Electronic Supplementary Material (ESI) for RSC Advances.

Quality by Design Approach for Green HPLC Method Development for Simultaneous Analysis of Two Thalassemia Drugs in Biological Fluid with Pharmacokinetic Study

Michel Y. Fares[a], Maha A. Hegazy[b], Ghada M. EL-Sayed[b *], Maha M. Abdelrahman[c], Nada S. Abdelwahab[a, c]

a: Pharmaceutical Chemistry Department, Faculty of Pharmacy, Nahda University, Sharq El-Nile, 62511 Beni-Suef, Egypt

b: Analytical Chemistry Department, Faculty of Pharmacy, Cairo University, Kasr El-Aini Street, Cairo 11562, Egypt

c: Pharmaceutical Analytical Chemistry, Faculty of Pharmacy, Beni-Suef University, Alshaheed Shehata Ahmad Hegazy St, 62514 Beni-Suef, Egypt

* ***Corresponding author:***

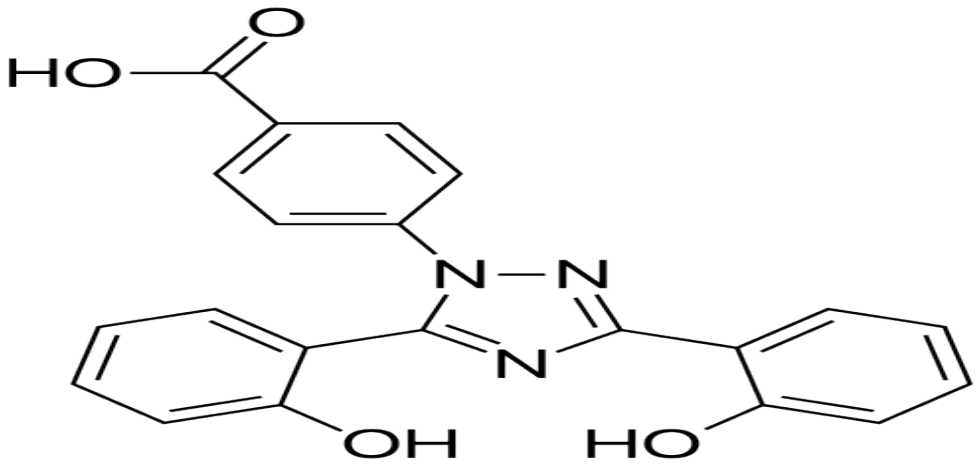
E-mail address: ghada.elsayed@pharma.cu.edu.eg ***(Ghada M. El-Sayed).***

Tel: +20-100-548-6038

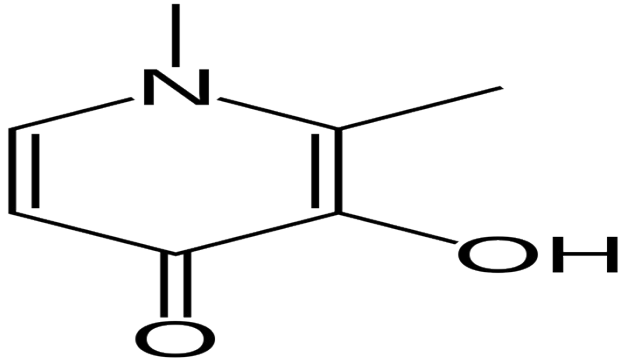
Abstract

This work implements a combined experimental approach of analytical Quality-by-design (AQbD) and green analytical chemistry (GAC) to develop an HPLC method for simultaneous determination of the two thalassemia drugs, deferasirox (DFX) and deferiprone (DFP) in biological fluid for the first time. This integration was designed to maximize efficiency and minimize environmental impacts, as well as energy and solvent consumption. To accomplish this goal, an analytical Quality-by-Design approach was performed, beginning with quality risk assessment and scouting analysis, followed by Placket-Burman design screening for five chromatographic parameters. Critical method parameters were thoroughly recognized and then optimized by using a two levels- three factors custom experimental design to evaluate the optimum conditions that achieved the highest resolution with acceptable peak symmetry within the shortest run time. The desirability function was used to define the optimal chromatographic conditions, and the optimal separation was achieved using an XBridge® HPLC RP-C18 (4.6× 250 mm, 5 µm) column with ethanol: acidic water at pH 3.0 adjusted by phosphoric acid in the ratio of (70: 30, v/v) as the mobile phase at a flow rate of 1 mL/min with UV detection at 225 nm at a temperature of 25 °C. Linearity was obtained over the concentration range of 0.30 –20.00 µg/mL and 0.20 –20.00 µg/mL for DFX and DFP, respectively, using 20.00 µg/mL of ibuprofen (IBF) as an internal standard. The greenness profile of the established method was evaluated and measured by using various assessment tools. On the validation of the developed method, FDA recommendations were followed, and all the results obtained met the acceptance criteria. The suggested method was successfully used to study the pharmacokinetic parameters of DFX and DFP in rat plasma.

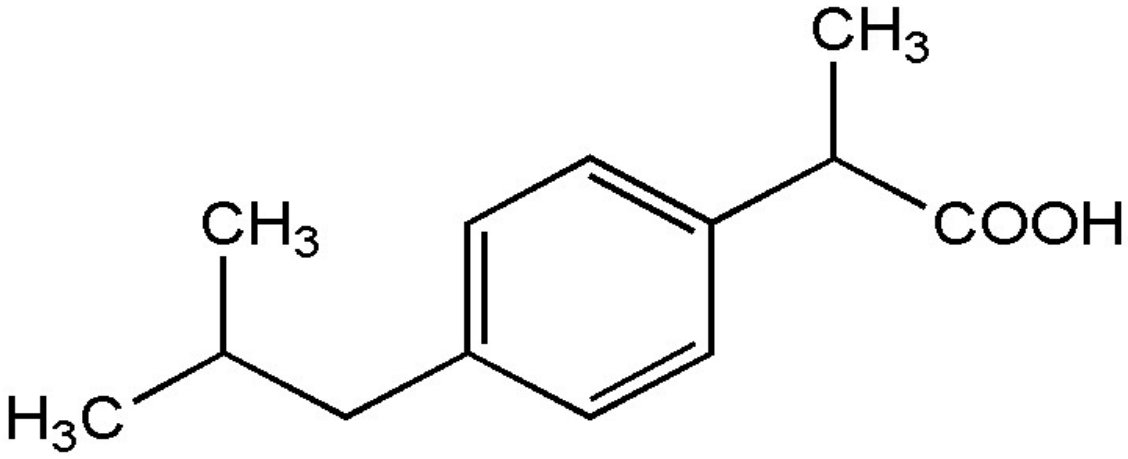
Keywords: Quality by Design; Green Profile; HPLC; Pharmacokinetic; Deferasirox; Deferiprone.



(a)



(b)



(c)

Fig. 1 S: Chemical structures of deferasirox (a), deferiprone (b), and Ibuprofen (c).

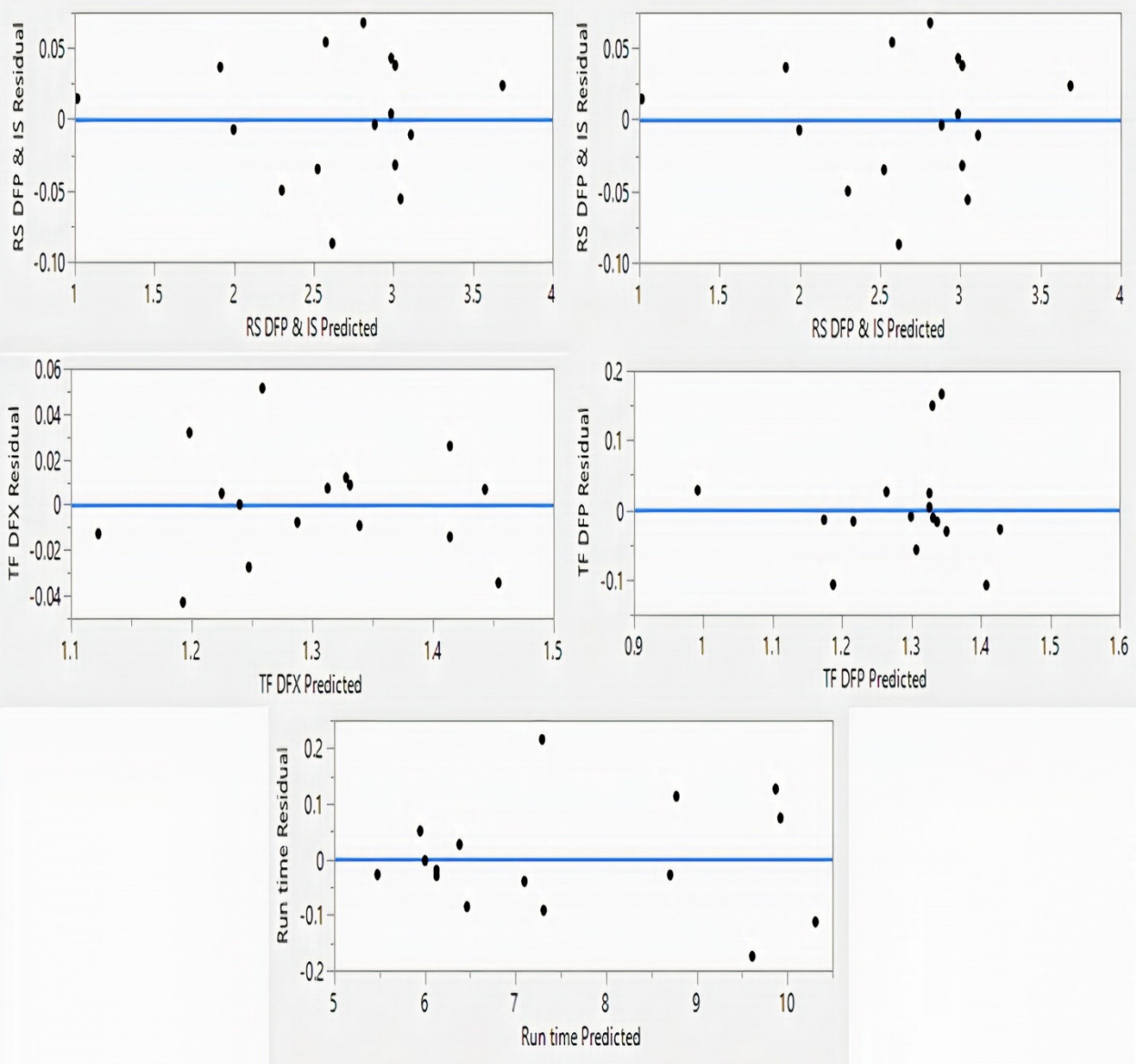


Fig. 2 S: Residuals by predicted plots.

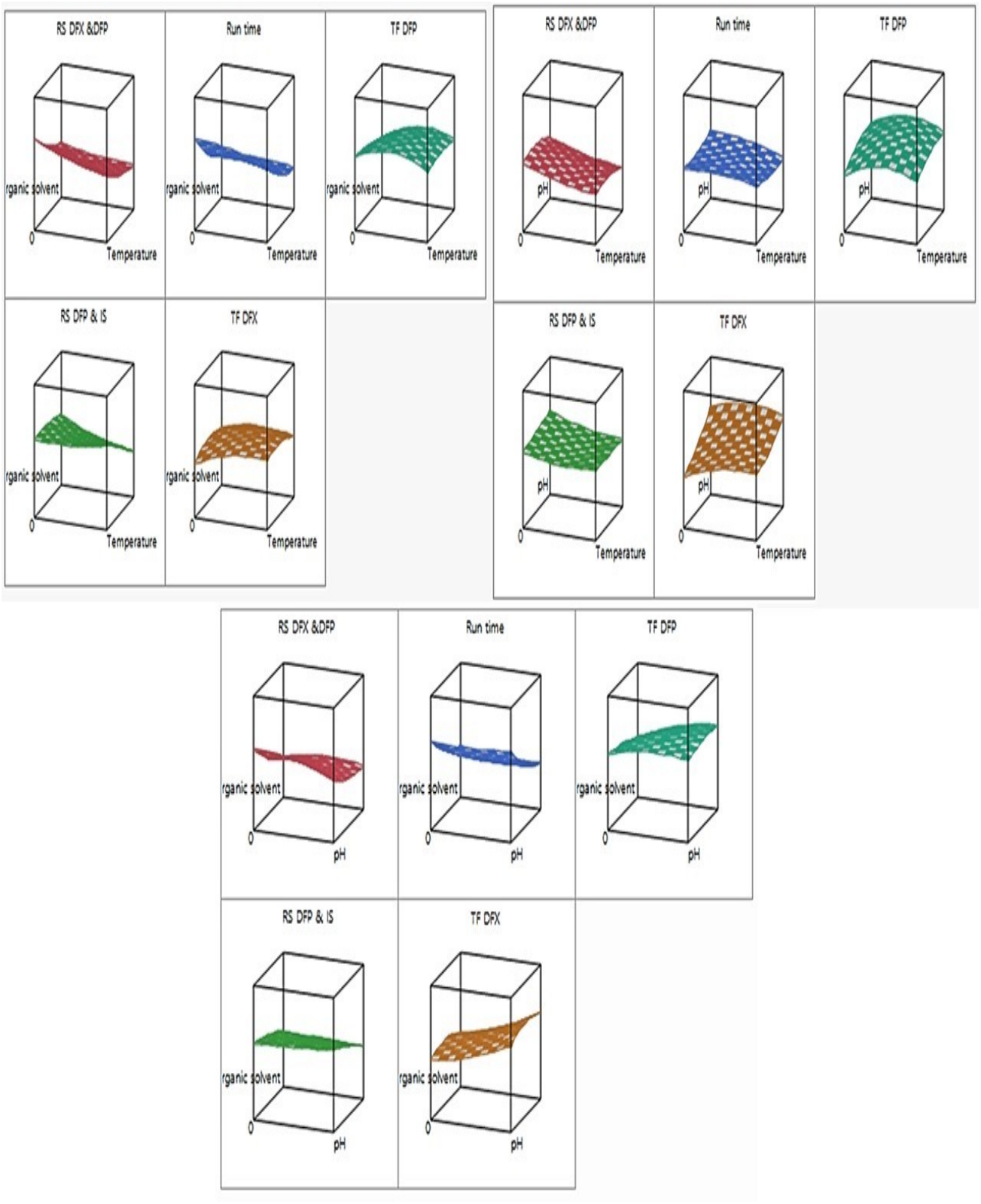


Fig. 3 S: Contour plots for the measured responses.

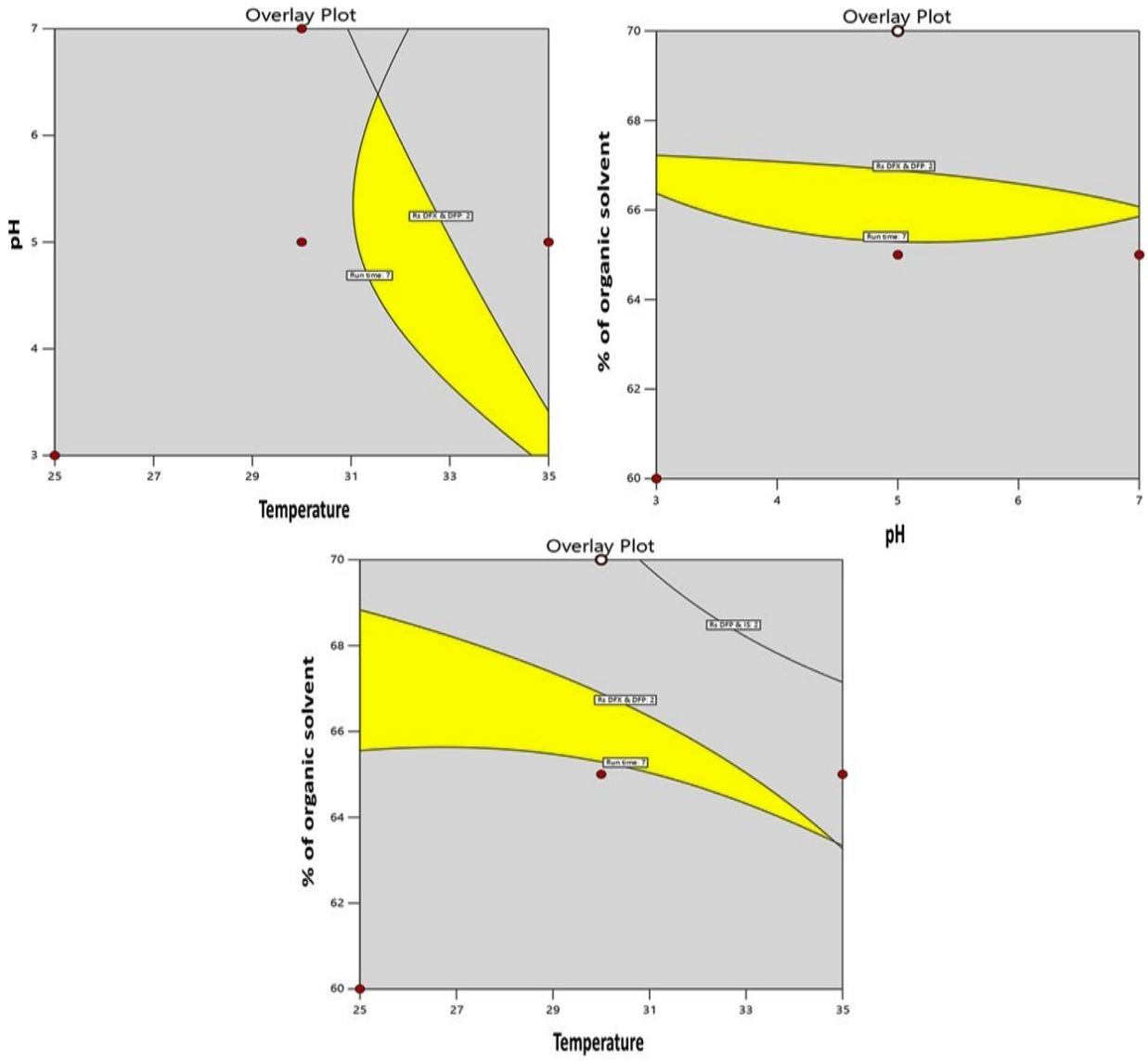


Fig. 4 S: Overlay contour plots describing the design spaces of the developed experimental design.

Table 1 S: Summary of parameters required for system suitability testing of the proposed HPLC method.

Parameters	DFX	DFP	Reference values
Retention time	4.01	4.78	-
Tailing factor (T)	1.11	1.02	< 2
Resolution (R_S)	2.21	2.25	> 2
Selectivity (α)	1.38	1.41	> 1
Capacity factor (K')	1.01	1.39	1-10
N	2858	1015	Increase with increase in column efficiency
HETP (cm)	0.009	0.025	The smaller the value, the higher the column efficiency

Table 2 S: The mean recoveries of deferasirox, deferiprone, and internal standard in rat plasma (n=7).

Compound	Added concentration (µg/mL)	% Recovery	RSD (%)
DFX	1.0	99.18 ± 1.88	2.19
	10.0	97.66 ± 2.19	2.14
	15.0	100.36 ± 1.97	1.86
DFP	1.0	98.87 ± 1.76	1.98
	10.0	97.89 ± 2.21	1.67
	15.0	100.18 ± 1.57	1.55
IS [a]	20.0	96.16 ± 2.09	2.11

[a] :Ibuprofen as an internal standard.

Table 3 S: Intra-day and inter-day precision of deferasirox and deferiprone in rat plasma (n=7).

Drug	Concentration (µg/mL)	Intra- day precision			Inter- day precision		
		Recovery % [c]	RE% [b]	RSD% [a]	Recovery % [c]	RE% [b]	RSD% [a]
DFX	1.0	98.05	0.79	0.89	101.13	-5.89	1.25
	10.0	100.94	-0.56	0.05	97.13	-3.46	0.12
	15.0	102.19	-0.83	0.56	99.84	-3.77	1.41
DFP	1.0	100.63	-6.01	0.83	99.43	-6.03	1.09
	10.0	97.87	1.84	0.06	99.93	-0.02	1.43
	15.0	99.84	9.78	0.18	102.94	8.94	0.61

[a] RSD %: Percentage of relative standard deviation.

[b] RE %: Percentage of relative error.

[c] Recovery %: Percentage of recovery.

Table 4 S: Stability results of deferasirox and deferiprone in rat plasma at different conditions.

Drug	Concentration added (µg/mL)	Remaining (%) ± SD			
		Room temperature for 2 h ± SD	auto-sampler for 24 h ± SD	Three freeze thaw cycles ± SD	- 80 °C for 6 weeks ± SD
DFX	1.0	98.55 ± 2.71	96.55 ± 1.99	97.50 ± 2.10	97.66 ± 1.98
	10.0	97.81 ± 2.01	97.65 ± 1.24	96.99 ± 2.09	96.70 ± 1.82
	15.0	96.22 ± 1.78	97.12 ± 1.65	98.87 ± 1.87	93.88 ± 3.21
DFP	1.0	98.10 ± 2.44	94.98 ± 2.44	97.88 ± 2.14	98.53 ± 1.66
	10.0	96.18 ± 2.79	97.30 ± 3.12	98.11 ± 2.23	95.81 ± 1.90
	15.0	95.99 ± 1.76	96.77 ± 2.05	98.86 ± 1.56	93.97 ± 2.87

Table 5 S: Statistical analysis of proposed HPLC and the reported methods for the determination of deferasirox and deferiprone in its dosage forms and results of standard addition technique.

Parameter	Proposed method		Reported method	
	DFX	DFP	DFX [c] [19]	DFP [d] [31]
Mean	96.92	97.25	95.06	96.49
SD	1.15	0.97	1.73	1.40
t-Test (2.228)[a]	2.19	1.09		
F-Value (5.050)[a]	2.29	2.08		
Standard addition (mean ± SD)[b]	97.31 ± 0.76	98.09 ± 0.98		

[a] The values between parentheses correspond to the theoretical values of t and F (p = 0.05).

[b] Standard addition was performed on three different levels and each was repeated three time.

[c] RP-HPLC method for determination of deferasirox in human plasma using a mobile phase of 50 mM ammonium acetate and 20 mM tetrabutyl ammonium hydrogen sulfate buffer (pH 6.3) –acetonitrile–methanol in the ratio of (33:22:45 v/v), and imipramine was used as an internal standard.

[d] RP-HPLC method for quantification of deferiprone in human plasma using a mobile phase of methanol-buffer (18:82, v/v), pH 3.5, and caffeine was used as an internal standard.

Table 6 S: The penalty points for the determination of deferasirox and deferiprone in rat plasma by the proposed method.

Reagents	Penalty points
(Ethanol: acidic water) (70: 30%) , F.R= 1.0 - **Distilled water** - **Phosphoric acid** **Subtotal PP= 1** (solvent <10 mL) **Signal word= Danger** (More severe hazard =2) = **2** **No. of pictogram= 1** (corrosive)  **PP of ethanol= subtotal PP * number of pictogram * signal word** **= 1*2*1= 2** - **Ethanol** **(run time* flow rate)= (6*1) = 6 mL.** **= (6*70)/100= 4.20 mL.** **Subtotal PP= 1** (solvent <10 mL) **Signal word= Danger** (More severe hazard =2) = **2** **No. of pictogram = 2** (flammable & irritant)  **PP of ethanol= subtotal PP * number of pictogram * signal word** **= 1*2*2= 4**	**0** **2** **4**
∑	**6**
Instruments	**Penalty points**
- **Energy:** (≤1.5 kWh per sample =1), HPLC= 1 - **Occupational hazard:** Analytical process hermetization: 0 - **Waste:** (1-10 mL= 3)	**1** **0** **3**
∑	**4**
Total penalty points =	**10**
Analytical Eco-Scale total score =	**90**

* Mobile phase consisted of ethanol: acidic water pH =3 (70:30, v/v).