RESEARCH ARTICLE

METHOD DEVELOPMENT AND VALIDATION FOR DETERMINATION OF IMPURITIES IN DEFERASIROX BY RP-HPLC TECHNIQUE

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ABSTRACT

A simple and isocratic reverse phase high performance liquid chromatography (RP-HPLC) method was developed and validated for determination of Deferasirox and its impurities. The method was validated as per International Conference on Harmonization (ICHQ2A (R1)) guidelines for system suitability, precision, linearity, specificity, solution stability, robustness and ruggedness, limit of detection and limit of quantitation. Deferasirox was analyzed by using Inertsil ODS-3V (150 mm X 4.6 mm, 5 µm) at 35°C column temperature, with isocratic elution. The analysis was performed at a wavelength of 245 nm using dual λ absorbance detector. Efficient UV detection at 245 nm enabled determination of Deferasirox without any interference of impurities Salicylic acid & Salicylamide. The retention time (RT) for Deferasirox was around 8.7 min. The impurities of Deferasirox are key starting materials. These impurities act as potential impurities. Thus identifying these impurities at low level is crucial. The calibration curves were linear over acceptable concentration range. The method developed was unique in determining the impurities even at low levels than that of specifications. The developed method was successfully applied to estimate the amount of Deferasirox.

Keywords: Deferasirox, HPLC, RP-HPLC, validation.

INTRODUCTION

Deferasirox is an iron chelating agent indicated in the treatment of chronic iron overload in patients who are receiving long term blood transfusions for conditions such as beta-thalassemia and other chronic anemias[1]. Its chemical name is [4-[(3Z,5E)-3,5-bis(6-oxo-1-cyclohexa-2,4-dienylidene)-1,2,4-triazolidin-1-yl] benzoic acid[2]. Deferasirox is a new tridentate oral iron chelator developed by computer remodeling recently approved by FDA for children above 2 years. It is the first oral medication approved in the USA for this purpose[1]. Ferric iron has six coordination sites, which need to be chelated completely to prevent the generation of free radicals. Deferasirox works in treating iron toxicity by binding trivalent (ferric) iron (for which it has a strong affinity), forming a stable complex which is eliminated via the kidneys[1]. Few HPLC and LC methods for determination of Deferasirox were reported in the literature review [5,6,7] and some reports mainly included the determination of Deferasirox in biological fluids[8,9]. The aim of this work is to develop a rapid, economical and sensitive RP-HPLC method for identifying these impurities at low level is very important. In order to develop a robustness method there is an immense need for developing a rapid, sensitive and validated analytical method for day- to-day analysis, in routine analysis in quality control laboratories of the drug and which can be even applicable for pharmaceutical dosage forms[4].

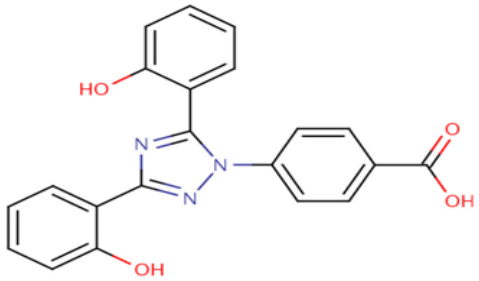

Figure 1: Structure of Deferasirox

MATERIALS AND METHODS

Materials and reagents

Deferasirox bulk drug (99.79% purity) and potential impurities Salicylic acid (85.00% purity) & Salicylamide (99.38% purity) were provided by quality standard labs. Acetonitrile (HPLC grade) were obtained from Merck.

Instrumentation

Chromatographic separation was performed with Shimadzu LC-2010 high performance liquid chromatography equipped with UV variable wavelength detector. Chromatograms and data were recorded by means of LC solutions software. A Mettler Toledo Analytical Balance (XP6), an Ultra Sonicator (Bandelin Sonorex EN-200US) was used in the study.

Preparation of solutions

Buffer Preparation

Weigh accurately 1.36 g of potassium di-hydrogen orthophosphate in 1000 ml of degassed and filtered Milli-Q-water and mixed thoroughly. Adjust the PH 3.0±0.05 with dilute H_3PO_4.

Mobile phase: Buffer: Acetonitrile (50:50v/v)

Diluent: Acetonitrile

Preparation of Deferasirox Standard Solution

Accurately weighed and transferred about 25 mg of the Deferasirox Standard into 50 ml volumetric flask. Dissolved in and make up to the volume with diluent. Further dilute 5 ml of above solution to 25 ml volumetric flask & make upto the mark with diluent (0.1mg/ml).

Preparation of Salicylic acid impurity (impurity-A) solution

Accurately weighed and transferred about 25 mg of the Impurity-A into 50 ml volumetric flask. Dissolved in and make up to the volume with diluent. Further dilute 5 ml of above solution to 25 ml volumetric flask & make upto the mark with diluent (0.1 mg/ml).

Preparation of Salicylamide impurity (impurity-B) solution

Accurately weighed and transferred about 25 mg of the Impurity-B into 50 ml volumetric flask. Dissolved in and make up to the volume with diluent. Further dilute 5 ml of above solution to 25 ml volumetric flask & make upto the mark with diluent (0.1 mg/ml).

Method validation

The proposed method was validated according to the International Conference on Harmonization (ICH) guidelines[3].

System suitability

The system suitability was assessed by the duplicate analysis of the drug which was spiked by all impurities at a concentration of 0.1% with respect to the test concentration of 0.1 mg/ml. The acceptance criteria were the resolution between salicylamide and salicylic acid should not be less than 1.5 and tailing factor for Deferasirox peak should not be more than 1.5. The theoretical plate count of Deferasirox should not be less than 2000.

Linearity (Calibration curve)

Level solutions

A series of solutions in 5 concentrations of 50 %, 80 %, 100 %, 120 % and 150 % were prepared using the standard stock solution (w.r.t 0.1 µg/ml). The linearity of the method was demonstrated by above prepared level concentration 50 %, 80 %, 100 %, 120 % and 150 % of the target concentration of 0.1 µg/ml. About 10.0 µl of each of the above prepared solutions was injected onto the chromatographic system and measured. The average peak area response was calculated.

The acceptance criteria were that the system suitability criteria should be meet and % Relative standard deviation for area of impurities should not be more than 5.0. The % RSD for the peak area of deferasirox in reference solution should not be more than 5.0. The regression coefficient for each impurity should not be less than 0.99 and % of Y- Intercept should be ± 5.0[14].

Determination of Limit of Detection and Limit of Quantitation

The limit of detection and limit of quantification of the impurities were determined by calculating the signal-to-noise ratio (S/N, i.e., 3.3 for LOD and 10 for LOQ) using the following equations designated by International Conference on Harmonization (ICH)[3].

Calculation

$$LOD = 3.3 \times \sigma/S$$

$$LOQ = 10 \times \sigma/S$$

Where σ = standard deviation

PRECISION

The precision was demonstration under three categories.

System Precision

Single individual preparation of Deferasirox drug substance was prepared with target concentration of about 0.1 mg/ml for system precision.

Procedure

Injected six replicate injections 10.0 µl of above preparation into the chromatographic system and recorded the chromatograms. The relative standard deviation of the replicate injections was calculated.

Method Precision

Six individual preparations of Deferasirox drug substance spiked with impurities were prepared for method Precision.

Procedure

Injected 10.0 µl of above preparations into the chromatographic system and recorded the chromatograms. Calculated the % relative standard deviations for the peak area of Deferasirox in reference solution and for the peak area of each impurity.

Intermediate precision [Ruggedness]

Demonstration of ruggedness was conducted by different analysts, systems, columns and in different days under similar conditions at different times. Six individual preparations of Deferasirox drug substance spiked with impurities were prepared and each was analyzed.

The acceptance criteria for system, method and intermediate precision were that the system suitability criteria should be meet and the % Relative standard deviation for the peak area of Deferasirox in reference solution from the six replicate injections should be not more than 5.0 %. The % Relative standard deviation for the peak area of each impurity should not be more than 5.0 and for Deferasirox should not be more than 2.0 %.

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Solution stability

The validity of the solutions was demonstrated for a period of 48 hrs by injecting the same solution at periodic intervals into the chromatographic system and the chromatograms were recorded.

Procedure

About 10 µl of the standard solution was injected at 0 hr, 24th hr and 48th hr into the chromatographic system and recorded the peak areas in the chromatograms.

Calculations

Observed the areas of the peaks of Deferasirox injected at the mentioned time intervals and the relative standard deviation of the injections was calculated.

The acceptance criteria were that the system suitability should pass as per the test method at variable conditions and % RSD for the peak area of Deferasirox should not be more than 2.0.

Specificity

The specificity of the method was demonstrated by interference check by injecting the diluent blank, Deferasirox and impurities solutions to determine whether any peaks in the diluent and impurities solutions are co-eluting with Deferasirox peaks.

RESULTS AND DISCUSSION

Method development and optimization

Deferasirox is soluble in methanol and acetonitrile[2]. The drug can be separated on a Inertsil ODS-3V column in reverse phase HPLC. The optimization of the method development was done by isocratic elution.

The peak shapes and symmetry of Deferasirox and impurities were good with above isocratic elution and peaks were resolved with greater than 1.5 resolution at a isocratic flow rate. The method developed was unique in determining the impurities even at low levels than that of specifications. The developed method was successfully applied to estimate the amount of Deferasirox.

Optimised chromatographic conditions:

Equipment	: HPLC Shimadzu
Detector	: UV-Vis detector
Column	: Inertsil ODS-3V: 150 X 4.6 mm; 5 µm
Flow rate	: 1.5 ml/ min
Wavelength	: 245 nm
Injection volume	: 10 µl
Column oven temp	: 35°C
Run time	: 20 min
Sample concentration	: 0.1 mg/ml

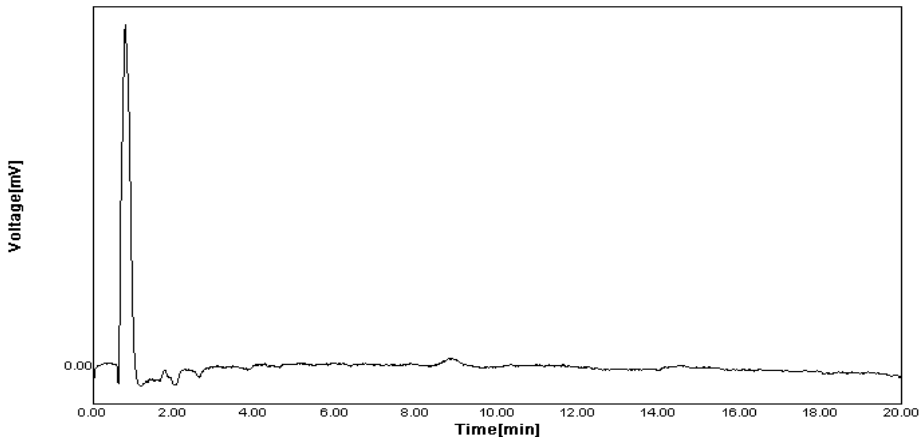

Figure 2: Blank Chromatogram of Deferasirox

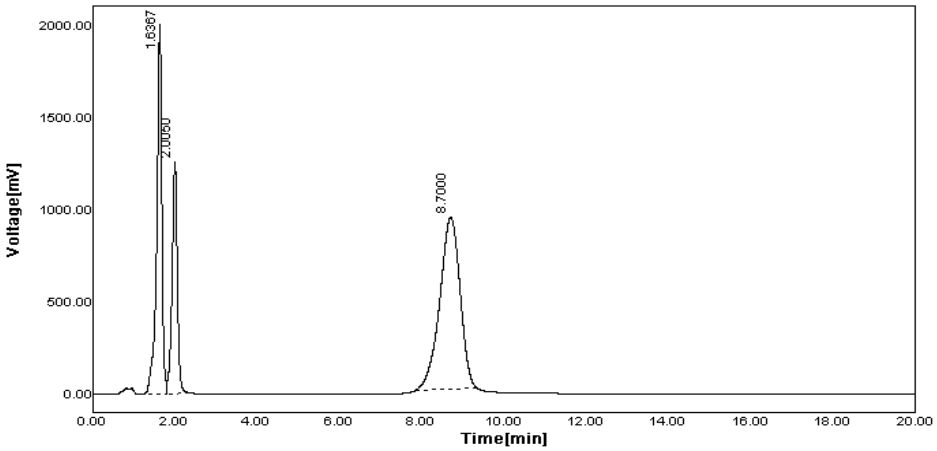

Figure 3: Optimized Chromatogram of Deferasirox sample spiked with impurities

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Method Validation

System Suitability

Resolution was not less than 1.5, number of theoretical plates was not less than 2000, and percentage relative standard deviation (%RSD) for Peak Area was not more than 2.0 % for Deferasirox peaks. The %RSD of peak area for the drug is within 2% indicating the suitability of the system (Table 1). The efficiency of the column as expressed by number of theoretical plates and the tailing factor was 0.90.

Table 1: System suitability studies

Injections	Resolution between salicylamide and salicylic acid	Theoretical plate count	Tailing factor
Inj-1	1.84	2017.03	0.89
Inj-2	1.77	2180.50	0.92
Average	1.80	2098.76	0.90

Acceptance criteria

Resolution was not less than 1.5, number of theoretical plates was not less than 2000, and percentage relative standard deviation (%RSD) for Peak Area was not more than 2.0 % for Deferasirox peaks as per ICH guidelines[3].

Linearity

The calibration curve constructed was evaluated by its correlation coefficient. The peak area of the impurities of the drug was linear in the range of 0.05 µg/ml to 0.15 µg/ml.

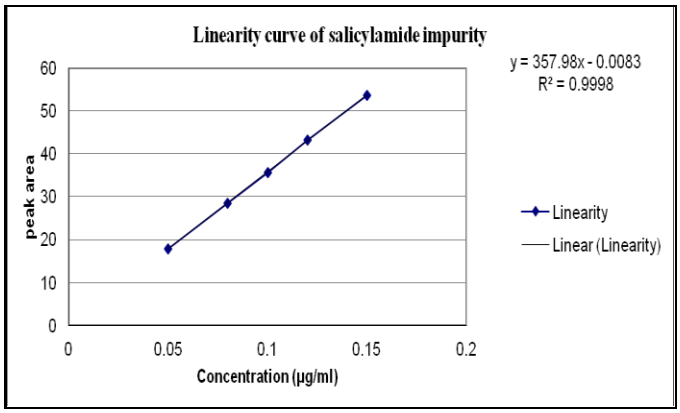

Figure 4: Calibration curve of salicylamide impurity

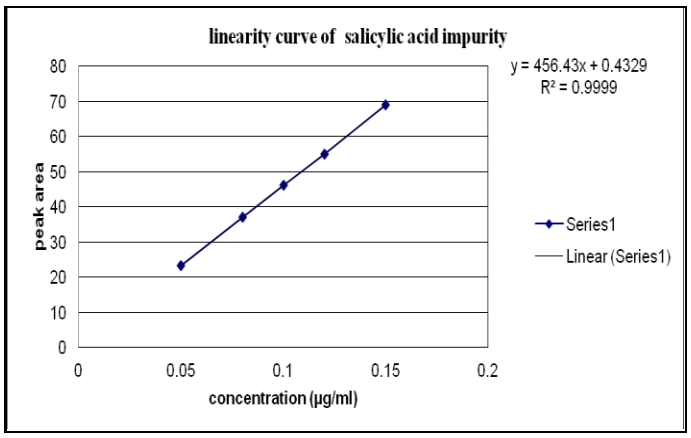

Figure 5: Calibration Curve of salicylic acid impurity

Table 2: Linearity values of salicylamide

Concentration	Peak area
0.05	17.92
0.08	28.55
0.10	35.64
0.12	43.29
0.15	53.55

Table 3: Linearity values of salicylic acid

Concentration	Peak area
0.05	23.22
0.08	37.01
0.10	46.21
0.12	54.95
0.15	68.99

Acceptance Criteria

Coefficient of correlation should be not less than 0.999

LOD and LOQ

The limit of detection and limit of quantification of the impurities is determined by taking the slope and intercept values from linearity studies. Limit of detection is calculated by using the formula LOD = 3.3 X σ/S and limit of detection is calculated by using the formula LOQ = 10 X σ/S. Limit of detection (LOD) for salicylamide was found to be 0.0060 µg/ml and Limit of quantitation (LOQ) for salicylamide was found to be 0.0182 µg/ml and LOD for salicylic acid was found to be 0.0028 µg/ml and LOQ for salicylic acid was found to be 0.0087 µg/ml.

Precision

The precision was demonstration under three categories.

For system precision Single individual preparation of Deferasiorx drug substance was prepared and six replicate injections 10.0 µl of above preparation into the chromatographic system and the chromatograms were recorded. The relative standard deviation of the replicate injections was calculated. For Method Precision six individual preparations of Deferasirox drug substance with impurities were injected 10.0 µl of above preparations into the chromatographic system and the chromatograms were recorded. Calculated the % Relative standard deviation for the peak area of Deferasirox in reference solution and for the peak area of each impurity. For ruggedness six individual preparations of Deferasirox drug substance with impurities were prepared and analyzed by different analysts, systems, columns and in different days under similar conditions at different times.

Table 4: System precision of Deferasirox

No. of Injections	Area (mV*s)
1	123698.52
2	124391.26
3	126397.90
4	125024.67
5	126461.02
Average	125194.67
Std. Deviation	887.4
% RSD	0.708

Acceptance criteria

The % RSD for the area of five standard injections of Deferasirox results should not be more than 2%.

Table 5: Method precision of Deferasirox

No. of Injections	Area (mV*s)		
	Deferasirox	Impurity-A	Impurity-B
1	121718.52	44.66	35.49
2	125790.37	44.37	34.44
3	125229.51	43.48	36.34
4	126942.31	43.76	35.83
5	125295.42	44.36	34.86
6	124867.03	44.12	34.99
Average	124973.86	44.12	35.16
Std. Deviation	1597	0.399	0.654
%RSD	1.24	0.90	1.80

Acceptance criteria

The % RSD for the area six standard injections of Deferasirox spiked with impurities results should not be more than 2%.

Table 6: Ruggedness

No. of Injections	Area(mV*s)		
	Deferasirox	Impurity - A	Impurity - B
1	123628.41	34.49	31.27
2	121445.21	34.27	32.59
3	122166.32	33.90	33.96
4	124297.37	34.05	32.14
5	124683.62	35.41	32.62
6	123557.76	35.53	31.70
Average	123296.31	34.61	32.38
Std. Deviation	1140	0.662	0.47
%RSD	0.92	1.90	1.48

Acceptance criteria

The % RSD for the area six standard injections of Deferasirox spiked with impurities results should not be more than 2%.

Solution stability

The validity of the solutions was demonstrated for a period of 48 hrs by injecting the same solution at 0 and 48 hrs intervals into the chromatographic system and the chromatograms were recorded.

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Procedure

About 10 µl of the standard solution was injected at 0 hr, 24th hr and 48th hr into the chromatographic system and recorded the peak areas in the chromatograms.

Calculations

Observed the areas of the peaks of Deferasirox injected at the mentioned time intervals and the relative standard deviation of the injections was calculated.

Specificity

The specificity of the method was demonstrated by interference check by injecting the diluent blank, Deferasirox and impurities solutions to determine whether any peaks in the diluent and impurities solutions are co-eluting with Deferasirox peaks.

Table 7: Stability data

Sample name	Area(mV*s)
Deferasirox - initial solution	124190.90
Deferasirox - after 24 hrs	126634.02
Deferasirox – after 48 hrs	156182.25

Table 8: Specificity of Deferasirox

S.No.	Name	RT[min]	Area[mV*s]	TP	TF	Resolution
1	Salicylamide	1.6367	17267.3625	2115.0	0.9666	0.0000
2	Salicylic acid	2.0050	11018.0711	2057.9	0.9784	1.8451
3	Deferasirox	8.6920	32084.5594	2102.2	0.9925	12.8081

Acceptance criteria

Resolution was not less than 1.5, number of theoretical plates was not less than 2000, and percentage relative standard deviation (%RSD) for Peak Area was not more than 2.0 % for Deferasirox peaks.

ROBUSTNESS

The Robustness studies were performed for the standard solutions and were presented in respectively (Table 9, 10 and 11). The values are within the limit which showed that the developed method is robust.

Table 9: Robustness studies for Deferasirox standard

Chromatographic condition	Retention time	Theoretical plate count	Tailing factor
Actual condition	8.70	2125	0.99
Flow rate 1.3 ml min^{-}	7.93	1944	0.96
Flow rate 1.7 ml min^{-}	9.08	2202	0.98
Acetonitrile 45%	8.55	2183	0.97
Acetonitrile 55%	8.59	2208	0.99
Column temperature 30ºC	8.81	2110	0.99
Column temperature 40ºC	8.80	2235	0.97
Buffer pH 2.8	9.41	2073	0.99
Buffer pH 3.2	8.44	2035	0.95

Table 10: Robustness studies for impurity - A

Chromatographic condition	Retention time	Theoretical plate count	Tailing factor
Actual condition	2.00	2125	0.97
Flow rate 1.3 ml min^{-}	2.20	2045	0.95
Flow rate 1.7 ml min^{-}	2.18	2287	0.99
Acetonitrile 45%	2.28	2089	0.93
Acetonitrile 55%	2.11	2208	0.99
Column temperature 30ºC	2.31	2100	1.01
Column temperature 40ºC	2.17	2066	1.00
Buffer pH 2.8	2.63	2302	0.98
Buffer pH 3.2	3.37	2777	1.13

Table 11: Robustness studies for impurity - B

Chromatographic condition	Retention time	Theoretical plate count	Tailing factor
Actual condition	1.63	2125	0.93
Flow rate 1.3 ml min^{-}	1.55	2044	0.91
Flow rate 1.7 ml min^{-}	1.52	2256	0.98
Acetonitrile 45%	1.60	2164	0.99
Acetonitrile 55%	1.48	2018	0.99
Column temperature 30ºC	1.61	2010	0.86
Column temperature 40ºC	1.54	2235	1.01
Buffer pH 2.8	1.63	2099	0.99
Buffer pH 3.2	1.57	2173	0.92

CONCLUSION

A simple and isocratic reverse phase high performance liquid chromatography (RP-HPLC) method was developed and validated for determination of impurities in Deferasirox. The method was validated as per International Conference on Harmonization (ICHQ2 (R1)) guidelines for system suitability, precision, linearity, specificity, solution stability, robustness and ruggedness, limit of detection and limit of quantitation. Deferasirox was analyzed by using Inertsil ODS-3V (150 X 4.6 mm; 5 µm) at 35ºC temperature with isocratic elution. The analysis was performed at a wavelength of 245 nm using UV-Vis detector. Efficient UV detection at 245 nm enabled determination of Deferasirox without any interference of impurities Salicylic acid and Salicylamide. The retention time (RT) for Deferasirox was around 8.7 min.

The impurities are potential impurities. Thus identifying these impurities at low level is crucial. The calibration curves were linear over a concentration range from 0.05 µg/ml to 0.15 µg/ml. Limit of detection (LOD) for salicylamide was found to be 0.0060 µg/ml and Limit of quantitation (LOQ) for salicylamide was found to be 0.0182 µg/ml and LOD for salicylic acid was found to be 0.0028 µg/ml and LOQ for salicylic acid was found to be 0.0087 µg/ml. The method developed was unique in determining the impurities even at low levels than that of specifications. The developed method was successfully applied to estimate the amount of Deferasirox. The proposed RP-HPLC method is precise, sensitive, accurate, specific and efficient and can be used in routine analysis in quality control laboratories.

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REFERENCES

1. www.drugs.com
2. www.chemblink.com
3. The International Conference on Harmonization Q2 (B), **"**Validation of Analytical Procedures Text and Methodology" 6th Nov 1996.
4. The International Conference on Harmonization Q2 (A), **"**Validation of Analytical Procedures Text and Methodology" 6th Nov 1996.
5. Ravikiran K, Surendranath KV, Radhakrishnanand P, Satish J, Satyanarayana PVV, "A Stability Indicating LC Method for Deferasirox in Bulk Drugs and Pharmaceutical Dosage Forms" Chromatographia, 2010, 72: (5), 441-446.
6. Chakravarthy VK, Gowrisankar D, "LC determination of Deferasirox in pharmaceutical Formulation" Journal of Global Trends in Pharmaceutical Sciences, 2010, 1(1), 37- 45.
7. Sambasivarao V, Phani RS, Seetharaman R., Lakshmi KS, "RP-HPLC Method Development for Analysis of Deferasirox in Formulation" IJPI's Journal of Analytical Chemistry, 2011, 1, 31-35.
8. Yang LP, Keam SJ, Keating GM, "Deferasirox: a review of its use in the management of transfusional chronic iron overload" Drugs, 2007, 67 (15), 2211–30.
9. Karnon J, Tolley K, Oyee J, Jewitt K, Ossa D, and Akehurst R, "Cost-utility analysis of Deferasirox compared to standard therapy with Desferrioxamine for patients requiring iron chelation therapy in the United Kingdom" Current Medical Research and Opinion, 2008, 24, 1609-1621.
10. Willard HH, Merit LL, Dean FA and Settle FA, "Instrumental Methods of Analysis" 7th ed. New Delhi: C. B.S Publishers; 2008. P. 614-615.
11. Snyder LR, Kirkland JJ, Joseph LG, "Practical HPLC Method Development" 2nd ed. New York: Wiley & sons; 1997. 46-51.
12. Chauzit E, Bouchet S, Micheau M, Mahon FX, Moore N, Titier K and Molimard MA, "Method to measure Deferasirox in plasma using HPLC coupled with MS/MS detection and its potential application" Chromatographia Ther Drug Monit. 2010; 32(4), 476-481.
13. United States Pharmacopoeia, 31st ed. United States Pharmacopeial Convention, Rockville USP (2008).

