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DEVELOP AND VALIDATE A SIMPLE, ACCURATE, PRECISE AND A ROBUST HPLC METHOD FOR THE QUANTITATIVE ANALYSIS OF DEFERASIROX IN RAW MATERIAL

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INTRODUCTION

Role of pharmaceutical analysis in drug development

Pharmaceutical analysis can be simply defined as analysis of a pharmaceutical compound or drug. Traditionally, pharmaceutical analysis referred to the chemical analysis of drug molecules. However, over the years, modern pharmaceutical analysis has evolved beyond this to encompass combination techniques, high-throughput technologies, chemometrics, microdosing studies, miniaturization and nanotechnology. These analytical advances are now being employed in all stages of drug discovery. With new, improved and evolving technologies, as well as new applications for existing technology, the search for new drugs for the prevention and treatment of human diseases continues. These analyses are needed in a variety of situations that range from an assay of the new chemical entity (NCE) in the presence of related compounds including optical isomers to complex determination of trace or ultratrace level of various related or transformation products.

METHODOLOGY

Method Validation

Preparation of phosphate buffer

Weigh accurately 1.3 gram of potassium hydrogen orthophosphate and transferred in to a 1000 ml standard flask and diluted to 1000 ml with the HPLC grade water. The pH was adjusted to 3.0 with phosphoric acid.

Preparation of mobile phase

65 volume of acetonitrile (65%) and 35 volumes of buffer (35%) with HPLC grade were transferred in to a 1000 ml standard flask and mixed well. After that, the mobile phase was degassed in Sonicator for 10 min.

Preparation of the standard & sample solution

Standard solution preparation

Weighed accurately 100mg of Deferasirox pure and transferred into a 100ml volumetric flask. Add 70ml of acetonitrile and sonicated to dissolve it completely and the volume was made up to the mark with the acetonitrile.

Sample solution preparation

Weigh accurately 100mg of deferasirox and transferred into a 100ml volumetric flask. Add 70ml of acetonitrile and sonicated to dissolve it completely and the volume was made up to the mark with the acetonitrile.

Selection of chromatographic method

The choice of chromatographic method is based on the nature of the sample (ionic or neutral molecule), its molecular weight and solubility. As drugs are polar in nature, the reverse phase chromatographic technique was selected for the present work.

Selection of wavelength (λmax)

In setting up the conditions for development of the assay method, the choice of the detection wavelength was based on the scanned absorption spectrum for deferasirox. The UV-spectrum of deferasirox were scanned in the wavelength range 200-400 nm against blank and 245 nm wavelength was selected for the analysis.

SELECTIVITY/ SPECIFICITY

Specificity will be evaluated to ensure that no other compounds that may be present interfere appreciably with the quantitation of the analyte. Specificity of the method shall be demonstrated by the ability to analyse Deferasirox from the finished product sample matrix. Different solutions such as a blank, standard and a batch sample of Deferasirox were analysed.

Preparations of standard solution

Weigh accurately about 100mg of sample in 100 ml of acetonitrile. Further diluted and make up to 1ml to 10ml with acetonitrile.

Preparation of sample

Weigh accurately about 100mg of sample in 100 ml of acetonitrile. Further diluted and make up to 1ml to 10ml with acetonitrile.

Table-4: Retention time.

Sample Id.	Retention Time (min.)
Standard	4.2717
Sample	4.2217

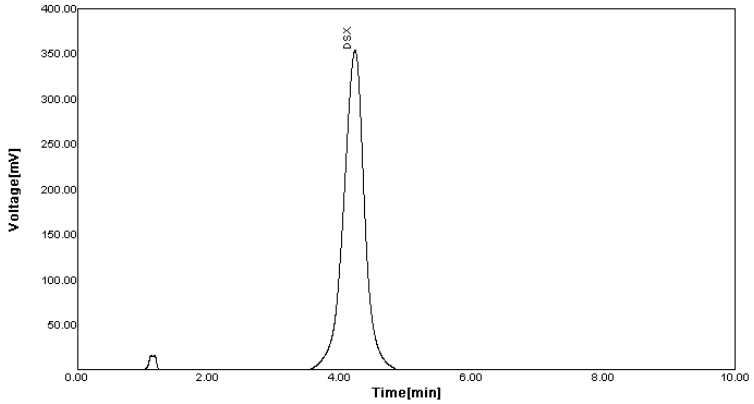

Fig. 10: Chromatogram for sample.

SYSTEM SUITABILITY

100 µg/ml of the standard stock solution was injected. From the chromatogram obtained for standard preparation the column efficiency was determined. System suitability parameters like asymmetry factor, tailing factor, capacity factor, HETP and %RSD were determined.

Table-5: System suitability.

S. No.	Ret.-Time	Peak Area
1	4.2033	8613.467
2	4.2017	8351.421
3	4.1967	8607.094
4	4.2083	8351.158
5	4.1917	8346.906
Std.Dev.	0.006361	142.6845
Mean	4.20034	8454.009
RSD	0.151451	1.687774

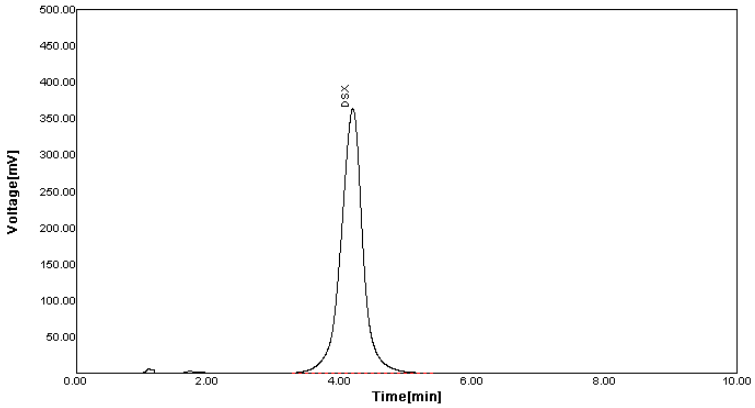

Fig. 11: Chromatogram for sample.

PRECISION

The Precision of an analytical method is determined by assaying a sufficient number of aliquots of a homogenous sample to be able to calculate statistically valid estimates of standard deviations or relative standard deviation (Coefficient of variation).

A) Standard preparation: Weighed accurately 10 mg of Deferasirox WS in to a 100ml volumetric flask and made up to volume with Acetonitrile.(100% i.e.100 mcg/ml)

B) Sample Preparation: Weighed accurately 10 mg of Deferasirox Sample in to a 100 ml volumetric flask and made up to volume with Acetonitrile. (100% i.e.100 mcg/ml)

Table-6: Standard response.

Sample Id.	Peak Response
Standard -1	8613.4672
Standard-2	8351.4211
Standard -3	8607.0938
Standard -4	8351.1578
Standard -5	8346.9063
AVG	8454.009
STDEV	127.6209
%RSD	1.5

Table-7: Sample response.

Sample Id.	Peak Response
Sample-inj.1	8247.1992
Sample-inj.2	8329.1141
Sample-inj.3	8312.3719
Sample-inj.4	8537.5641
Sample-inj.5	8619.8766
Sample-inj.6	8348.0852
AVG	8399.035
STDEV	132.9227
%RSD	1.58

INTERMEDIATE - PRECISION

The Intermediate Precision of an analytical method is determined by assaying a sufficient number of aliquots of a different preparation of sample in different day to be able to calculate statistically valid estimates of standard deviations or relative standard deviation (Coefficient of variation).

Table-8: Standard Response.

Sample Id.	Peak Response
Standard -1	8699.6406
Standard-2	8961.9891
Standard -3	8978.8047
Standard -4	8852.0250
Standard -5	8677.3039
AVG	8833.953
STDEV	126.7128
%RSD	1.43

Table -9: Sample response.

Sample Id.	Peak Response
Sample prep-1	9491.2437
Sample prep-2	9228.6164
Sample prep-3	9298.8586
Sample prep-4	9081.8844
Sample prep-5	9094.9258
Sample prep-6	9249.6187
AVG	9240.853
STDEV	137.145
%RSD	1.48

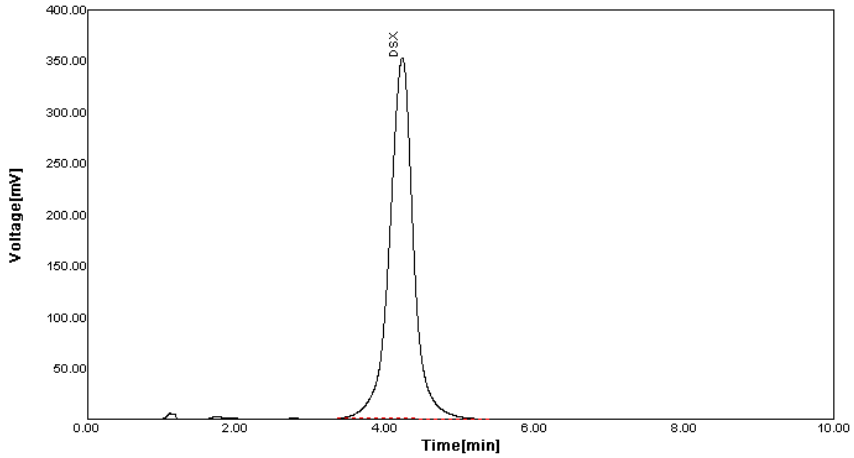

Fig. 12: Chromatogram for precision.

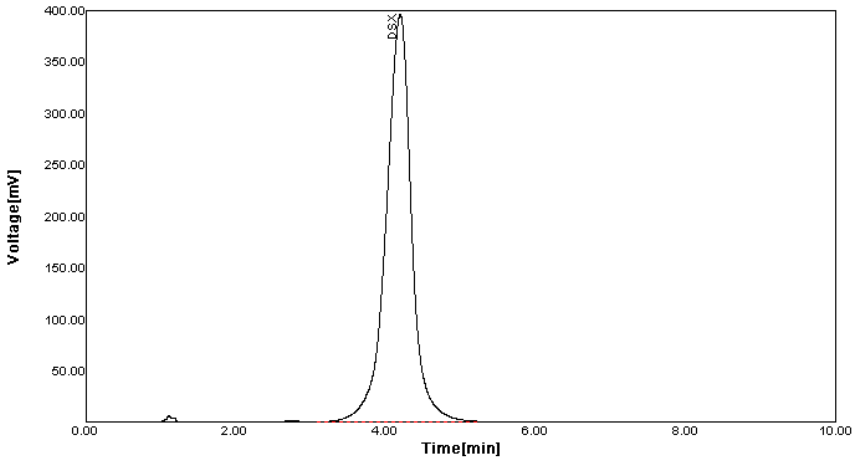

Fig. 13: Chromatogram for intermediate precision.

LINEARITY

The Linearity of an analytical method is determined by mathematical treatment of test results obtained by analysis of samples with analyte concentrations across the claimed range of the method.

Five standard dilution concentrations are prepared across the range at 80, 90, 100, 110 and 120 percent of the test concentration. Test concentration at 100 % is 100 mcg/ml.

Standard preparation is prepared as follows:

A) Accurately weighed about 8 mg of Deferasirox standard in to a 100ml volumetric flask and made up to volume with mobile phase (80% i.e.80 mcg/ml).

B) Accurately weighed about 9 mg of Deferasirox standard in to a 100ml volumetric flask and made up to volume with mobile phase (90% i.e.90 mcg/ml).

C) Accurately weighed about 11 mg of Deferasirox standard in to a 100ml volumetric flask and made up to volume with mobile phase (110% i.e.110 mcg/ml).

D) Accurately weighed about 12 mg of Deferasirox standard in 100ml volumetric flask and made up to volume with mobile phase (120% i.e.120 mcg/ml).

Table-10: Linearity.

S. No	Concentration	Peak area
1	80%	6793.0484
2	90%	7430.9914
3	100%	8297.4203
4	110%	8984.64
5	120%	10012.73

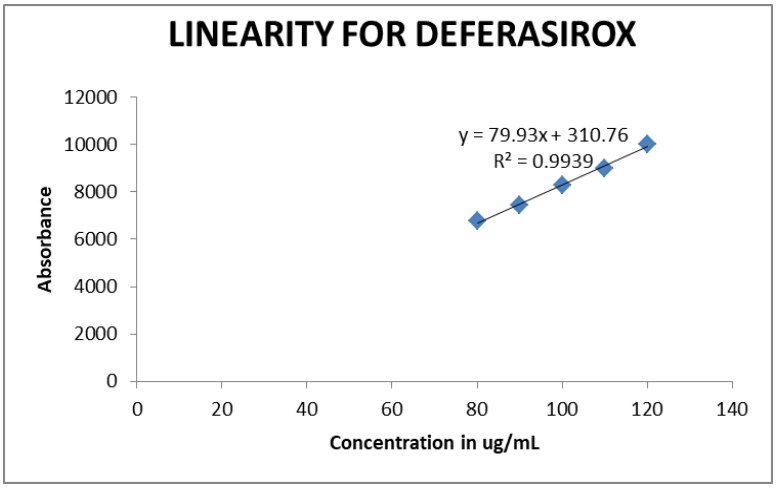

Fig. 14: Chromatogram for linearity.

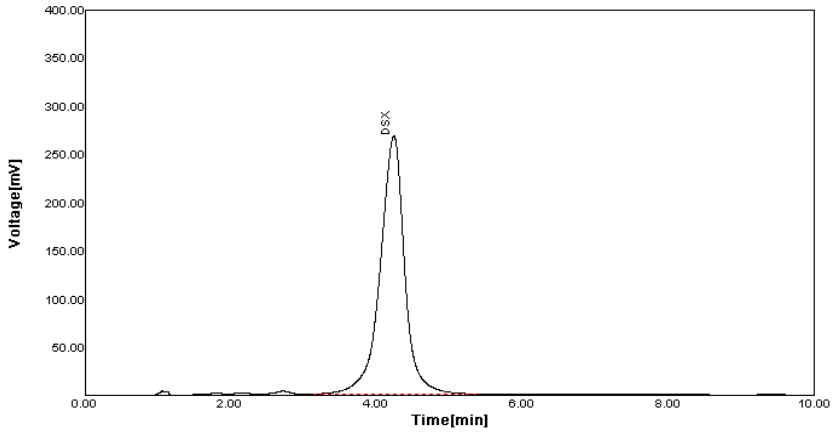

Fig. 15: Chromatogram for 80% linearity.

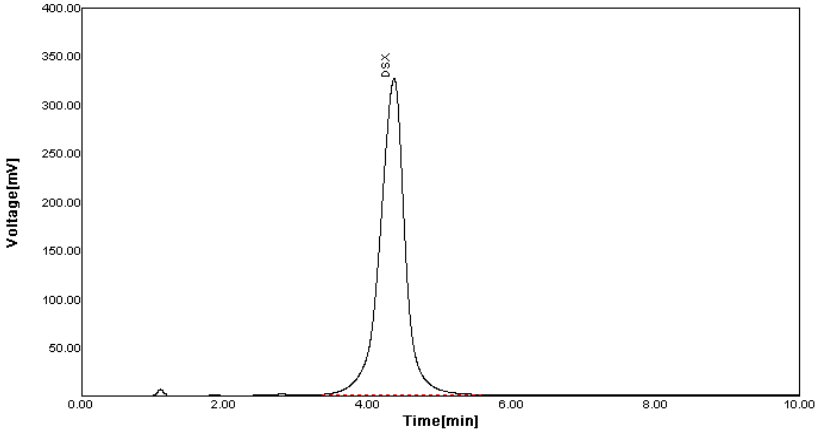

Fig. 16: Chromatogram for 90% linearity.

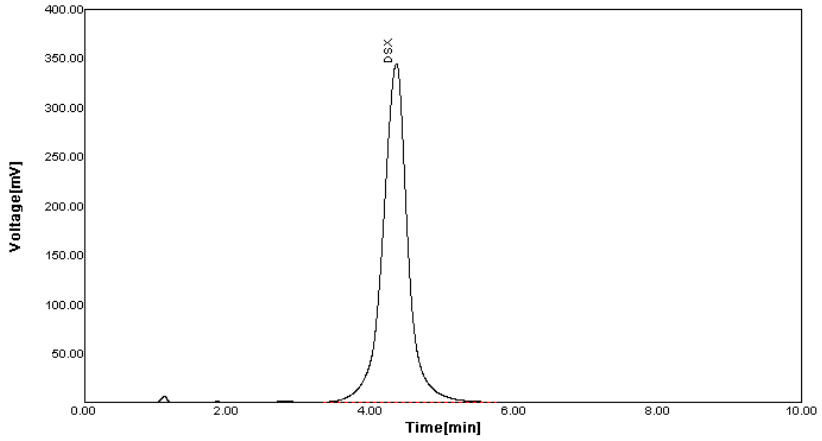

Fig. 17: Chromatogram for 100% linearity.

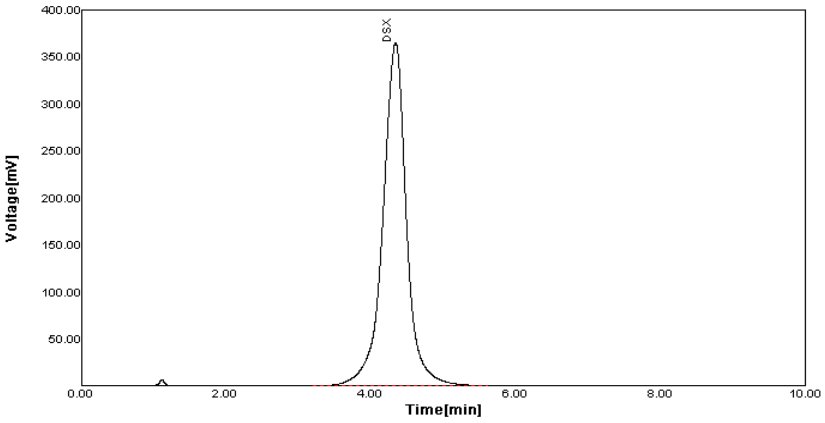

Fig. 18: Chromatogram for 110% linearity.

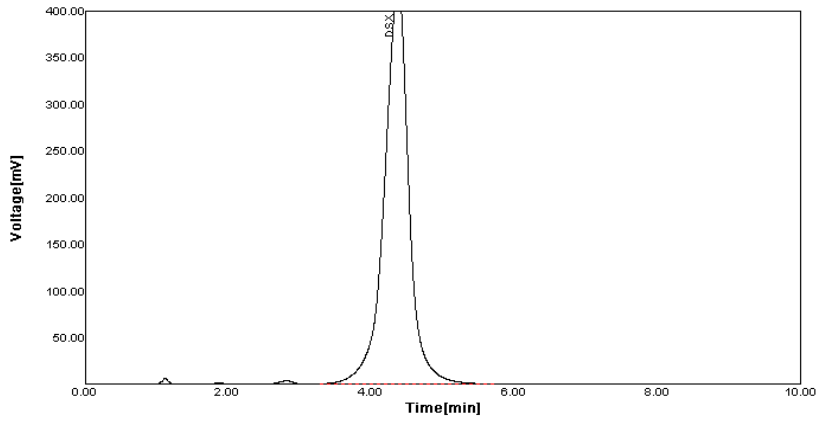

Fig. 19: Chromatogram for 120% linearity.

RANGE

The range of the method is the interval between the upper and lower levels of analyte that have been determined with precision, and linearity.

The range of the method is validated by verifying that the analytical method provides acceptable precision, and linearity when applied to samples containing analyte at the extreme of the range as well as within the range.

Three Sample dilution concentrations were prepared across the range at 80, 100 and 120 percent of the test concentration; Test concentration being 100 mcg/ml.

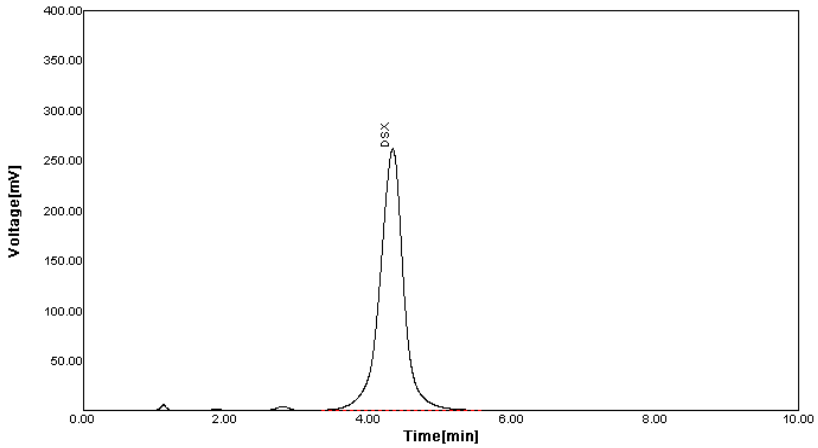

Fig. 20: Chromatogram for 80% range.

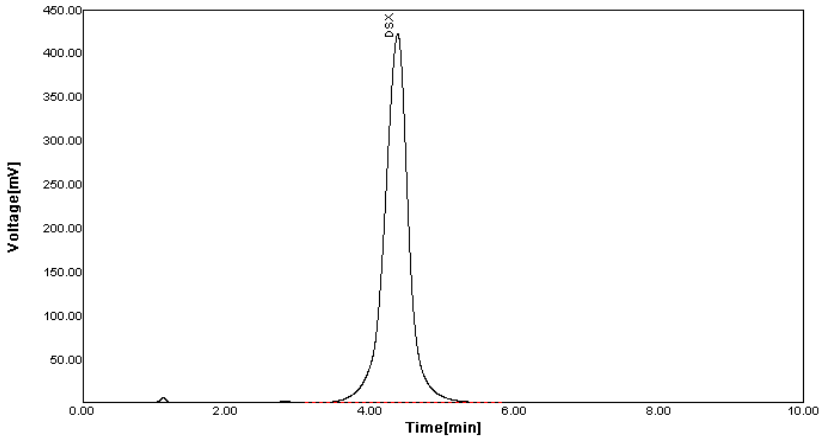

Fig. 21: Chromatogram for 120% range.

LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION

The Limit of Detection and Limit of Quantification of analyte is determined by equation

$$LOD = \frac{3.3 \text{ X STEYX}}{\text{SLOPE}} \qquad LOQ = \frac{10 \text{ X STEYX}}{\text{SLOPE}}$$

Preparation of 80% solution

Pipette out 0.8 ml of standard stock solution in to a 10ml volumetric flask, and make up with acetonitrile up to the mark.

Preparation of 90% solution

Pipette out 0.9 ml of standard stock solution in to a 10ml volumetric flask, and make up with up Acetonitrile up to the mark.

Preparation of 100% solution

Pipette out 1.0 ml of standard stock solution in to a 10 ml volumemetric flask, and make up with Acetonitrile up to the mark.

Preparation 110% solution

Pipette out 1.1 ml of standard stock solution in to a 10 ml volumemetric flask, and make up with Acetonitrile up to the mark.

Preparation of 120% solution

Pipette out 1.2 ml of standard stock solution in to a 10 ml volumemetric flask, and make up with Acetonitrile up to the mark.

Table- 11: LOD & LOQ.

Conc.(mcg/mL)	Peak Area
80	673.1544
90	800.4121
110	978.8321
120	1084.698
C.C.	0.9979
STEYX	14.3814
SLOPE	100.1507
LOD	0.5
LOQ	1.4

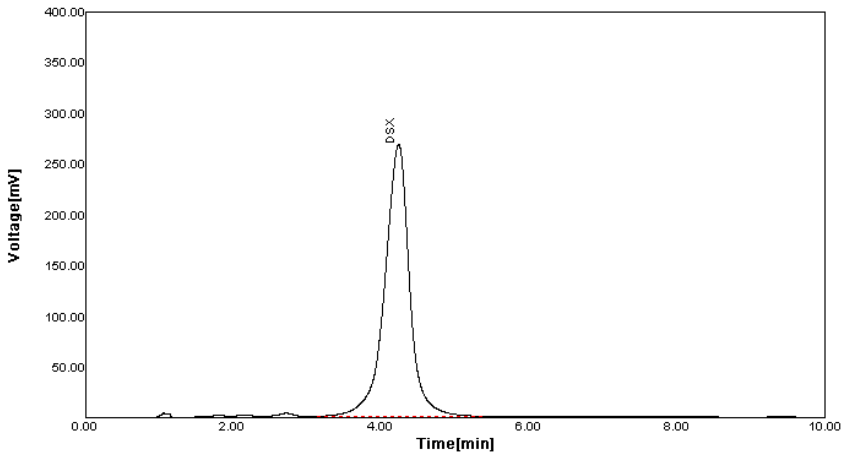

Fig. 22: Chromatogram for 80% solution.

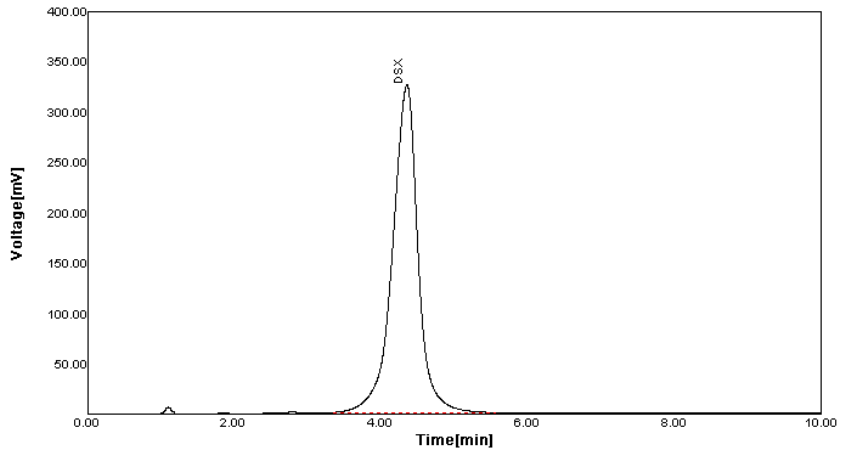

Fig. 23: Chromatogram for 90% solution.

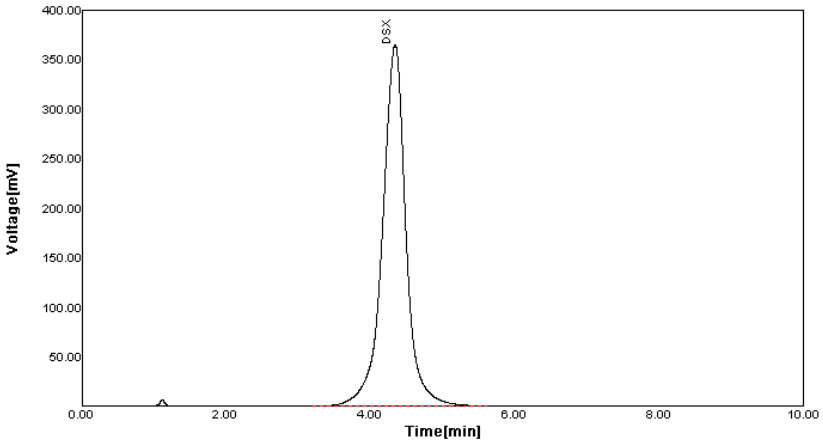

Fig. 24: Chromatogram for 110% solution.

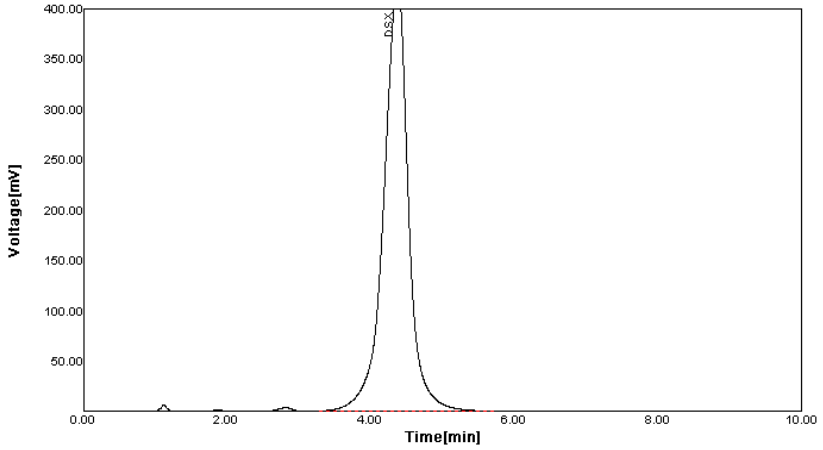

Fig. 25: Chromatogram for 120% solution.

ROBUSTNESS

The Robustness of an analytical method is determined by assaying a sufficient number of aliquots of a different preparation of sample at deliberate changes to calculate statistically valid estimates of standard deviations or relative standard deviation (Coefficient of variation).

Table-12: Temperature 243 &247nm.

Chromatogram Details	243 nm		247 nm	
	Retention time	Peak Response	Retention Time	Peak Response
Standard -1	4.2650	6160.2648	4.1400	6043.3875
Standard-2	4.2550	7023.5289	4.1383	6721.3102
Standard -3	4.2583	6854.5078	4.1300	6973.9688
%RSD	Less than 10%			

Table-13: Temperature 23^0C & 27^0.

Chromatogram Details	23C		27 C	
	Retention Time	Peak Response	Retention Time	Peak Response
Standard -1	4.1467	6391.8250	4.0983	6834.6359
Standard-2	4.1500	6940.3414	4.1033	7150.0617
Standard -3	4.1483	6775.3992	4.1150	6170.0578
%RSD	Less than 10%			

Table-14: Flow rate at 0.9 & 1.1mL/min.

Chromatogram Details	0.9 mL/min.		1.1 mL/min.	
	Retention Time	Peak Response	Retention Time	Peak Response
Standard -1	4.1900	6327.3687	3.9717	5433.3430
Standard-2	4.2200	6995.4836	4.0817	5986.1219
Standard -3	4.2450	7054.5820	4.1467	5833.8523
%RSD	Less than 10%			

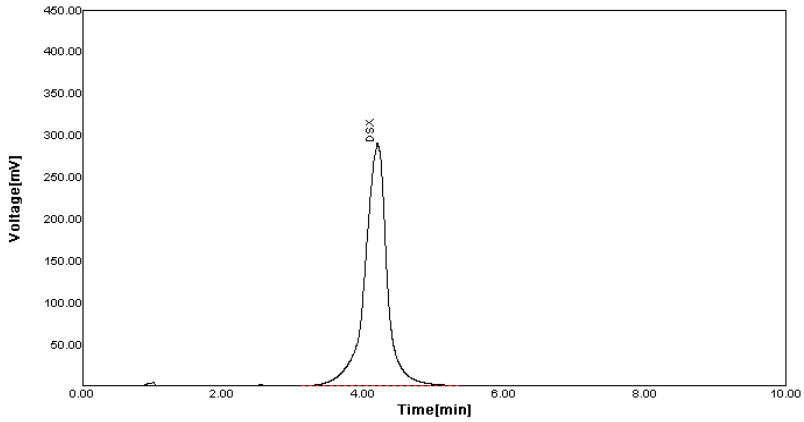

Fig. 26: Chromatogram for 0.9 Ml/ min.

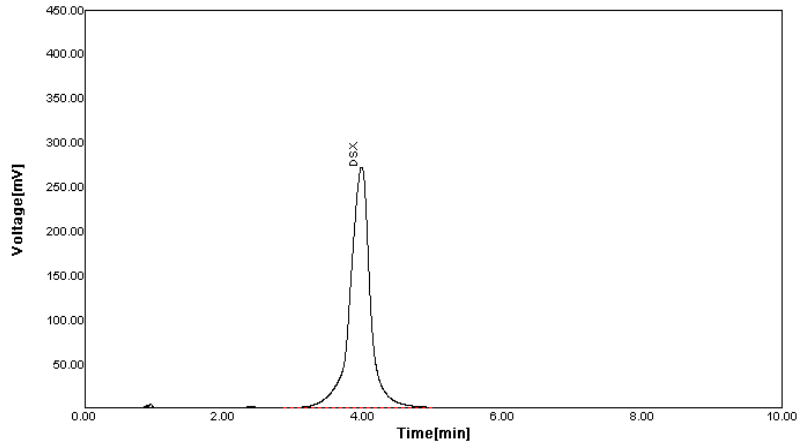

Fig. 27: Chromatogram for 1.1 Ml/ min.

Assay

Inject 20 µl of standard and sample solution into the chromatographic system and measure the area of deferasirox and calculate the percentage assay by using the following formula.

$$\frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{AVG.WT}{Label\ claim} \times 100$$

AT = Peak area of test preparation

AS = peak area of standard preparation

WS = weight of working standard in mg

WT = weight of sample taken in mg

DS = dilution of standard solution

DT = dilution of test solution

P = percentage potency of working standard. (% W/W)

Table -16: Assay Results.

	S. No.	Area of Deferasirox
Standard	1.	8351.421
	2.	8607.094
	3.	8351.158
	Avg	8436.558
Sample	1.	8359.611
	2.	8406.264
	3.	8451.258
	Avg	8405.711
	%Assay	**99.63437**

8. RESULTS AND DISCUSSION

With potassium dihydrogen orthophosphate (pH 3) and Acetonitrile in the ratio 65:35% v/v, flow rate of 1.0ml/min and symmetry C_{18} inertsil ODS column, showed good separation, sharp peak and without tailing. The chromatogram thus obtained was selected as the optimised chromatogram and the conditions were selected as the optimised chromatographic conditions. The optimised chromatogram was shown in figure 9. The various trials were performed and the chromatographic conditions were mentioned in method development. The retention time in the optimised chromatogram was found to be 4.2min.

With the optimised chromatographic conditions, stock solutions of Deferasirox was prepared by using mobile phase (potassium dihydrogen orthophosphate buffer of pH 3 and Acetonitrile in the ratio 65:35) and various concentrations were prepared in the range of 80-120µg/ml and injected individually. Deferasirox was found to obey the Beers law in the concentration range of 80-120µg/ml. The chromatograms were recorded at 245 nm. The chromatograms were shown in figures 15, 16, 17, 18 and 19. The values obtained in linearity study was shown in table 10.

The calibration curve was plotted using concentration against peak area. The correlation coefficient was found to be 0.993 which indicates that the concentration of Deferasirox has good linearity.

The limit of detection and the limit of quantification were determined based on the signal to noise ratio. The limit of detection was found to be 0.5µg/ml and the limit of quantification was found to be 1.4µg/ml. The chromatogram for blank, LOD and LOQ were shown in figures, 22, 23, 24 and 25 respectively. The values were given in table 11.

Precision study was done with Deferasirox standard. 70µg/ml solution was prepared and injected five times and the area of five replicate injections were recorded in HPLC. The %RSD for the area of five replicate injections was found to be 1.58 which was found to be within limits. It shows that the drug was having good precision and the chromatograms were shown in figures 12. The results were shown in table 6&7.

Intermediate precision study was done with Deferasirox standard. 70µg/ml solution of Deferasirox was prepared and injected five times and the area of five injections were recorded in HPLC. The %RSD was found to be 1.48% which was found to be within the limits. It shows that the intermediate precision was within the specified limits which were shown in table 8&9. The chromatograms were shown in figure 13.

Robustness was performed by changing the flow rate and by changing the organic composition of the mobile phase. The chromatograms were shown in figures 26 and 27. The results were shown in the table 12, 13, and 14. It shows that there was no change in the values even after making deliberate change in the analytical procedure.

The assay method of Deferasirox was found to be within the limits of 99.63437.

All the above parameters combined with the simplicity and ease of operation ensured that the application of proposed method was useful in the assay of drug in pharmaceutical dosage form. Hence the RP-HPLC method may be applied for the estimation of Deferasirox.

SUMMARY AND CONCLUSION

- A simple, precise and accurate RP-HPLC method was developed for the analysis of Deferasirox in pure raw material using the mobile phase consisting of potassium dihydrogen orthophosphate buffer(pH3) : acetonitrile in the ratio 65:35%v/v.
- The chromatographic condition was set at a flow rate of 1.0ml/min with the UV characteristics and has also been validated.
- It is evident that the responses for Deferasirox was found to be linear in the studied concentration range from 80 – 120 µg/ml and the correlation coefficient was found to be 0.993.
- The low % RSD values (NMT 2%) indicated that the method was sufficiently precise.
- Allowable variation in flow rate and mobile phase ratio which indicated that the method was robust enough.
- Thus it was concluded that the proposed method has been evaluated for accuracy, linearity, precision, intermediate precision, LOD, LOQ and robustness and proved to be simple, rapid, convenient and effective for the routine analysis of Deferasirox raw material.

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