ESTIMATION OF DORAVIRINE IN TABLETS BY RP-HPLC THROUGH QUALITY BY DESIGN APPROACH AND ITS APPLICATION TO STRESS DEGRADATION STUDIES

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Abstract

As per the present regulatory scenario the method for Quality Control of Doravirine (DRN) was optimized with Analytical Quality by design (AQbD) approach. Experimental data is procured by Full factorial design in Design Expert software with three critical process parameters flow rate, aqueous mobile phase ratio and wavelength. The dependent critical quality attribute for the above process parameters were theoretical plates, retention time and capacity factor. The optimized chromatographic conditions were BDS (150×4.6 mm, 5µm) kromasil column, mobile phase of mixture of buffer-potassium hydrogen phosphate and acetonitrile (65: 35 v/v) with flow rate 1.0ml/min. The ICH guidelines were considered for method validation.. The method was linear between concentration and peak area with the range of concentration 5-30 µg/ml. The detection and quantitation limits were found to be 0.02 µg/ml and 0.07 µg/ml respectively. The DRN was retained at 2.2 min with maximum absorbance wavelength 272.0 nm. Several stress conditions like hydrolytic (acid, base, neutral), oxidative, thermal and photolytic were exposed on the drug to study the degradation pattern. Degradation was more in basic condition. So the developed method was robust, accurate, sensitive and stability indicating and the developed method can be used for routine analysis. The method was extended to analyse the drug in the presence of degradants.

Keywords: QbD, Doravirine, stress degradation.

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INTRODUCTION

HIV stood as a great world public health issue, by claiming 40.4 million [32.9–51.3 million] cases up to now with continuos transmission in all countries globally. Doravirine is a non-nucleoside inhibitor of reverse transcriptase enzyme of human immunodeficiency virus type 1 (HIV-1). It is a derivative of pyridinone used to treat HIV infection. Orally Doravirine non-competitively inhibits HIV-1 reverse transcriptase enzyme, which results in inhibiting HIV-1 viral replication. The enzyme Reverse transcriptase generates complementary DNA (cDNA) with HIV to its RNA genome - this cDNA is then inserted into the host cell genome, where it can be transcribed into viral RNA for the purposes of replication. Doravirine inhibits HIV-1 replication by inhibiting HIV-1 reverse transcriptase non-competitively. However, Doravirine does not inhibit the human cellular α, ß, DNA polymerases and γ mitochondrial DNA polymerase.

Literature survey states that there was no QbD based method reported for analysis of DRN. There is much need for Analytical QBD methods in Pharma Industry as they took less time and also cost effective. So the present research work was based on the QbD method.

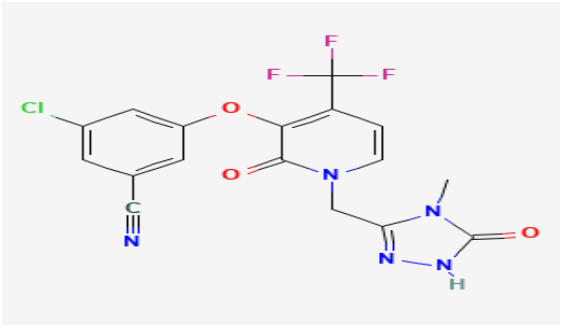

Figure-1: Structure of Doravirine

EXPERIMENTAL

Materials

The HPLC 2695 SYSTEM (WATERS) of Empower 2 Software with PDA detector was chosen for the estimation of drug. Weighing was done with Electronic Balance (Denver). pH values were recorded with Digital pH meter 7007 (Digisun Electronics Hyderabad). The mobile phase was degassed with Ultra sonicator Labman. UV-VIS spectrophotometer integrated with UV win 6 Software, Vacuum pump of Crompton), Hot Air Oven of Serve well Instrument PVT LTD, Bangalore were taken to perform the research work. Design expert software of version 11 was used for method development.

Acetonitrile and water of HPLC grade were purchased from Merck chemical division, Mumbai. Potassium dihydrogen ortho phosphate, Ortho-phosphoric acid of AR grade were procured from Rankem, avantor performance material India limited. Hetero Labs Ltd, Hyderabad supplied Doravirine pure drug as gift sample.

Method development

Preparation of Standard stock solution:

10mg of Doravirine pure drug was accurately weighed and transferred into a 50ml clean dry volumetric flask and 10ml of diluent was added. Sonication was done for 10 minutes and finally volume was made to 50 ml with diluents (200 µg/ml of Bupropion). From the above stock solution 1ml was taken into a 10ml volumetric flask and volume was made upto 10ml. (20 µg/ml of Doravirine) [1].

Mobile phase Preparation:

1.36 gm of Potassium dihydrogen Ortho phosphate (0.01N KH_2PO_4) was accurately weighed and transferred into a 1000ml of Volumetric flask and about 900ml of milli-Q water was added and degassed with sonicator and finally volume was made with water and pH was adjusted with Ortho phosphoric acid. (pH 4.8).

Potassium dihydrogen Ortho phosphate buffer and Acetonitrile was uesd in the ratio of 65:35 (v/v)

Software implementation:

Design expert software of version 11.1.0.1 was used. Response surface method with randomised study method was utilised. 20 runs obtained from Central composite design (CCD) were performed experimentally (table 1). Build time of 19 ms was used in Quadratic model. Effects of several parameters were studied at once in this approach[2]. Risk assessment suggested that flow rate, aqueous mobile phase ratio and wavelength as input variables. Input variables influenced responses of retention time, theoretical plates and capacity factor. The runs given by central composite design were performed as Chromatographic trails. These runs are of different compositions of input variables for the optimisation of the method at maximum absorbance wavelength 272.0 nm. Interactions between variables were studied by Correlation graphs. Fraction of design space statistics gives the data of Prediction based metrics. If Condition number is 1.33 then the method is less collinear.

Standard error of design was used to study interaction between factors. Data was collected from various graphs like correlation graphs,

correlation matrices, FDS graphs, perturbation graphs and interaction graphs[4].

Optimised conditions of the developed method:
After performing experiments with the given trails by the design the optimised conditions were found to be Flow rate of 1ml/min, Detectors wave length was 272.0 nm and aqueous mobile phase ratio was 65%. Column temperature was 30°C with Injection volume of 10.0µL. The doravirine drug was detected at 2.2 min with Run time of 5.0 minutes.

Validation of the method
Method to determine the purity of Dosage form:
Equivalent weight of 50 mg of the powder sample was transferred into a 100ml volumetric flask. 50ml of diluent was added and sonicated for 25 min, further the volume was made up with diluent and the solution is filtered by milli-Q filters (1000µg/ml Doravirine). 0.4ml of filtered sample stock solution was taken in 10ml volumetric flask and made up with diluent. (20µg/ml Doravirine) [5].

The amount of drug in the tablets was determined by calibration curve method through regression equation. Label Claim of Pifeltro tablets is 100 mg of Doravirine and the assay data is in table 13.

Linearity:
Various concentrations like 5, 10, 15, 20, 25, 30 µg/ml were prepared from standard stock solution of Doravirine. Peak areas were recorded. These peak areas are directly proportionate with the concentration values and hence showed linear values. From that peak area values, Regression equation and correlation coefficient (R^2) were determined[6].

Precision:
Intraday precision and inter day precision were performed for determining method precision. Intra-day study was done by analysing the six different concentrations of drug for three times in the same day. Inter-day Precision was accomplished by analysing the same six different concentrations of drugs for three days in a week and the data is tabulated in table 10.

Accuracy:
Three different levels i.e. 50%, 100%, 150% of the pure drug was taken to assess the accuracy of the method. The recovery studies was carried out by adding known amount of standard solution of the drug to the pre analysed tablet solutions. The resulting solutions were then reanalysed by regression equation methods and the data is in table 11[7].

*Limits of detection (*LOD) *& Quantification (LOQ):*
The limit of Detection and the limit of quantification were determined by formula method. The formula is LOD=3.3 SD/slope and for LOQ=10 SD /slope. (SD= standard deviation)

Robustness:
Peak areas were found by deliberately changing the experimental conditions. Flow rate, aqueous mobile phase ratio and wavelength parameters were changed slightly within the method operable design region and the values were recorded in the table 12.

Method of Degradation[3]
Hydrogen Peroxide degradation:
1 ml of stock solution of Doravirine was taken and 1 ml of 20% hydrogen peroxide (H_2O_2) was added. The duration of the observation was 30 min and the solution is maintained at 60^0c. The resultant solution was diluted to obtain 20µg/ml solution and 10 µl were injected into the HPLC system and the chromatograms were studied to assess the stability of sample.

Acid Degradation:
1 ml of stock solution of Doravirine was taken and 1 ml of 2N HCl was added. The duration of the observation was 30 min and the solution is maintained at 60^0c. The resultant solution was diluted to obtain 20µg/ml solution and 10 µl were injected into the HPLC system and the chromatograms were studied to assess the stability of sample.

Base Degradation:
1 ml of 2N sodium hydroxide was added to 1 ml of Doravirine stock solution and refluxed for 30mins at the temperature of 60^0c. The above solution was diluted to obtain 20 µg/ml solution and 10 µl was injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation:
The Doravirine stock solution was placed in an oven at 105°C for a period of 6 hours. From the above stock solution 20 µg/ml dilution was prepared and 10µl solution was injected into the system and the obtained chromatograms were recorded to observe the stability of the sample[9].

Photo Degradation:

The photochemical stability of Doravirine was also studied by exposing the 200 µg/ml solution to UV Light by keeping the beaker in UV Chamber for 7days or 200Watt hours/m^2 in photo stability chamber. The resultant solution was diluted to obtain 20 µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral Degradation:

The drug was refluxed in water for 6 hours at a temperature of 60ºC. For HPLC study, the resultant solution was diluted to 20µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of drug[10]. The data regarding the degradation studies is in table 14.

RESULTS AND DISCUSSION

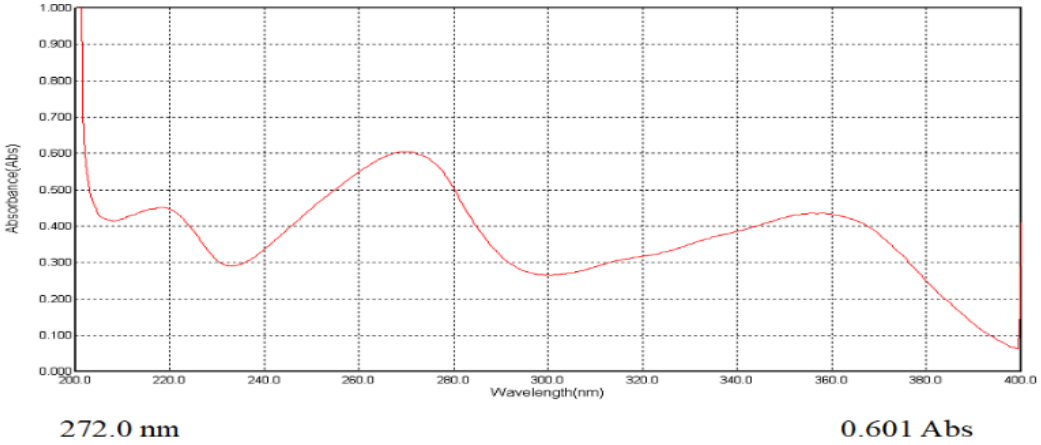

Figure-2: UV Spectrum of Doravirine

Method development by QbD Approach

Table-1: Central Composite Design for 20 runs

		Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3
Std	Run	A:FR	B:MP(aqueous)	C:Wavelenght	RT	NTP	KF
		min/ml	%	num	min	num	num
1	12	0.9	60	270	2.306	7464.6	1.31
2	20	1.1	60	270	1.898	4682.1	0.9
3	13	0.9	70	270	2.536	6668.5	1.54
4	17	1.1	70	270	2.054	7341.1	1.05
5	10	0.9	60	274	2.301	5661.8	1.3
6	3	1.1	60	274	1.881	4686.2	0.88
7	18	0.9	70	274	2.524	6165.8	1.52
8	4	1.1	70	274	2.057	7853	1.06
9	9	0.831821	65	272	2.567	8264.1	1.57
10	11	1.16818	65	272	1.853	7398.1	0.85
11	14	1	56.591	272	2.051	6169.8	1.05
12	5	1	73.409	272	2.391	8679.1	1.39
13	16	1	65	268.636	2.165	4271.1	1.16
14	6	1	65	275.364	2.165	4080.9	1.17
15	15	1	65	272	2.152	7931	1.15
16	8	1	65	272	2.153	7910	1.15
17	2	1	65	272	2.156	7987	1.16
18	1	1	65	272	2.159	7909	1.16
19	7	1	65	272	2.166	8017	1.17
20	19	1	65	272	2.168	8014	1.17

Table-2: Build Information

File Version	11.1.0.1		
Study Type	Response Surface	**Subtype**	Randomized
Design Type	Central Composite	**Runs**	20
Design Model	Quadratic	**Blocks**	No Blocks
Build Time (ms)	19.00		

Table-3: Input Factors (Critical Process Parameters)

Factor	Name	Units	Type	Minimum	Maximum	Coded Low	Coded High	Mean	Std. Dev.
A	FR	min/ ml	Numeric	0.8318	1.17	-1 ↔ 0.90	+1 ↔ 1.10	1.000 0	0.08 48
B	MP (aqueous)	%	Numeric	56.59	73.41	-1 ↔ 60.00	+1 ↔ 70.00	65.00	4.24
C	Wavelength	num	Numeric	268.64	275.36	-1 ↔ 270.00	+1 ↔ 274.00	272.0 0	1.70

Table-4: Responses (Critical Quality Attributes)

Respo nse	Name	Units	Observ ations	Analysis	Minimum	Maxim um	Mean	Std. Dev.	Ratio	Transform	Model
R1	RT	min	20	Polynomial	1.853	2.567	2.19	0.2047	1.39	None	Quadra tic
R2	NTP	num	20	Polynomial	4080.9	8679.1	6857.71	1464.83	2.13	None	Quadra tic
R3	KF	num	20	Polynomial	0.85	1.57	1.19	0.2053	1.85	None	Quadra tic

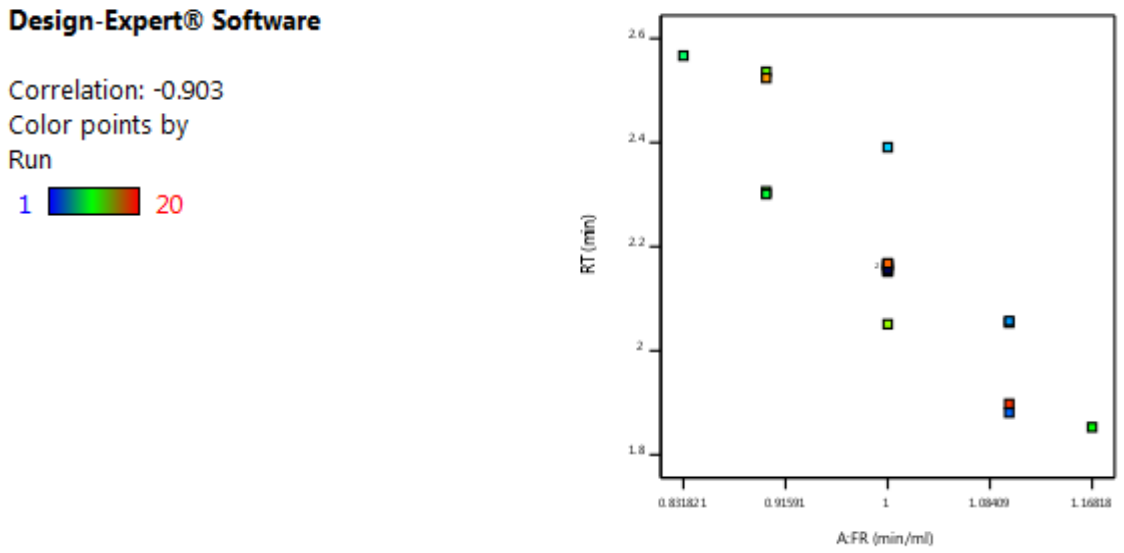

Figure-3: Correlation graph

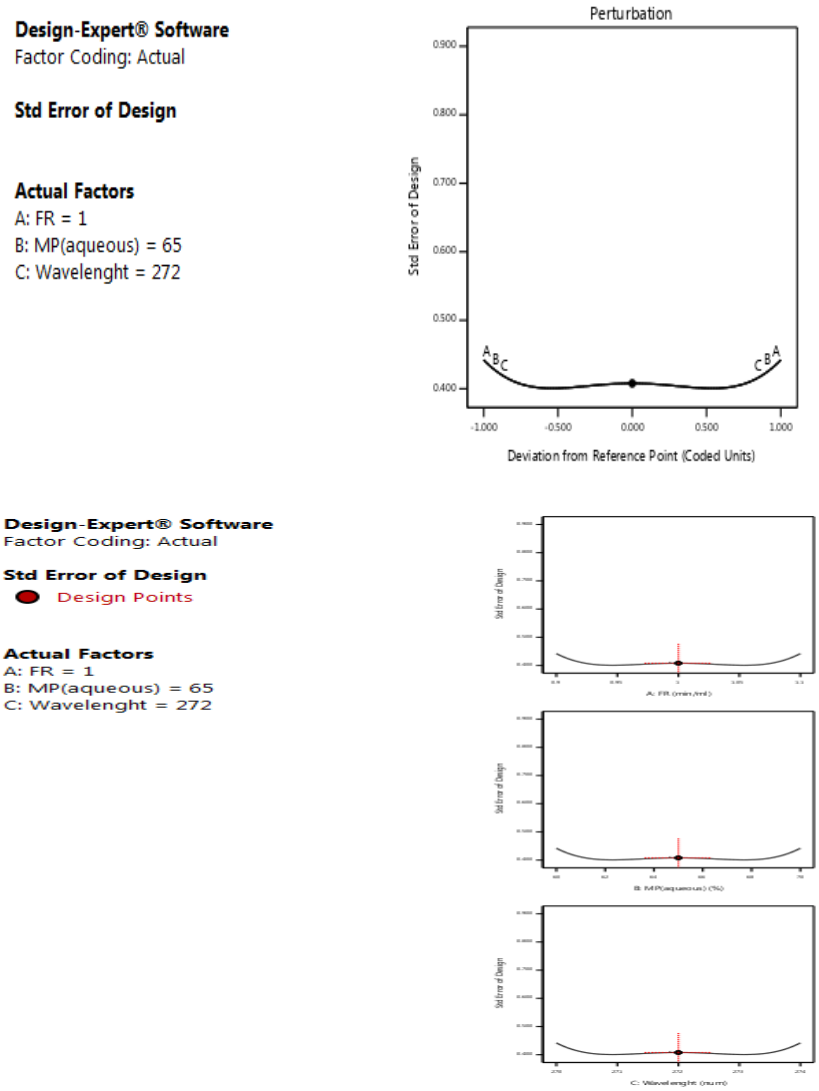

Figure-4: Perturbation graphs

The maximum absorbance wavelength of Doravirine showed at 272nm in UV Spectrum. The data of 20 runs was obtained with various combinations of 3 input factors namely flow rate, aqueous phase of mobile phase and wavelength through central composite design(CCD). The CCD given highest and lowest values of three variables. The lowest and highest values of Flow rate are 0.8318 ml/min and 1.17 ml/min respectively. The highest and lowest values for aqueous phase are 73.41% and 56.59% respectively. The highest and lowest values For wavelength are 275.36 nm and 268.64 nm respectively.

The influence of flow rate on retention time was studied by correlation graph. The above correlation graph shows the optimum retention time (response factor) where there is less influence of flow rate on retention time. The perturbation graphs showed the values of standard error of design for the input factors. These graphs showed the deviation of input factors flow rate, aqueous phase and wavelength from the reference points 1ml/min, 65%, 272 nm.

Fit Summary data

Response 1: Retention Time

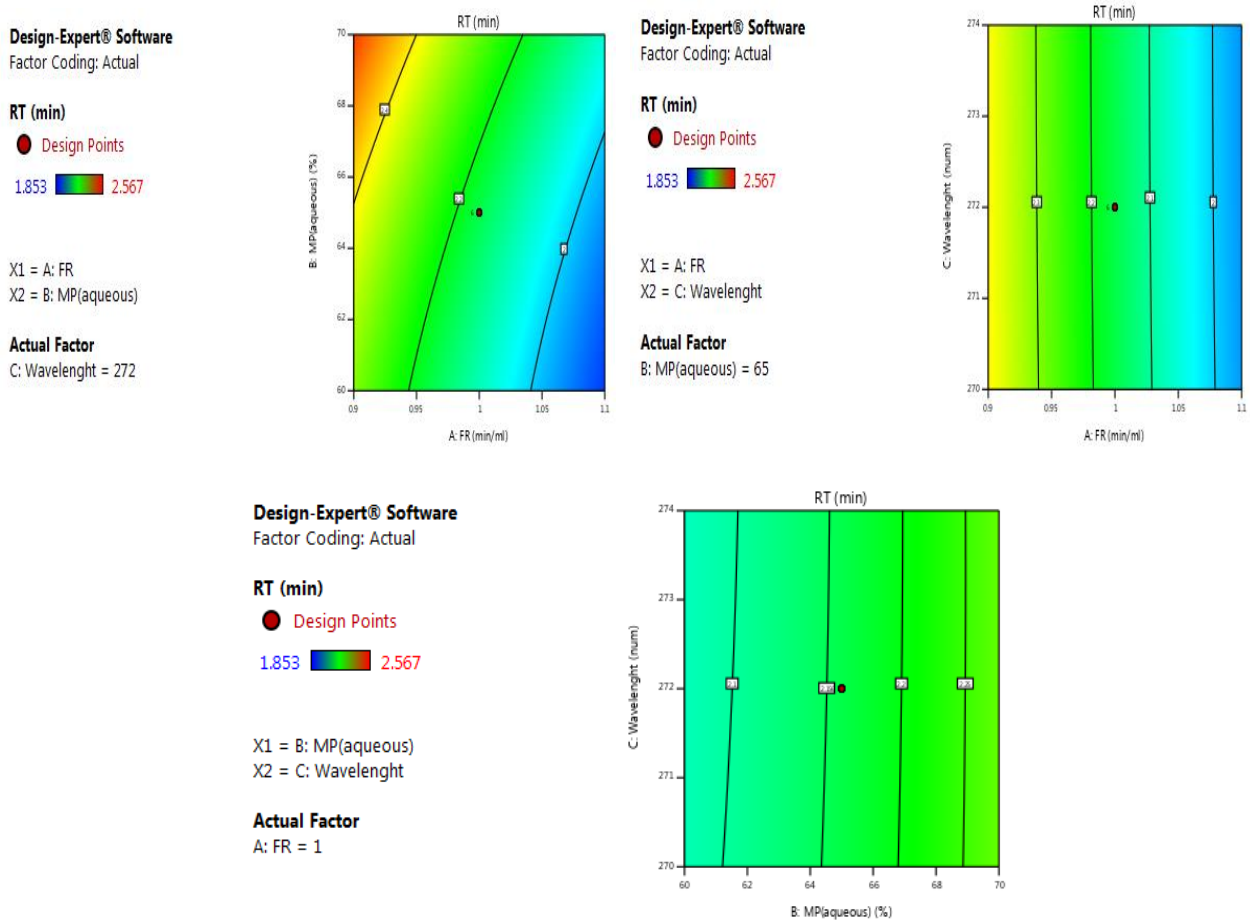

Figure-5: Contour graphs

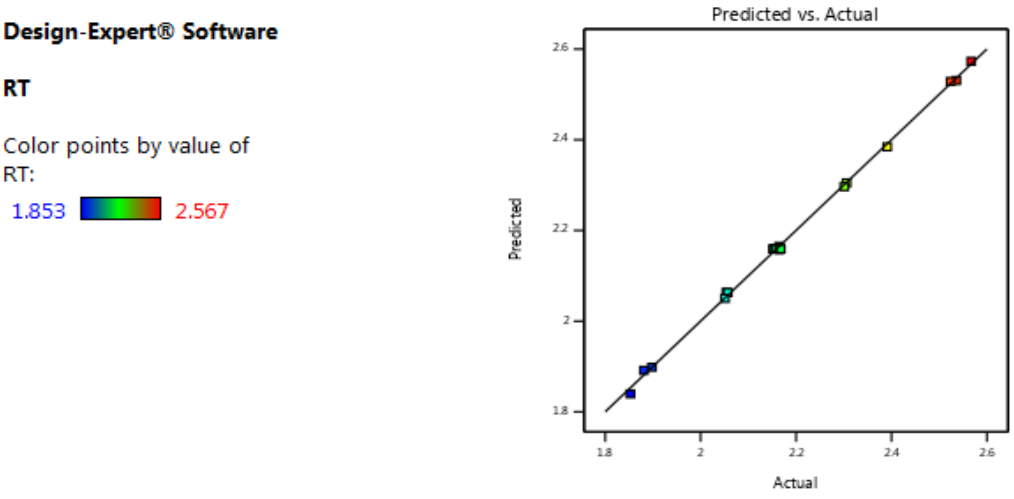

Figure-6: Linearity graph between predicted and actual values

The above contour graphs displayed the best responses (retention time) with the input factors combination. Flow rate should be 1 ml/min and aqueous phase should be 65% for the retention time of approximately 2.1min. At this retention time the influence of input factors is less. The contour graph showed the method operable design region for flow rate that is between 0.9 to 1.1 ml/min and for the aqueous phase it is between 60 to 70% and for the wavelength it is between 270 to 274 nm. The above plot showed the linearity between the actual responses and predicted responses.

Fit Summary data

Response 2: *Theoretical Plates*

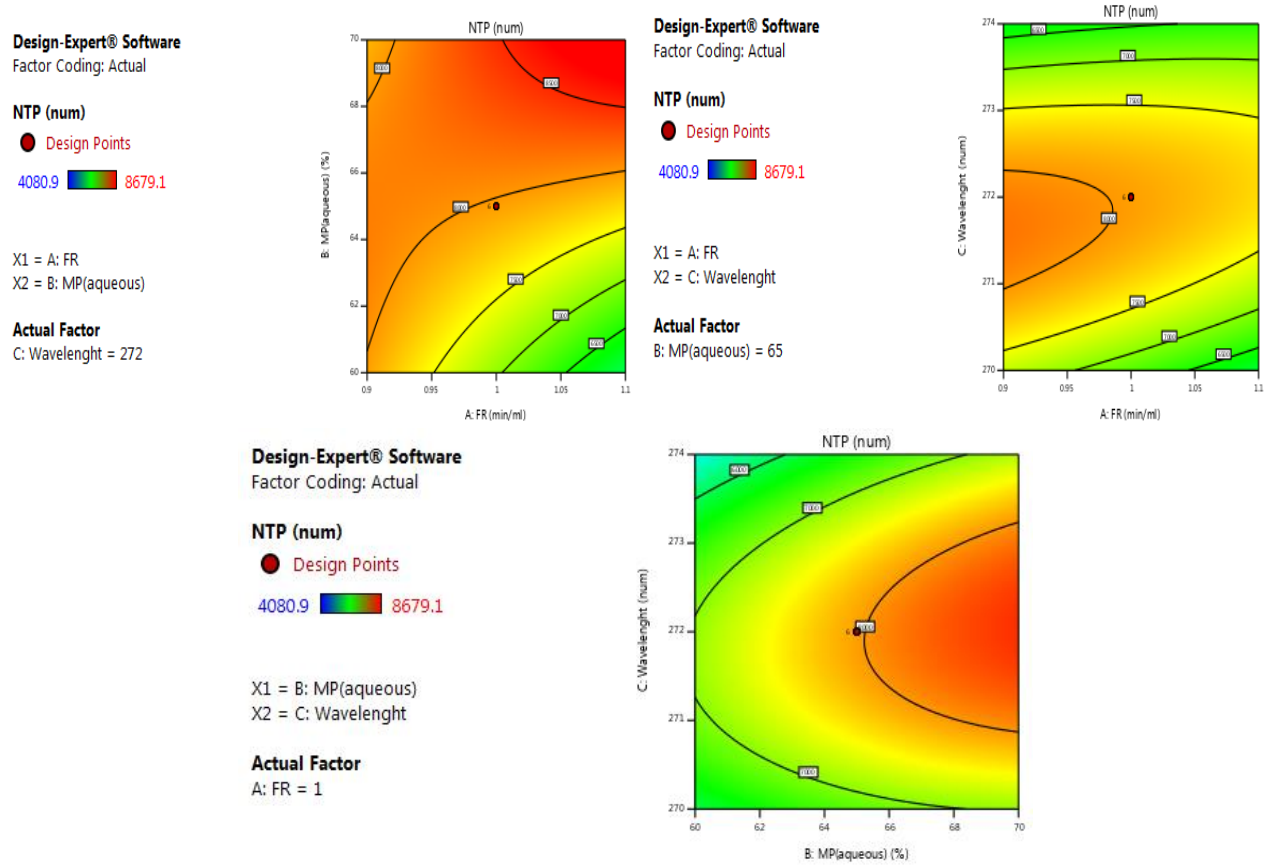

Figure-7: Contour graphs

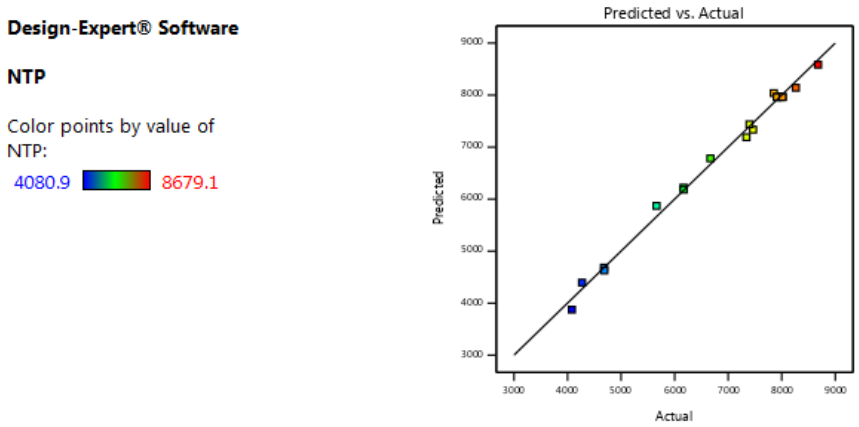

Figure-8: Linearity graph between predicted and actual values

The above contour graphs given the best responses (theoretical plate number) with the input factors combination. Flow rate should be 1 ml/min and aqueous phase should be 65% for the theoretical plates of approximately 6857. At this theoretical plate number the influence of input factors is less. The above plot showed the linearity between the actual responses and predicted responses. The contour graph showed the method operable design region for flow rate that is between 0.9 to 1.1 ml/min and for the aqueous phase it is between 60 to 70% and for the wavelength it is between 272 to 274nm. The above plot showed the linearity between the actual responses and predicted responses.

Response 3: Capacity Factor

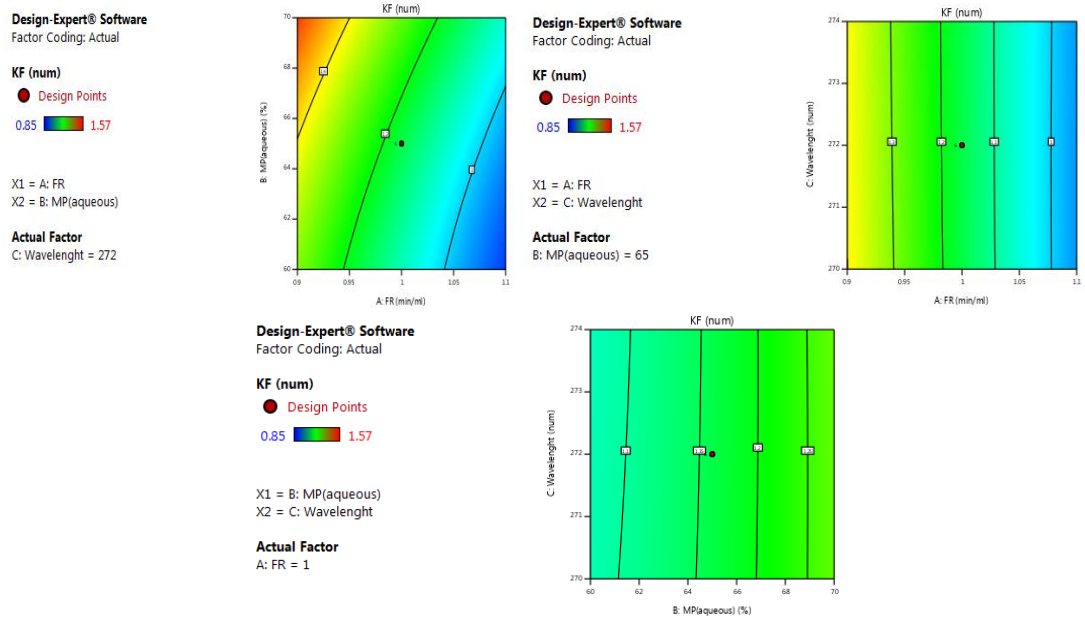

Figure-9: Contour graphs

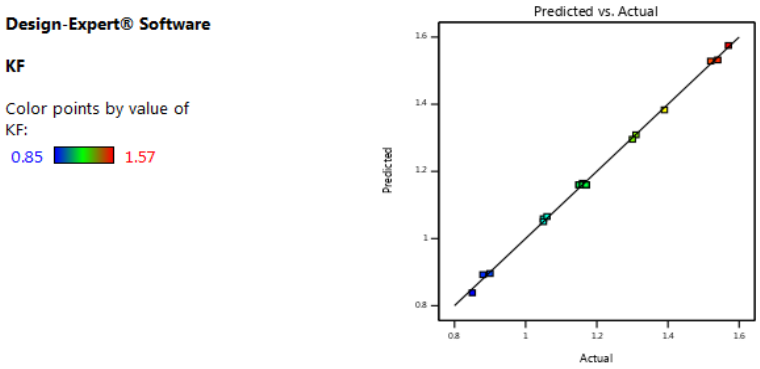

Figure-10: Linearity graph between predicted and actual values

The above contour graphs showed the best responses (capacity factor) with the input factors combination. Flow rate should be 1 ml/min and aqueous phase should be 65% for the capacity factor of approximately 1.1. At this capacity factor the influence of input factors is less. The above plot showed the linearity between the actual responses and predicted responses. The contour graph showed the method operable design region for flow rate that is between 0.9 to 1.1 ml/min and for the aqueous phase it is between 60 to 70% and for the wavelength it is between 270 to 274 nm. The above plot showed the linearity between the actual responses and predicted responses.

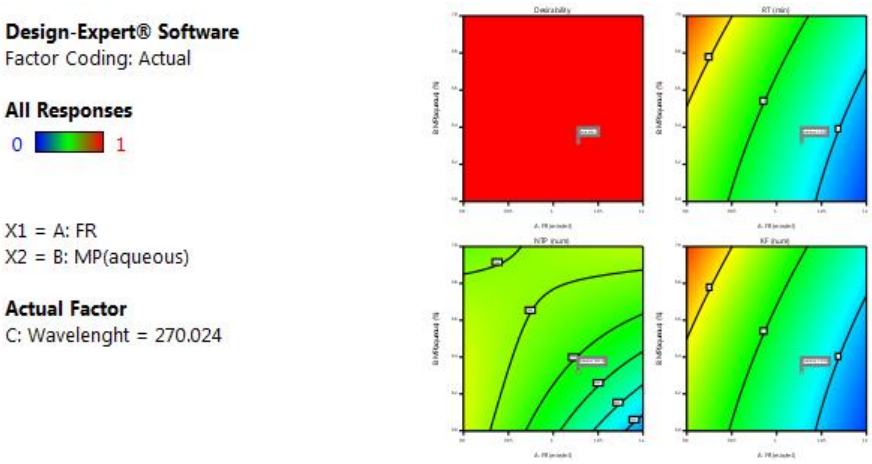

Figure-11: Desirability graph and contour graphs

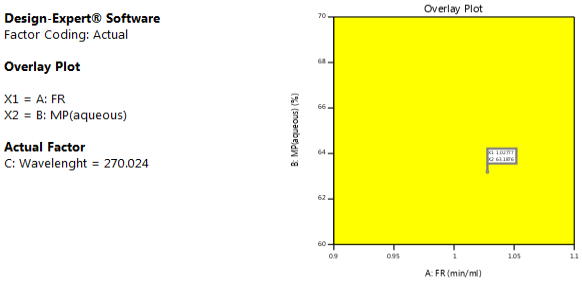

Figure-12: Overlay plot

The above desirability graph showed the point of flow rate value and aqueous phase value for the best responses. The contour graphs of three responses showed the desirable values of input factors. The contour graph of capacity factor showed the desirability point of flow rate 1.02 ml/min and aqueous phase of 63.1%. The overlay plot showed the optimum values of flow rate that is 1.02 ml/min and aqueous phase of 63.1%.

Table-5: ANOVA for Retention Time

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	0.7953	9	0.0884	1041.78	< 0.0001	significant
Lack of Fit	0.0006	5	0.0001	2.79	0.1425	not significant

The Model F-value of 1041.78 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. Model terms are significant if p-value is less than 0.005. The Lack of Fit F-value of 2.79 implies the Lack of Fit is not significant.

Table-6: ANOVA for Theoretical Plate number

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	4.054E+07	9	4.504E+06	193.52	< 0.0001	significant
Lack of Fit	2.199E+05	5	43982.33	17.15	0.0036	significant

The Model F-value of 193.52 implies the model is significant. P-values less than 0.0001 indicate model terms are significant. The Lack of Fit F-value of 17.15 implies the Lack of Fit is significant.

Table-7: *ANOVA for Capacity Factor*

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	0.7997	9	0.0889	745.66	< 0.0001	significant
Lack of Fit	0.0008	5	0.0002	1.98	0.2358	not significant

The Model F-value of 745.66 implies the model is significant. P-values less than 0.0001 indicate model terms are significant. The Lack of Fit F-value of 1.98 implies the Lack of Fit is not significant.

Table-8: Confirmation Location

Flow rate	Organic phase	Wavelength
1.02777	63.1876	270.024

Validation data

Table-9: Linearity data of Doravirine

S.No	Concentration (µg/ml)	Peak area
1	5	126365
2	10	242607
3	15	347972
4	20	474588
5	25	597207
6	30	712715

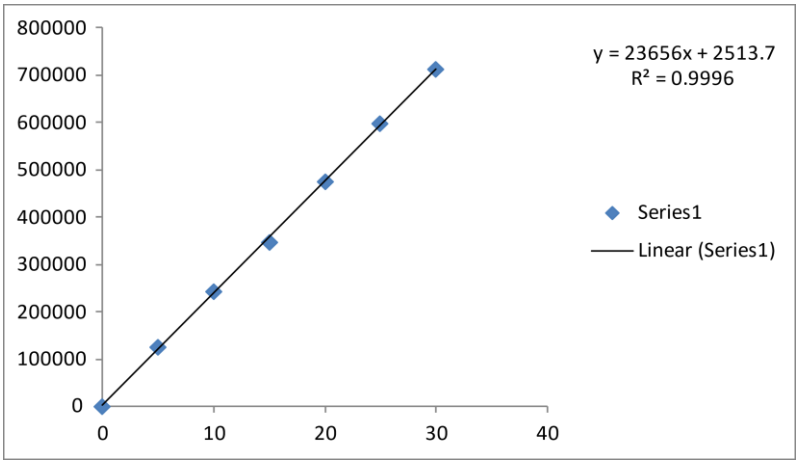

Figure-13: Calibration curve

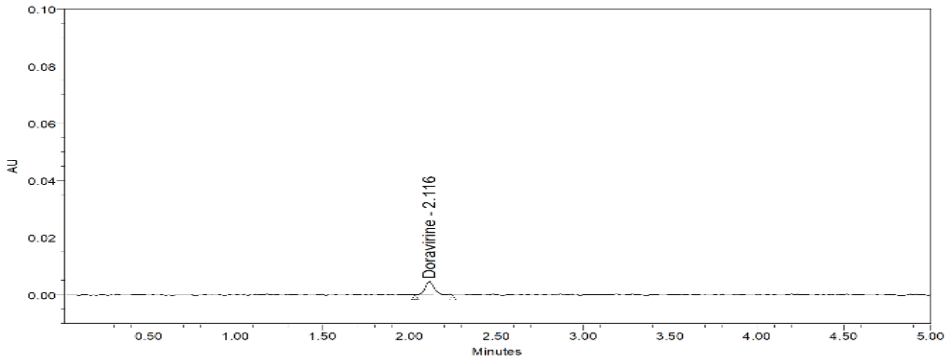

Figure-14: LOD of Doravirine

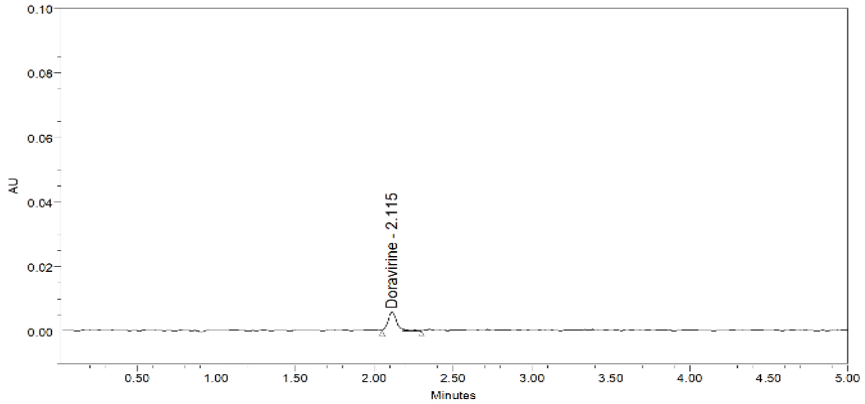

Figure-15: LOQ of Doravirine

Table-10: Precision data of Doravirine

S.No	Peak area of Initial concentration	Peak areas of Intraday precision	Peak areas of Interday precision
1	471881	475523	464753
2	475357	477219	462013
3	476729	474047	463476
4	473067	473739	463330
5	474704	469137	460702
6	477737	475110	467878
Avg	474913	474129	463692
SD	2196.1	2739.8	2473.3
%RSD	0.5	0.6	0.5

Table-11: Data of Recovery studies

S.No	Recovery level	Amount of sample drug (µg/ml)	Amount of pure drug (µg/ml)	Total amount (µg/ml)	%Recovery	%RSD
1	50%	20	10	29.90	99.04	0.4
2	100%	20	20	39.92	99.61	0.5
3	150%	20	30	49.70	99.01	0.4

Table-12: Robustness data

	High flow rate	Low flow rate	High aqueous phase	Low aqueous phase	High wavelength	Low wavelength
Peak area	432999	509875	496169	492368	500673	450234
SD	3196.4	1949.6	5742.6	4754.2	2087.4	1491.8
%RSD	0.4	0.7	1	1.2	0.3	0.4

Table-13: Assay Data

Drug	Lable claim (Pifeltro tablets)	Amount found	%Recovery	%RSD
Doravirine	100 mg	104.6 mg	104.6	0.6

The analytical concentration range was 5-30 (µg/ml). The correlation coefficient R^2 is 0.9996. The intraday and inter day precision RSD values were 0.6 and 0.5 respectively. Amount of drug recovered in recovery studies was 99.04%, 99.61% and 99.01% at 50%, 100% and 150% levels respectively. The robustness %RSD **values** were 0.4 and 0.7 at higher and lower levels of flow rate respectively. The %RSD of robustness were 1.0 and 1.2 at higher and lower levels of aqueous phase respectively. The robustness %RSD values were 0.3 and 0.4 at higher and lower levels of wavelength respectively. The amount of drug recovered in assay was found to be 104.6%.

Table-14: Degradation data of Doravirine tablet

	Peak area		% Drug	% Degradation
	Initial	After Degradation		
Acid	474913	461736	97.03	2.97
Base	474913	430664	90.50	9.50
Peroxide	474913	474913	98.86	1.14
Thermal	474913	471071	98.99	1.01
UV	474913	469037	98.57	1.43
Water	474913	472654	99.33	0.67

The degradation was found to be high in basic condition among all the stress conditions and the degradation of dosage form was 9.5%.

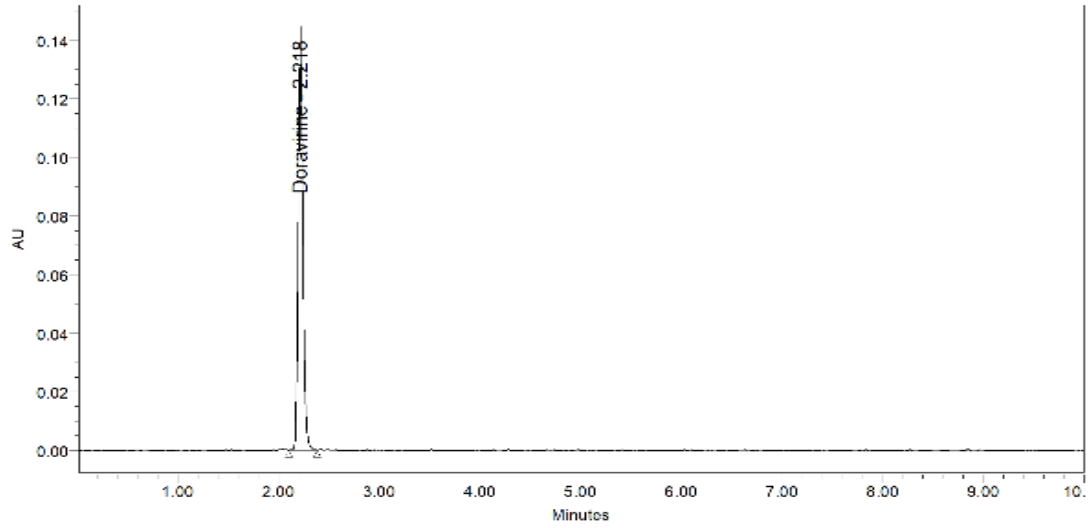

Figure-16: Degradation of Doravirine under oxidation

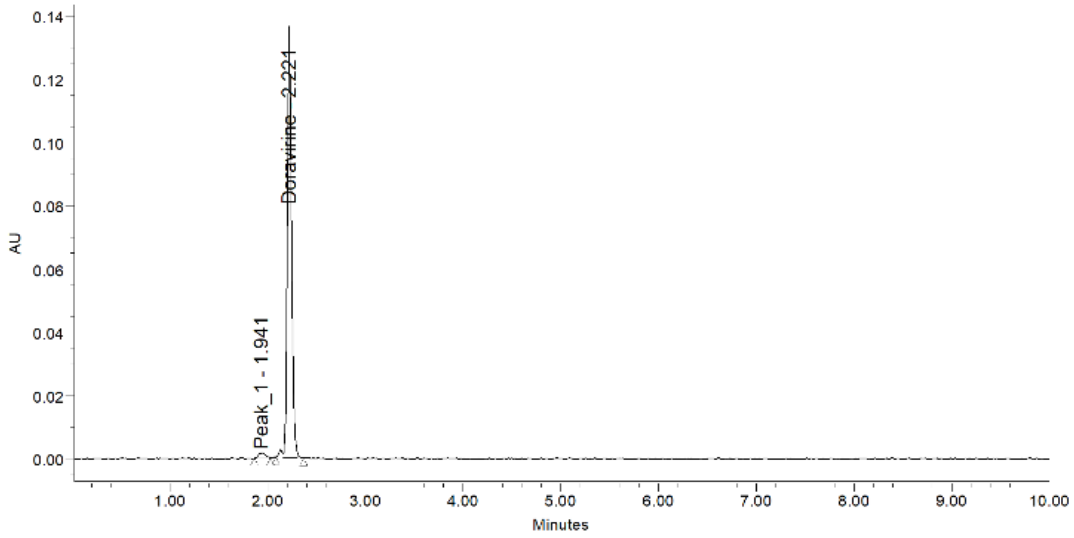

Figure-17: Basic degradation of Doravirine

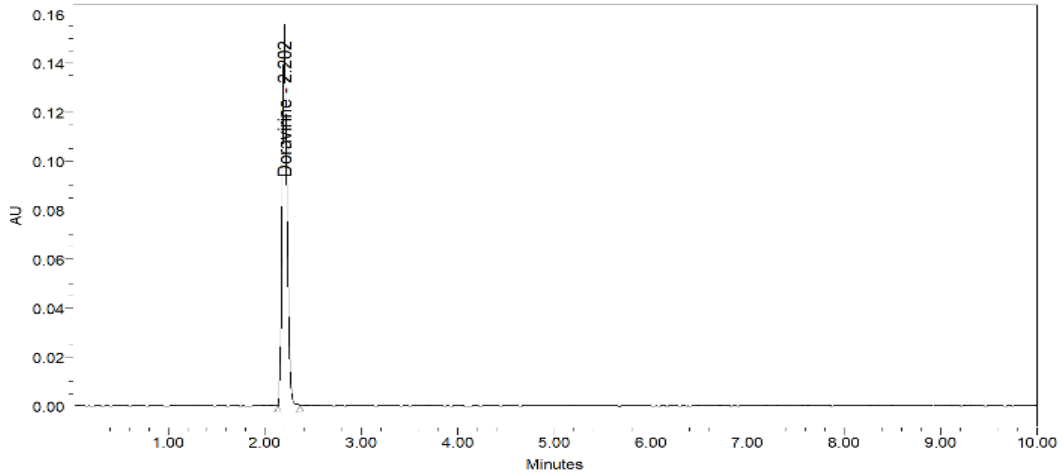

Figure-18: Thermal degradation of Doravirine

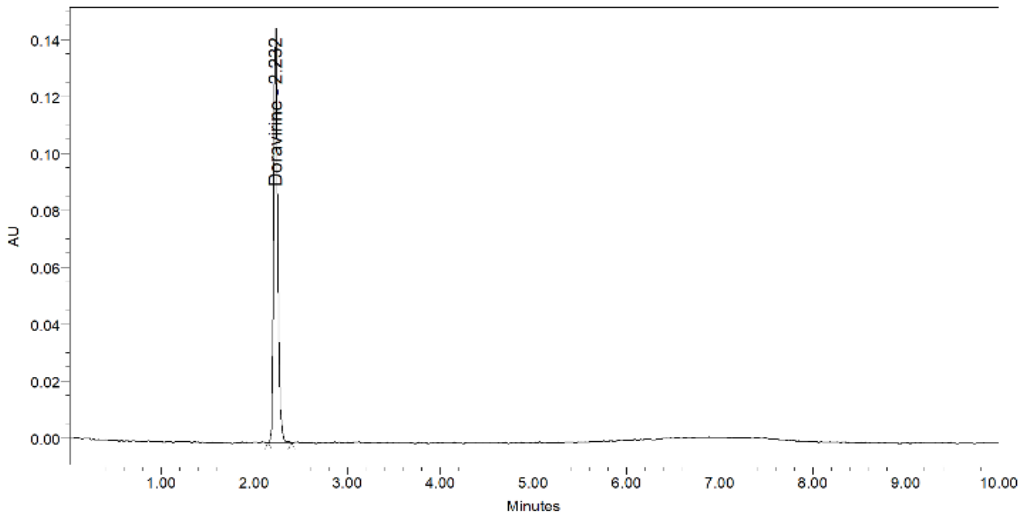

Figure-19: Acidic degradation of Doravirine

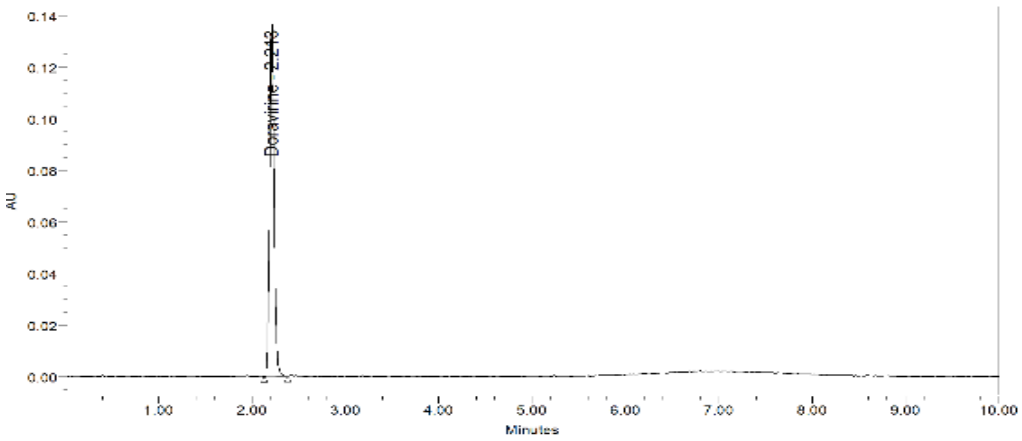

Figure-20: Photo degradation of Doravirine

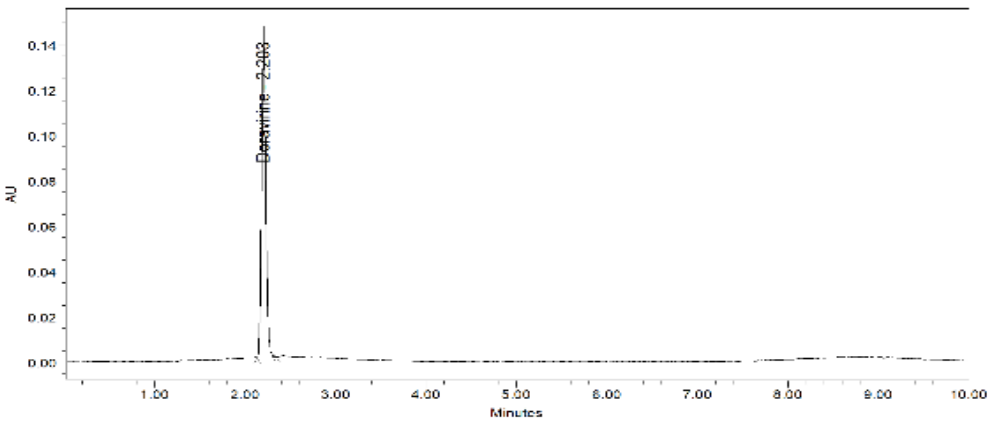

Figure-21: Hydrolytic degradation of Doravirine

CONCLUSION

In this article a detailed analytical approach was expressed to quantify Doravirine in tablets. Experimental runs were conducted by central composite design through design expert software which could save time, reagents and other resources. The predicted responses have been verified by actual responses from experimental results. There was good linearity between actual responses and predicted responses. So the developed method is flexible and transferable. The above method is simple, sensitive and robust for

routine analysis of Doravirine in dosage forms even in the presence of degradants.

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CONFLICT OF INTEREST

The authors declare no conflict of interest

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