ANALYTICAL QUALITY BY DESIGN METHODOLOGY FOR BEXAGLIFLOZIN ASSESSMENT IN FORMULATION IN THE PRESENCE OF DEGRADANTS USING RP-HPLC

K. Swaroopa Rani[1], B. Ramya Kuber[*2]

Abstract

Bexagliflozin (BXN) inhibits sodium-glucose co-transporter 2 used to induce glycemic control in patients with type 2 diabetes mellitus. Evaluation of its quality is the necessary factor of these days. Present work is based on analytical quality by design (AQbD) approach as it became the necessary regulatory requirement. Central Composite design was selected for obtaining experimental combinations in Design Expert software. The three critical process parameters namely flow rate, aqueous mobile phase ratio and temperature were influencing the dependent critical quality attributes (CQA) nothing but responses namely theoretical plates, retention time and tailing factor. Responses were studied by analysis of variance. The optimized method conditions were mobile phase of mixture containing buffer-0.1% ortho phosphoric acid and acetonitrile (60: 40 v/v) pumped with a flow rate of 0.8 ml/min on Agilent column150×4.6 mm, 5µm. The method validation was accomplished on the basis of the ICH guidelines. The method is linear with the range of concentration is 0.5-30 µg/ml. The detection and quantitation limits were to be 0.05 µg/ml and 0.14 µg/ml respectively. The BXN was retained at 2.2 min showing maximum absorbance wavelength at 220.0 nm with run time of 6 min. The information from the contour graphs of responses was harmonized with the experimental results. So this method was apt for quantifying the dosage form and also the method was used for estimating the drug in the presence of degradants.

Keywords: Quality by Design, Bexagliflozin, degradants.

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INTRODUCTION

Diabetes a metabolic disorder is a chronic condition that appears either when the pancreas unable to secrete sufficient insulin or if the body lost the capacity to use the insulin it produces. Bexagliflozin is a very specific inhibitor of sodium–glucose co-transporter 2 (SGLT2). It is in the proximal renal tubule of kidney where more reabsorption occurs, and they send glucose and sodium from the tubular lumen to the epithelium. Bexagliflozin inhibits SGLT2 and it decreases glucose reabsorption in the kidney and increases glucose excretion in urine. So, in type 2 diabetes mellitus (T2DM) patients, bexagliflozin reduces blood glucose levels without depending on insulin sensitivity. Besides having glycemic control, Bexagliflozin may also reduces body weight, systolic blood pressure, and albuminuria. The mechanisms for for these other effects are not explained clearly. There is a chance that they depend on the natriuresis by bexagliflozin, followed by a change in tissue sodium handling.[4]

Literature survey asserts that there was no analytical method published for the analysis of BXN. There is ample prerequisite for Analytical QBD techniques in Pharma Industries as they require less time and also cost effective. So the present research work was based on the QbD method. There is a requisite for the development of analytical method via quality by design which reduces the time of experimental runs and cost for drug analysis. AQbD put forward method operable design region for CQA so as to achieve ruggedness and robustness in a method which results in continuos improvement. AQbD

involves less trails and gives sound science about the interaction effects in the method.

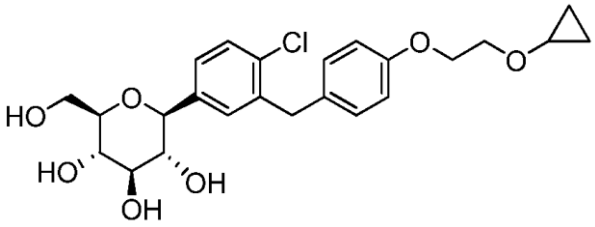

Figure-1: Structure of Bexagliflozin

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 Digit pH meter 7007 (Digisun Electronics Hyderabad). The mobile phase was degassed with Ultra sonicator Labman. UV-VIS spectrophotometer 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 bought from Merck chemical division, Mumbai. Ortho-phosphoric acid of AR grade were procured from Rankem, Avantor performance material India limited. BMR Chemicals, Hyderabad supplied pure Bexagliflozin drug as gift sample. Marketed formulation Brenzavvy was obtained from local pharmacy.

Method development

Preparation of Standard stock solution:

Bexagliflozin pure drug of 10mg was accurately weighed and poured into a 50ml clean dry volumetric flask and diluent of 10ml was added. Finally volume was made to 50 ml with diluents (200 µg/ml of Bexagliglozin). From the above stock solution 1ml was taken into a 10ml volumetric flask and made up the volume whose concentration is 20 µg/ml of Bexagliglozin[1]. Sonication was done for 10 minutes.

Mobile phase Preparation:

Potassium dihydrogen Ortho phosphate (0.01N KH_2PO_4) of 1 gm was accurately weighed and poured into a 1000ml of Volumetric flask and finally volume was made with water and Ortho phosphoric acid is used to adjust the pH(pH 4.8). Potassium dihydrogen Ortho phosphate buffer and Acetonitrile were mixed in the ratio of 60:40 (v/v) and degassed with sonicator[2].

Software implementation:

software of Design expert with version 11.1.0.1 was used. Randomised study of Response surface method was utilised. 20 runs obtained from Central composite design (CCD) were performed experimentally (table 1). 2 ms was Build time used in Quadratic model. Effects of several parameters were studied at once in this approach[3].

Risk assessment revealed that flow rate, organic phase ratio in mobile phase and temperature as input variables. These input variables influenced the responses of retention time, theoretical plates and tailing factor. The combination of input variables given by central composite design were performed experimentally as Chromatographic trails. These runs are of different compositions of input variables for the optimisation of the method at maximum absorbance wavelength 220.0 nm. Interactions between variables were studied by Correlation graphs. Fraction of design space statistics given 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. Information was obtained 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 trails from design the optimised conditions were found to be flow rate of 1.09ml/min, organic mobile phase ratio was 42.3% and Column temperature was 31.5°C with Injection volume of 10.0µL. The BXN drug was detected at 2.2 min with Run time of 6.0 minutes.

Validation of the method

Method to determine the purity of Dosage form:

From the tablet powder, equivalent weight of 50 mg was taken into a 50ml volumetric flask. The volume was made up with solvent and allowed for sonication for 25 min. The solution is filtered through milli-Q filters (1000µg/ml Bexagliflozin). 0.2ml of filtered sample stock solution was taken in 10ml volumetric flask and made up with solvent. (20µg/ml Bexagliflozin) [5].The concentration of pure drug in the tablets was determined by calibration curve method through regression equation. Label Claim of Brenzavvy tablets is 20

mg of Bexagliflozin and the assay data is in table 13.

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

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

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

*Detection Limit(*LOD) *& Quantification Limit (LOQ):*
Detection Limit & Quantification Limit were found by formula method. The formula is LOD=3.3 SD/slope and for LOQ=10 SD /slope. (SD= standard deviation)

Robustness:
Results are gathered by deliberate change in the experimental conditions. Flow rate, organic mobile phase ratio and temperature were changed slightly within the method operable design region and the results were tabulated in table 12[8].

Method of Degradation[3]
Hydrogen Peroxide degradation:
20% hydrogen peroxide (H_2O_2) and Bexagliflozin stock solution were prepared. 1 ml of solution was collected from each solution. The resultant solution was maintained at 60^0c for 30 min. 20µg/ml dilution was prepared from above solution and analysed for the stability.

Acid Degradation:
2N hydrochloric acid and Bexagliflozin stock solution were prepared. 1 ml of solution was collected from each solution. The resultant was maintained at 60^0c for 30 min. 20µg/ml dilution was prepared from above solution and analysed for the stability[9].

Base Degradation:
2N sodium hydroxide and Bexagliflozin stock solution were prepared. 1 ml of solution was collected from each solution. The resultant was maintained at 60^0c for 30 min. 20µg/ml dilution was prepared from above solution and analysed for the stability[10].

Dry Heat Degradation:
The stock solution of Bexagliflozin was placed in an oven at 105°C for 6 hours. 20µg/ml dilution was prepared from above solution and analysed for the stability[12].

Photo Degradation:
200 µg/ml solution was prepared from stock solution of Bexagliflozin and beaker containing above solution was kept in UV Chamber for 7days or 200Watt hours/m^2 in photo stability chamber. 20µg/ml dilution was prepared from above solution and analysed for the stability[13s].

Neutral Degradation:
10 mg of drug was refluxed in 50ml water for 6 hours at a temperature of 60ºC. 20µg/ml dilution was prepared from above solution and analysed for the stability. The degradation results were tabulated in table 16.

RESULTS AND DISCUSSION

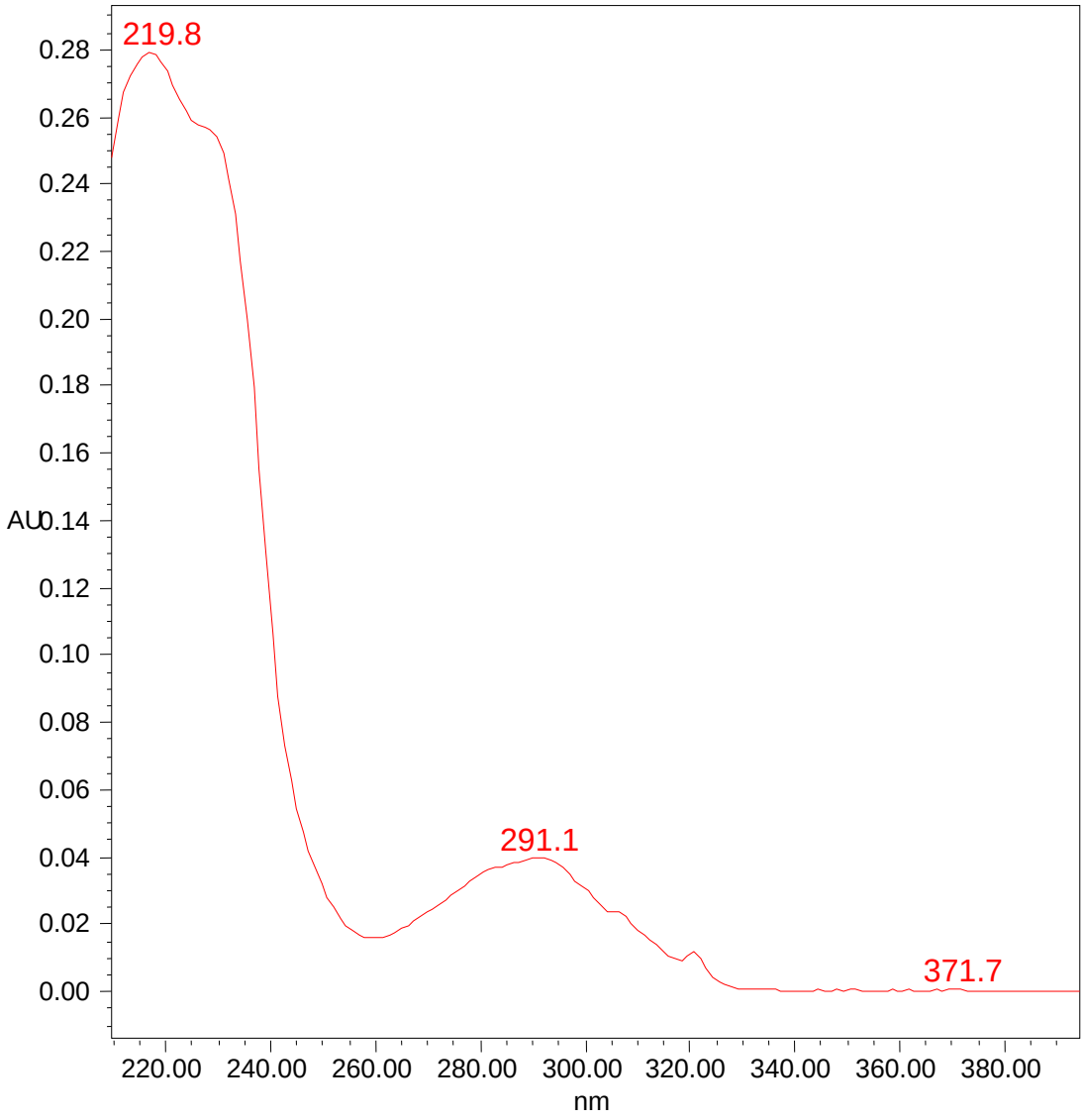

Figure-2: UV Spectrum of Bexagliflozin

Development of method through Quality by Design

Table-1: Central Composite Design for 20 runs

Std	Run	A:Flow Rate	B:Mobile Phase(aqueous)	C:Temperature	Retention Time(RT)	Number of Theoretical Plates(NTP)	Tailing factor(TF)
		min/ml	%	°c	min	number	num
1	15	0.9	60	27	2.893	9180	1.46
2	14	1.1	60	27	2.335	8618	1.35
3	8	0.9	70	27	2.997	8617	1.33
4	3	1.1	70	27	2.425	8693	1.36
5	17	0.9	60	33	2.321	9278	1.24
6	5	1.1	60	33	1.938	8693	1.26
7	19	0.9	70	33	2.408	7912	1.3
8	6	1.1	70	33	1.991	7825	1.45
9	11	0.831821	65	30	2.835	9674	1.34
10	9	1.16818	65	30	2.031	9177	1.37
11	2	1	56.591	30	2.3	8574	1.33
12	7	1	73.409	30	2.458	7437	1.36
13	18	1	65	24.9546	2.939	9124	1.41
14	13	1	65	35.0454	1.965	8341	1.3
15	1	1	65	30	2.357	9304	1.34
16	20	1	65	30	2.364	9341	1.35
17	10	1	65	30	2.37	9362	1.34
18	12	1	65	30	2.371	9372	1.34
19	4	1	65	30	2.371	9303	1.34
20	16	1	65	30	2.372	9316	1.34

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)	2.00		

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	1.0000	0.0848
B	MP (aqueous)	%	Numeric	31.59	48.41	-1 ↔ 35.00	+1 ↔ 45.00	40.00	4.24
C	temperature	°c	Numeric	24.95	35.05	-1 ↔ 27.00	+1 ↔ 33.00	30.00	2.54

Table-4: Responses (Critical Quality Attributes)

Response	Name	Units	Observations	Analysis	Minimum	Maximum	Mean	Std. Dev.	Ratio	Transform	Model
R1	RT	min	20	Polynomial	1.938	2.997	2.40	0.3092	1.55	None	Quadratic
R2	NTP	num	20	Polynomial	7437	9674	8857.05	605.93	1.30	None	Quadratic
R3	TF	num	20	Polynomial	1.24	1.46	1.35	0.0526	1.18	None	Quadratic

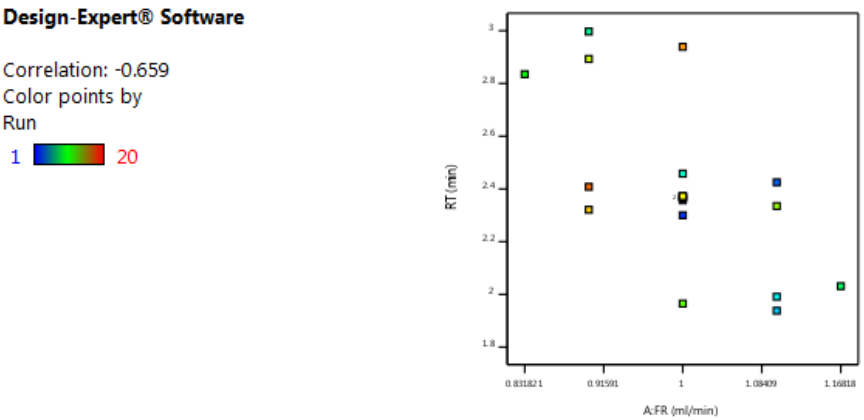

Figure-3: Correlation graph

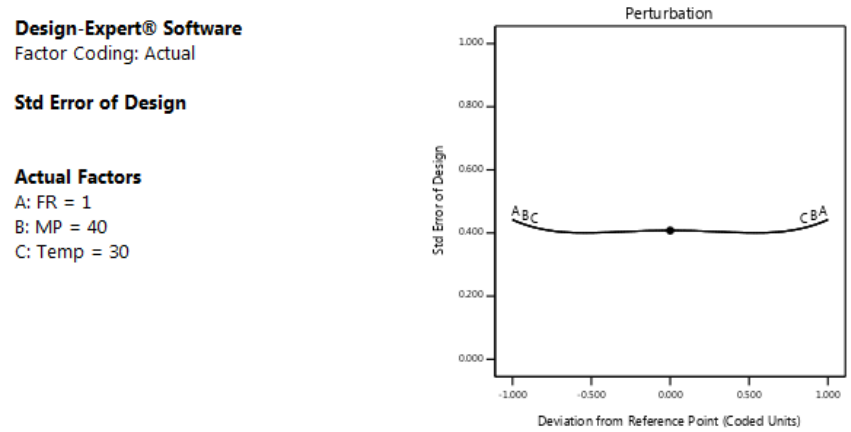

Figure-4: Perturbation graph of three input factors

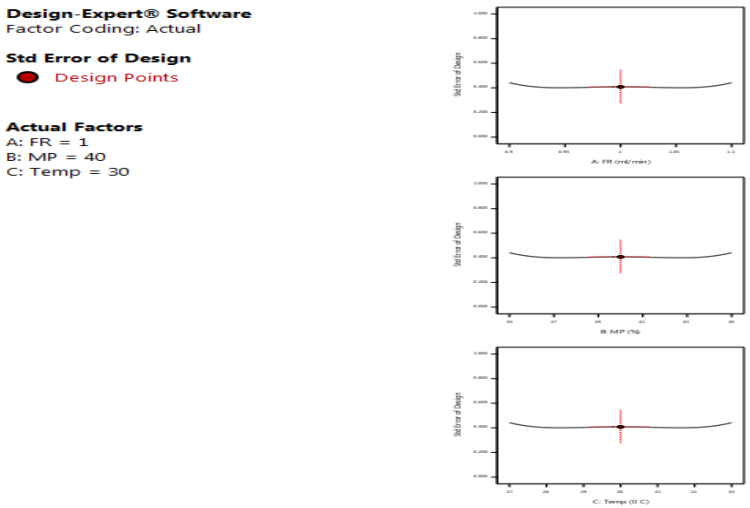

Figure-5: Perturbation graphs

The maximum absorbance wavelength of Bexagliflozin is 220nm in UV Spectrum. The information of 20 runs was obtained with different interactions of three input variables namely flow rate, aqueous phase of mobile phase and temperature through central composite design (CCD). The CCD produced high and low values of three variables. The low and high values of flow rate are 0.8 ml/min and 1.1ml/min respectively. The low and high values of organic phase are 31.4% and 48.4% respectively. The low and high values of column temperature are 24.9^{o}C and 35.0^{o}C respectively.

The effect of flow rate on retention time was understood by correlation graph. The above correlation graph showed the optimum retention

time (response) at that point there is minimum effect of flow rate on retention time. The perturbation graphs given standard error value for the input variables. These plots given the deviation of input variables flow rate, organic phase and temperature from the reference points 1.09 ml/min, 42.3%, 31.5^{o}C.

Fit Summary information

Response 1: Retention Time

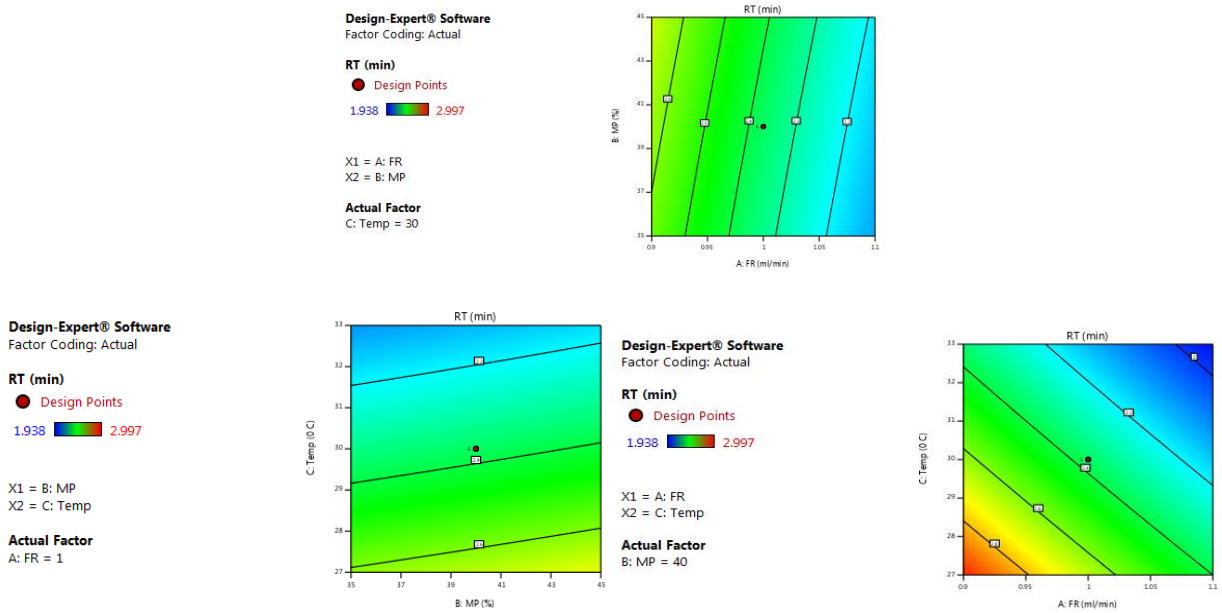

Figure-6: Method operable design regions of input factors for retention time

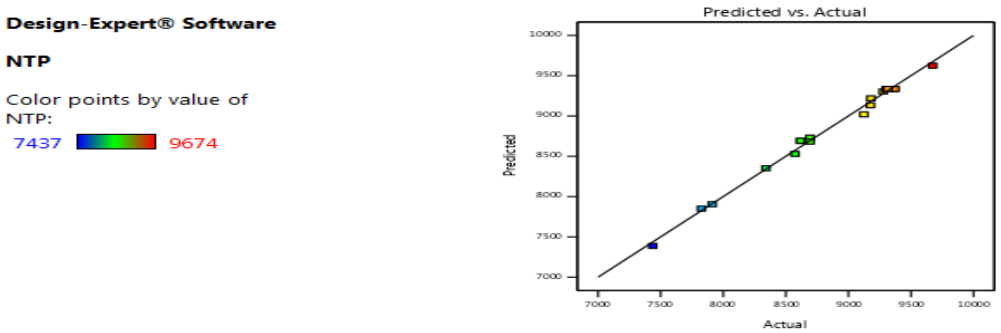

Figure-7: Plot between predicted and actual values

The above Method operable design regions showed the good response (retention time) with the input variable interaction. Flow rate of 1 ml/min and aqueous phase of 60% is apt for the retention time of around 2.4min. At that point of retention time input factors effect is less. The method operable design region for flow rate is between 0.9 to 1.1 ml/min and for the organic phase it is between 35 to 45% and for the temperature it is between 27^{o}C to 33^{o}C. The above plot showed the good linearity between the actual and predicted responses.

Fit Summary data

Response 2: *Theoretical Plates*

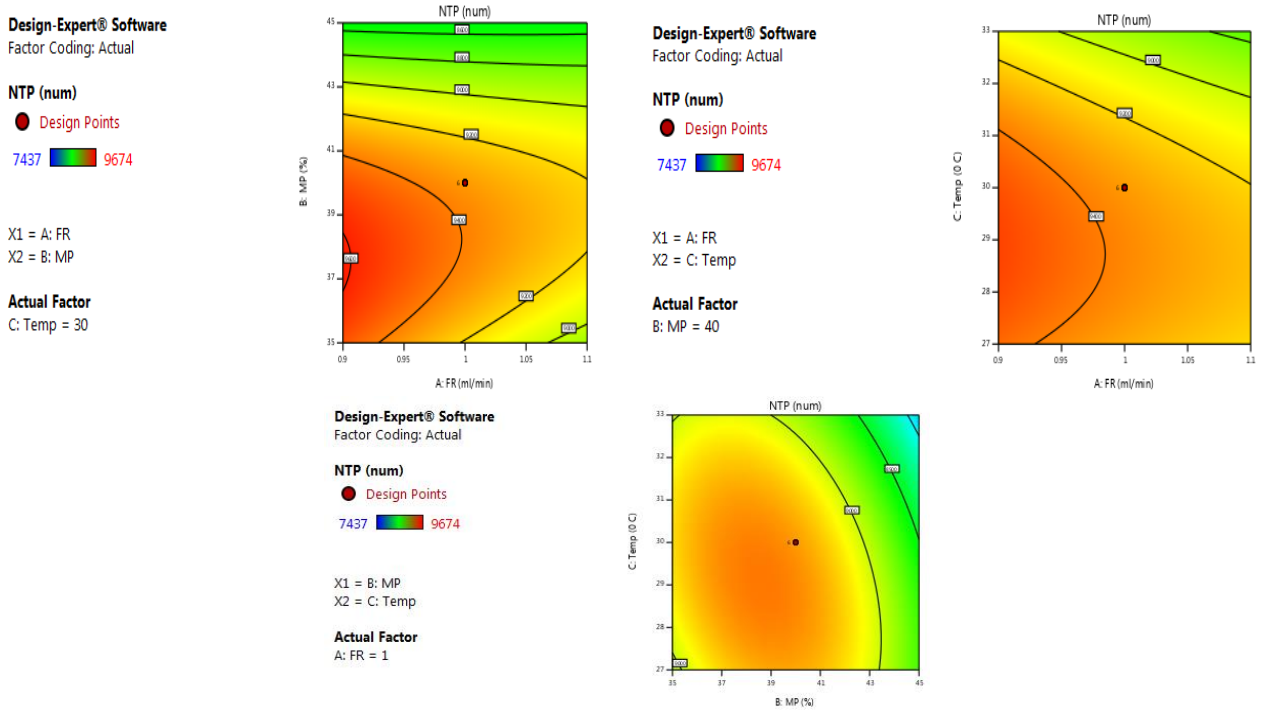

Figure-:8 Method operable design regions of input factors for number of theoretical plates

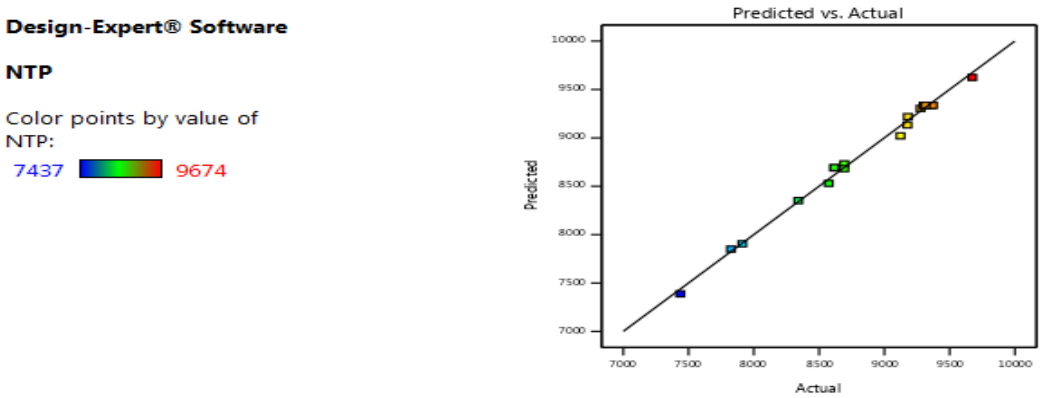

Figure-9: Plot between predicted and actual values

The above contour graphs showed the best theoretical plate number with the input factors interactions. Flow rate of 1 ml/min and organic phase of 40% is apt to get theoretical plates of approximately 8779. At this point of theoretical plate number the impact of input factors is less. The above plot produced the good linearity between the actual 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 organic phase it is between 35 to 45% and for the temperature it is between 27 ^{o}C to 33 ^{o}C. The above plot showed the good linearity between the actual and predicted responses.

Response 3: Tailing Factor

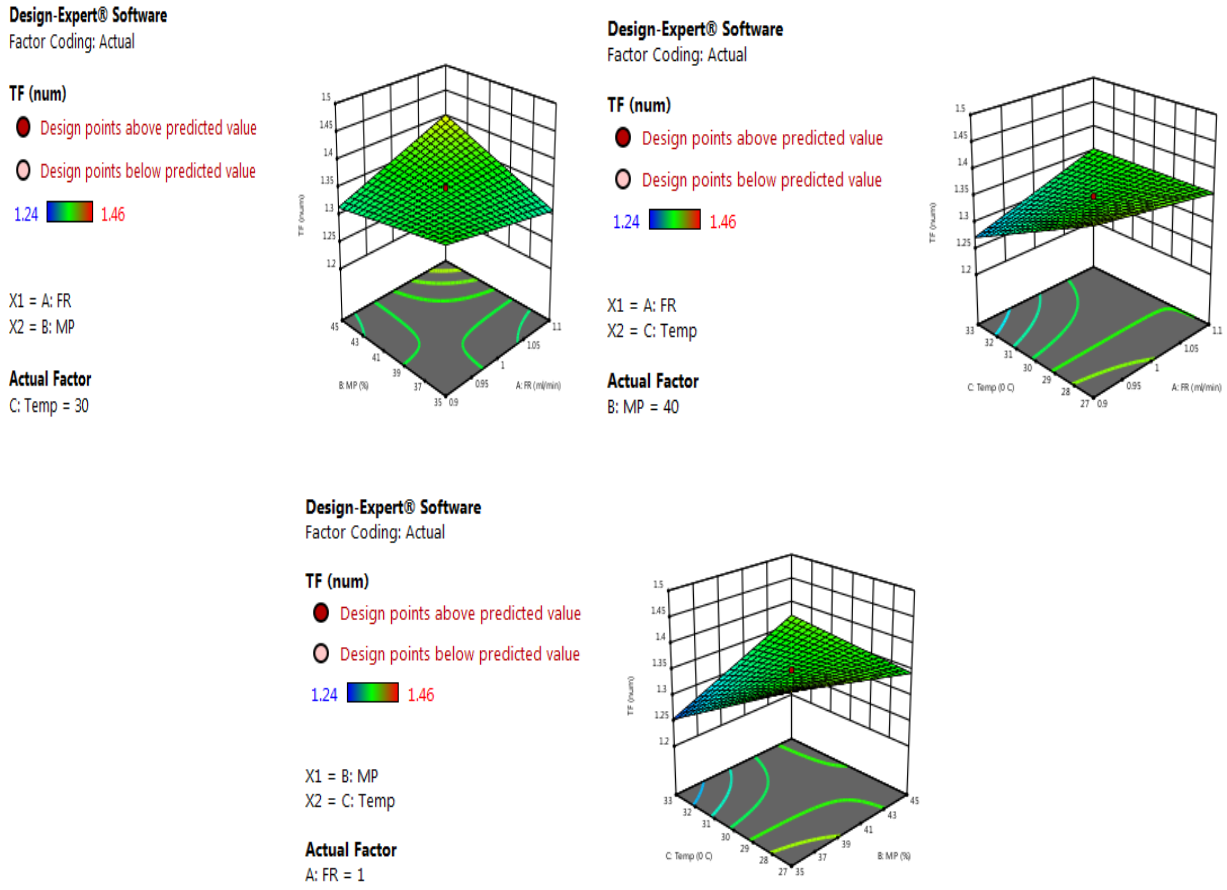

Figure-10: Contour graphs for tailing factor as a function of input factors

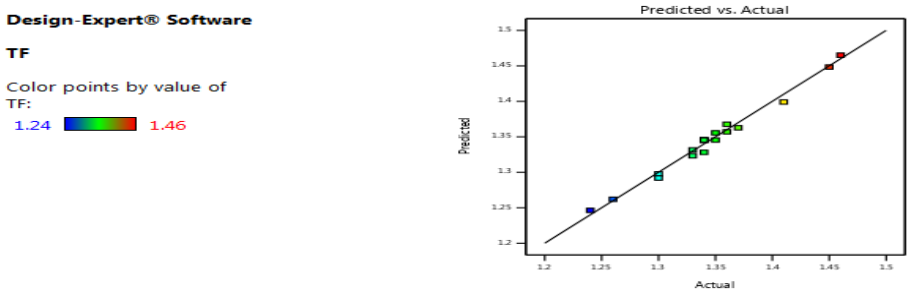

Figure- Figure -11: Linearity plot between predicted and actual values

The above contour graphs showed the best responses (tailing factor) with the input factors combination. Flow rate should be 1 ml/min and organic phase should be 40% for the tailing factor of approximately 1.3. At this tailing factor the influence of input factors is less. The above plot showed the good 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 organic phase it is between 35 to 45% and for the temperature it is between 27 ^{o}C to 33^{o}C. The above plot showed the good linearity between the actual responses and predicted responses.

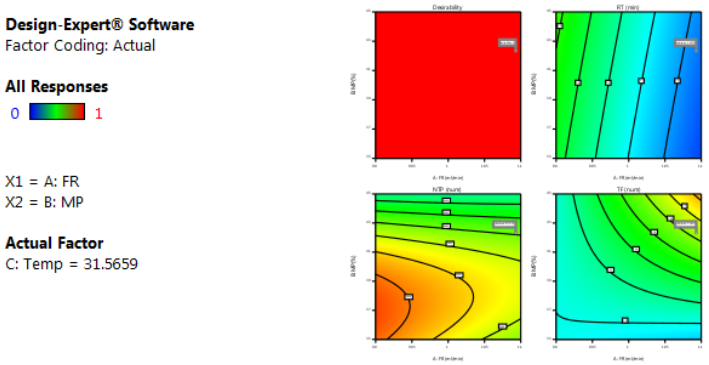

Figure-12: Desirability graph and contour graphs

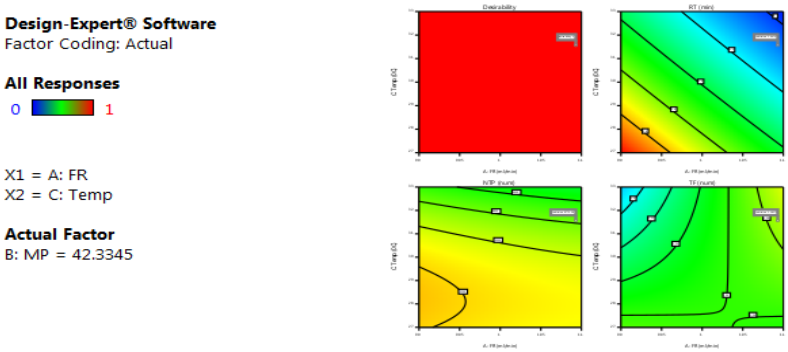

Figure-13: Desirability graph and contour graphs

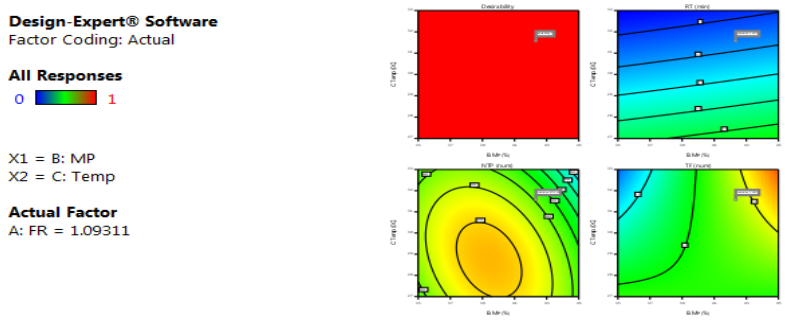

Figure-14: Desirability graph and contour graphs

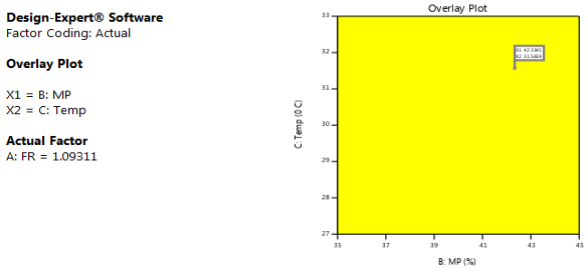

Figure-15: Overlay plot of Temperature and MP

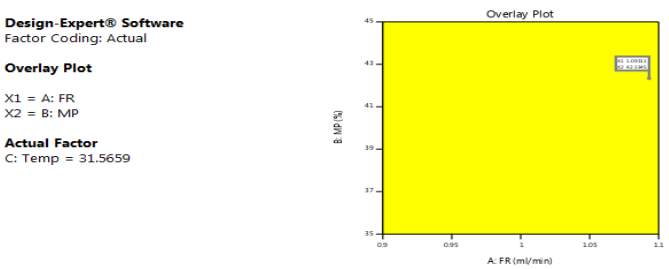

Figure-16: Overlay plot of MP and FR

The above desirability graph showed the point of flow rate value and organic 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 1.09 ml/min and organic phase of 42%. The overlay plot showed the optimum values of flow rate that is 1.02 ml/min and organic phase of 42%.

Table-5: ANOVA for Retention Time

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	1.81	9	0.2011	325.53	< 0.0001	significant
Lack of Fit	0.0060	5	0.0012	34.61	0.0007	significant

Table-6: ANOVA for Theoretical Plate number

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	6.936E+06	9	7.707E+05	195.02	< 0.0001	significant
Lack of Fit	35064.30	5	7012.86	7.87	0.0205	significant

Table-7: ANOVA for Tailing Factor

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	0.0517	6	0.0086	144.96	< 0.0001	significant
Lack of Fit	0.0007	8	0.0001	5.17	0.0434	significant

The Model F-value of 325.53 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 34.61 implies the Lack of Fit is significant. Significant lack of fit is bad.

The Model F-value of 195.02 implies the model is significant. P-values less than 0.0001 indicate model terms are significant. The Lack of Fit F-value of 7.87 implies the Lack of Fit is significant which is bad. We want the model to fit.

The Model F-value of 144.96 implies the model is significant. P-values less than 0.0001 indicate model terms are significant. The Lack of Fit F-value of 5.17 implies the Lack of Fit is significant which is bad. We want the model of good fit.

Table-9: Confirmation of responses

Solution 1 of 100 Responses	**Predicted Mean**
RT	2.06757
NTP	8779.38
TF	1.38575

Validation data

Table-10: Linearity data of Bexagliflozin

S.No	**Concentration (µg/ml)**	**Peak area**
1	5	51164
2	10	103532
3	15	153010
4	20	204620
5	25	255450
6	30	303210

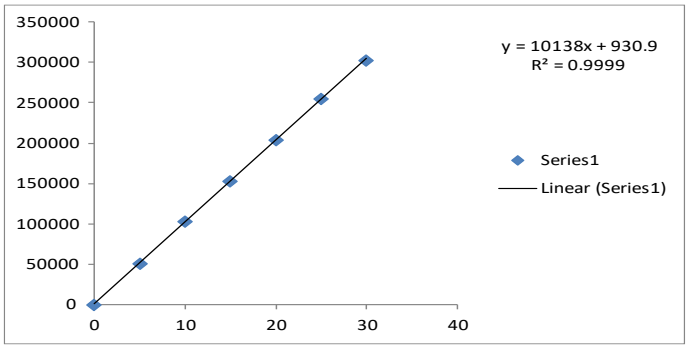

Figure-17: Standard curve

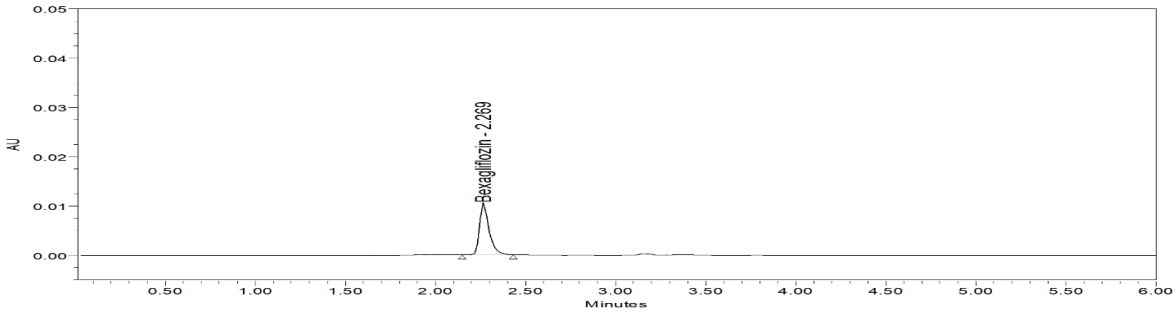

Figure-18: LOD of Bexagliflozin

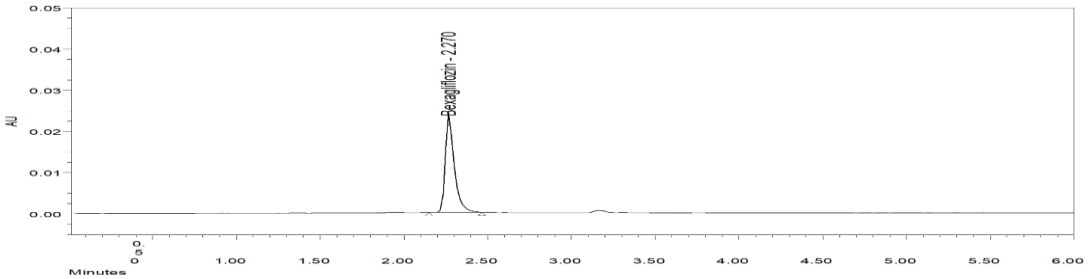

Figure-19: LOQ of Bexagliflozin

Table-11: Precision data of Bexagliflozin

S.No	Initial Peak areas	Peak area of Intraday precision	Peak area of Inter day precision
1	205329	204889	464753
2	205647	204111	199446
3	205619	205581	199214
4	204569	205644	199272
5	205272	205696	201266
6	205958	206277	200119
Avg	205399	205366	199615
SD	475.9	757.0	778.4
%RSD	0.2	0.4	0.4

Table-12: 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	29.93	99.88	0.5
2	100%	20	20	39.90	99.54	0.4
3	150%	20	30	49.78	99.28	0.2

Table-13: Robustness data

	High flow rate	**Low flow rate**	**High aqueous phase**	**Low aqueous phase**	**High Temperature**	**Low Temperature**
Peak area	**214755**	**207615**	**212470**	**198072**	**200064**	**211185**
SD	1402.8	1124.7	346.6	357.2	695.8	1868.0
%RSD	0.7	0.5	0.2	0.2	0.3	0.9

Table-14: Assay Data

Drug	**Lable claim (Brenzavvy tablets)**	**Amount found**	**%Recovery**	**%RSD**
Bexagliflozin	20 mg	19.98 mg	99.9	0.5

Table-15: System suitability data

S.No	**Parameter**	
1	Chromatographic column	Agilent C18 (150 x 4.6 mm, 5µ)
2	Mobile phase	Buffer (Potassium dihydrogen ortho phosphate): Acetonitrile (60:40%V/V)
3	Flow rate	1 ml/min
4	Detector wavelength	220 nm
5	Retention time	2.2 min
6	Peak area	205804
7	Theoretical plates	8955
8	Tailing factor	1.35

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-16: Degradation data of Bexagliflozin tablet

	Peak area		**% Drug**	**% Degradation**
	Initial	After Degradation		
Acid	205399	193998	94.26	5.74
Base	205399	193881	94.20	5.80
Peroxide	205399	197888	96.15	3.85
Thermal	205399	201287	97.80	2.20
UV	205399	201462	97.89	2.11
Water	205399	203976	99.11	0.89

Table-17: Degradation data of Bexagliflozin pure drug

	Peak area	**% Drug**	

	Initial	After Degradation		**% Degradation**
Acid	205399	196006	95.24	4.76
Base	205399	196234	95.35	4.65
Peroxide	205399	198821	96.60	3.40
Thermal	205399	201454	97.88	2.12
UV	205399	202875	98.57	1.43
Water	205399	204856	99.54	0.46

The degradation was found to be high in basic condition in formulation where as in pure drug degradation was more in acidic condition.

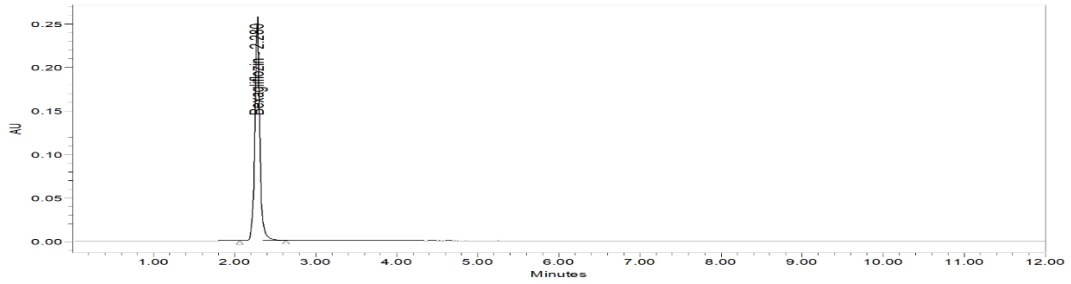

Figure-20: Degradation of Bexagliflozin under oxidation

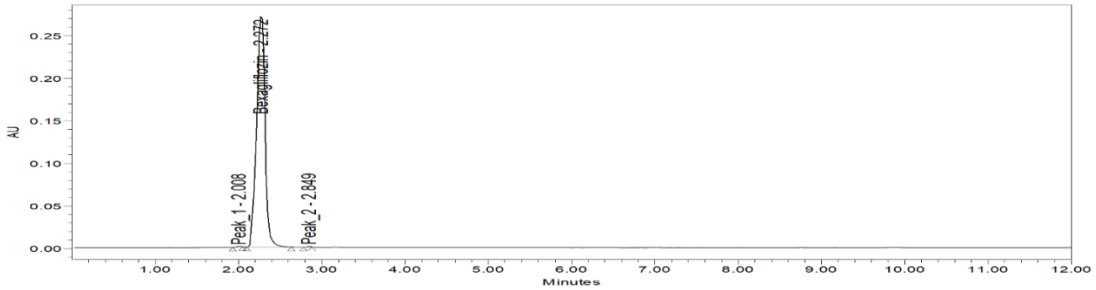

Figure-21: Basic degradation of Bexagliflozin

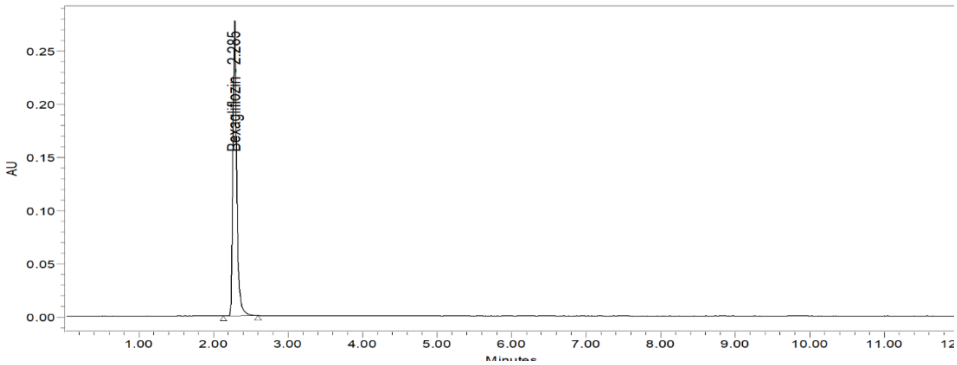

Figure-22: Thermal degradation of Bexagliflozin

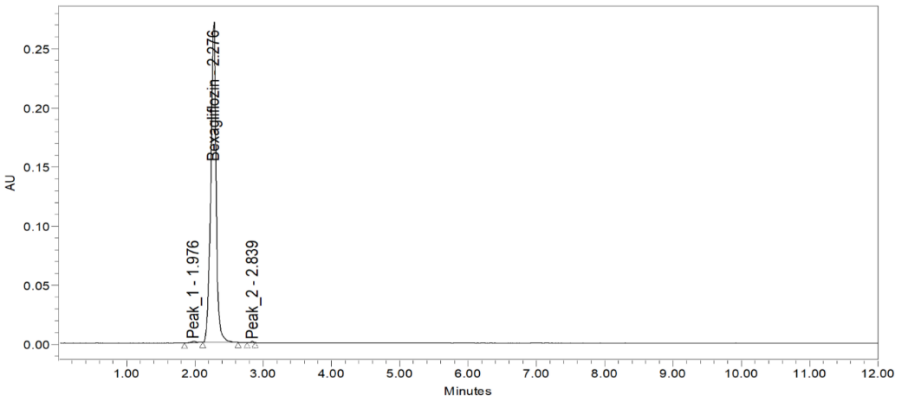

Figure-23: Acidic degradation of Bexagliflozin

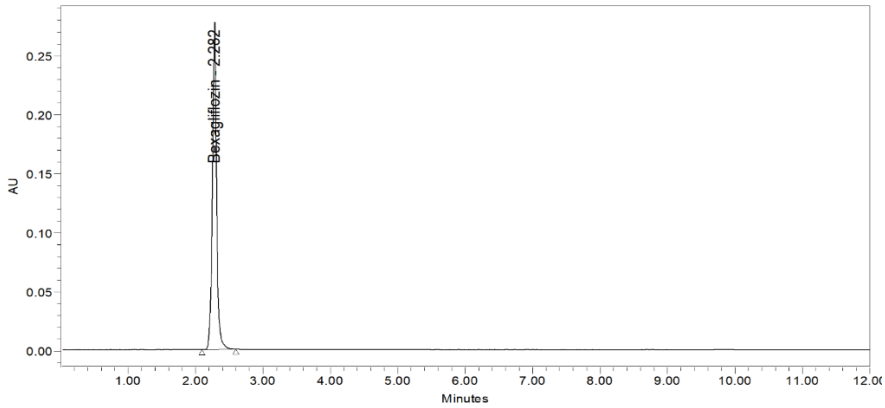

Figure-24: Photo degradation of Bexagliflozin

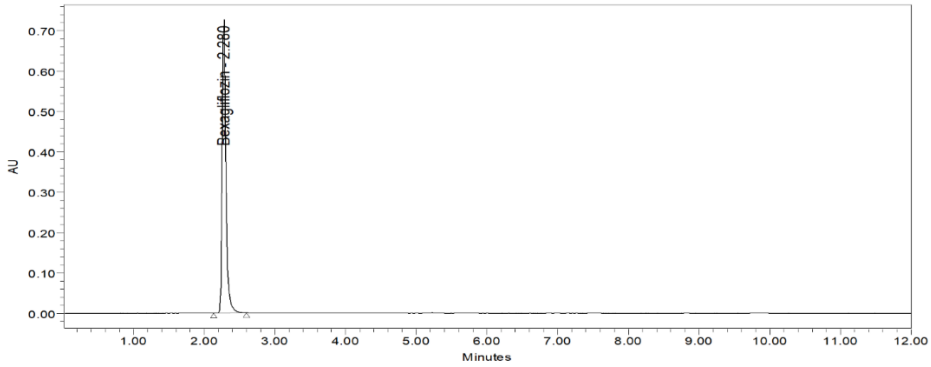

Figure-25: Hydrolytic degradation of Bexagliflozin

CONCLUSION

In this article a detailed analytical approach was expressed to quantify Bexagliflozin 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 Bexagliflozin 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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