50	6954494	20	6956371
R^2	0.999	R^2	0.998
	%RSD	0.070	

LOD and LOQ

The limit of detection and limit of quantitation were calculated on the basis of standard deviation of the accuracy response and slope of the linearity calibration curve.

Formulas;

$$LOD = 3.3 \times \frac{SD}{Slope}$$

Forced Degradation Study: In order to establish the forced degradation study, the standard API was subjected in to the various stress conditions as follows:

Result of forced degradation study

Sr. No.	Stress Condition	% degradation
1	Alkaline condition	6.21%
2	Acidic condition	1.17%
3	Oxidative condition	3.18%
4	Thermal condition	3.54%

RESULT, DISCUSSION AND CONCLUSION

The present study included development of stability indicating RP-HPLC method for estimation of Deferiprone and the results obtained were found to be satisfactory. The work was started with solubility testing; the Deferiprone was soluble in water, methanol and acetonitrile. Then the wavelength was selected by UV spectrophotometric method in methanol: water (80:20 v/v), the Deferiprone was shown □max at 218 nm, and this 218 nm was selected as the detector wavelength in a HPLC.

For the selection of mobile phase the different trials were taken, the trail -1 was not selected due to the broad peak observed with 100 % methanol in the recorded chromatogram. By reducing the methanol: water ratio in (90:10 v/v) in trail-2, the recorded chromatogram was shown the solvent peak too close to the principal peak of Deferiprone, In trial-3 the methanol: water (80:20 v/v) was selected as mobile phase due to sharp peak obtained in chromatogram, which fulfilled the acceptance criteria, therefore trial-3 chromatographic condition was optimized for the analysis of Deferiprone.

For estimation of drug in marketed formulation, drug solution equivalent to -----
The validation of the developed method was carried out as per ICH guidelines and the results were found to be complying with the acceptable limit.

Results of validation

Sr. No.	Parameter	Result	Acceptance criteria
1	Linearity	10-50µg/mL, R^2 = 0.999	R^2 ≥0.999
2	System suitability test	T.Plates=8089, A. factor=	T.Plates≥2000, A. factor<1.75
3	Accuracy	99.74	98-102%
4	Interday precision	0.15%	%RSD < 2%
5	Intraday precision	0.10%	%RSD < 2%
6	Specificity	No Interference	No Interference

7	Robustness	Change in wavelength: 0.28%, Change in flow rate:0.10%, Change in composition: 0.13%	%RSD < 2%
8	Ruggedness	0.070	%RSD < 2%
9	LOD and LOQ	0.14 and 0.45	LOQ is three times of LOD

The forced degradation study was carried out in acid, base, peroxide and thermal stress condition. In alkaline condition 6.21% drug was degraded may be due to the breakdown of benzene ring. In acidic condition 1.17% drug was degraded may be due to the double bond break in benzene ring and in oxidative drug was 3.18% degraded may be due to the oxidation of nitrogen. In thermal degradation, 3.54% of drug was degraded at 60^0C.

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