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RP-HPLC Method Development and Validation for the Determination of Pemigatinib using Design of Experiments Approach

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Authors' contributions

This work was carried out in collaboration among all authors. Author CHS designed the study, performed the statistical analysis and wrote the first draft of the manuscript. Authors KSN, AKMP provided the mentorship and reviewed the data. Author KH helped in analysing the data. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To develop and validate a simple, precise, accurate and robust RP-HPLC method for the determination of Pemigatinib by using Design of Experiments (DoE) approach.
Study Design: A 2^3 Factorial design consisting of three factors at two levels was considered for the experimental plan initially to select the initial chromatographic conditions and optimization was done using Box-Behnken Design. The critical method parameters selected for optimization were % Organic phase composition, pH of the buffer and flow rate. The critical quality attributes investigated were retention time, theoretical plates and tailing factor.
Methodology: Chromatographic separation was achieved on Agilent Zorbax XDB C18 (250×4.6 mm, 5 µm) column maintained at ambient temperature and PDA-UV detection set at 262nm. The optimized and predicted data from the Design Expert® (12.0.12.0) modelling software (Stat-Ease Inc., Minneapolis, MN, USA) consisted of mobile phase 0.1% OPA pH 2.5 buffer (60%): Acetonitrile

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(40%) pumped at a flow rate of 1.06ml/min gave the highest desirability.
Results: The retention time of the drug was found to be 3.258 min. The developed method was linear over the concentration range of 25-150 µg/mL with correlation coefficient of 0.999. The optimized method was validated as per ICH Q2 (R1) guidelines.
Conclusion: Based on the ANOVA results, the selected models for the responses retention time and tailing factor were found to be significant with P=0.05. 2D Contour plots were used to visualize the effect of factors and their interactions on the responses. Design validation was done using predicted vs. actual plots for the responses. The results of the validation parameters were within the acceptable limit. The stability of the drug was examined under different stress conditions forcibly and significant degradation was found in reductive condition.

Keywords: Design of Experiments; BBD; desirability; ANOVA; Pemigatinib.

1. INTRODUCTION

Pemigatinib (PGB) marketed under the brand name Pemazyre is a drug approved for the treatment of adults with cholangiocarcinoma, a type of biliary duct cancer that is locally advanced or spread to other parts of the body and cannot be treated by surgery. The drug is mainly used in the adults who have already received a previous treatment or whose tumour has a certain type of abnormal FGFR2 (Fibroblast growth factor receptor) gene. PGB is a small molecule kinase inhibitor that exerts anti-tumour activity through inhibition of FGFRs [1-5]. IUPAC name is 11-(2,6-difluoro-3,5-dimethoxyphenyl)-13-ethyl-4-(morpholin-4-ylmethyl)-5,7,11,13-tetrazatricyclo [7.4.0.0^{2,6}]trideca-1,3,6,8-tetraen-12-one and chemical structure was shown in Fig. 1.

Since this drug was granted accelerated approval by FDA in April 2020, till now no analytical methods were reported for the determination of PGB in bulk and pharmaceutical dosage form. Only phase studies were conducted to study the safety and efficacy of the drug [6-8]. Hence the present work is aimed at development and validation of RP-HPLC method for the determination of PGB by using Design of Experiments approach. Analytical Quality by Design (AQbD) approach which uses good experimental designs, risk assessment, ruggedness and robustness testing is much vigorous when compared with the traditional

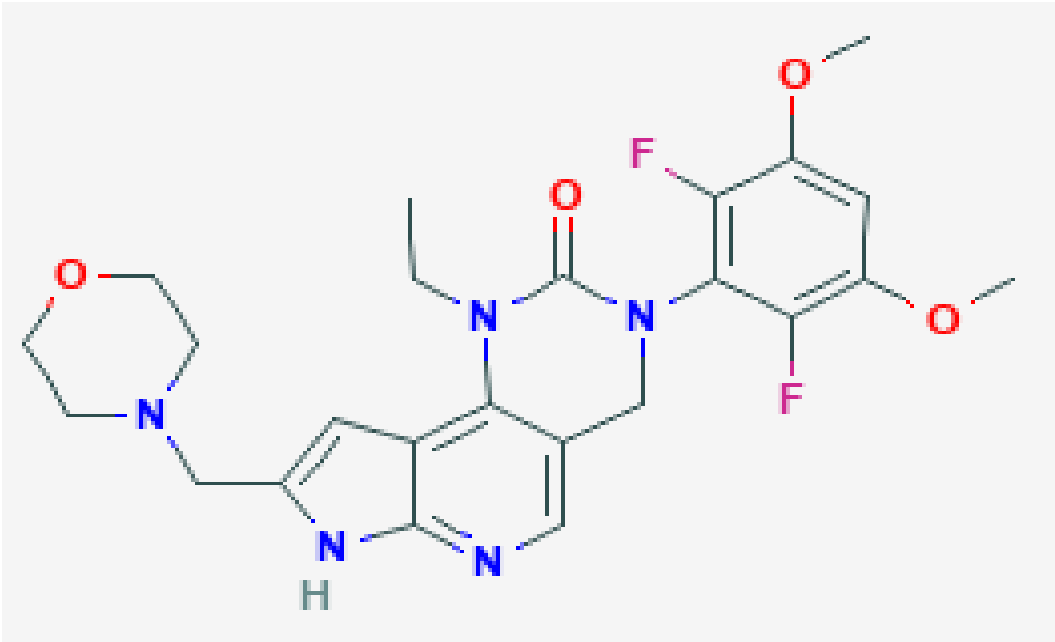


Fig. 1. Structure of Pemigatinib

methods developed by one-factor-at-a-time (OFAT) approach. For the selection of initial chromatographic conditions a 2^3 Factorial design consisting of three factors at two levels was considered requiring minimum number of runs. Optimization was done using Box-Behnken design. BBD usually have fewer design points than Central composite design (CCD) and less expensive to run with same number of factors. BBD is a statistical experimental design used in Response surface methodology (RSM). Response surface is a geometrical representation of response variables plotted as a function of independent variables using 2D Contour and 3D Surface plots. Statistical analysis of the results was done using Analysis of Variance (ANOVA). Predicted versus actual plots and Normal plot of residuals are used for design validation. Optimization of the method was done by applying the desirability function.

2. MATERIALS AND METHODS

2.1 Chemicals

HPLC grade Acetonitrile and Methanol were purchased from Fischer scientific, HPLC grade water obtained from Merck milli-Q water purification unit. Potassium dihydrogen orthophosphate and ortho phosphoric acid (OPA) were purchased from Merck India Pvt. Ltd, Mumbai, India. The other reagents used in this research were analytical grade. API of PGB (99.9% purity) was obtained as a gift sample from Zydus Cadila, Ahmedabad, India.

2.2 Equipment

FT-IR/ATR (BRUKER ALFA) spectrophotometer and UV-VIS spectrophotometer (Shimadzu - 1800, Japan) were used for the authentication of drug sample. HPLC study was carried out on WATERS HPLC 2695 system with photo diode array (PDA) Detector. Software used is Empower 2 for HPLC method development and validation. Design Expert® (12.0.12.0) modelling software (Stat-Ease Inc., Minneapolis, MN, USA) was used for RSM.

2.3 Authentication and Identification of Sample

By UV-VIS Spectra: 100µg/mL concentration of Pemigatinib was prepared using acetonitrile and UV spectrum was recorded. The absorption maxima was found to be 262nm as shown in the Fig. 2.

By IR spectra: Pemigatinib was scanned in FT-IR spectrometer (Bruker- ALFA) from 4000 to 400 cm^{-1} and characteristic absorption peaks of functional groups were found at 3171, 2835, 1428, 1060 cm^{-1}. The corresponding IR spectra is shown in the Fig. 3.

2.3.1 Preparation of mobile phase

Mobile phase was prepared by using HPLC grade Acetonitrile and 0.1% ortho phosphoric acid (pH 2.5) in 40:60 ratio.

2.3.2 Preparation of standard stock solution

Accurately weighed 100mg of Pemigatinib was transferred to 100ml volumetric flask, 3/4th of final volume was filled with mobile phase and sonicated to dissolve completely. Final volume was made upto 100ml and labelled as standard stock solution (1000µg/ml of Pemigatinib). 1 ml of the above stock solution was pipetted into 10ml volumetric flask and made up to volume with mobile phase to get 100 µg/ml and this concentration was used for the optimization study.

2.3.3 Preparation of sample solution

Synthetic mixture was prepared by mixing 9mg of Pemigatinib and 16mg of placebo (povidone). The amount of drug equivalent to 10mg was transferred to 10ml clean dry volumetric flask, mobile phase was added to dissolve the drug and sonicated for 30mins. Then the volume was made up to the mark with mobile phase. It is the stock solution having concentration of 1000µg/ml of Pemigatinib. Then it is filtered through 0.45µm membrane filter. Further 1ml of above solution was pipetted into 10ml volumetric flask and diluted up to the mark with mobile phase to get 100 µg/ml.

2.3.4 Screening for the selection of initial chromatographic conditions

For the selection of initial chromatographic conditions like stationary phase, organic phase and buffer a 2^3 Factorial design consisting of 3 factors at 2 levels was selected [9]. The selected 2^3 factorial design results in 8 trial runs suggesting various combinations for the factors chosen.

2.3.5 Optimization of the method by RSM-BBD

Many types of response surface designs are used for optimization like Central composite, Doehlert, and Box–Behnken. BBD is preferable to the Central composite and Doehlert designs because it requires fewer test runs, rotatable and it does not contain any points at the extremes of the cubic region. In the present investigation BBD was used to optimize the method for the selected drug Pemigatinib by RP-HPLC because the design provides three levels for each factor and requires fewer runs in the three-factor case than Central composite and Doehlert design [10].

2.4 Method Validation

The final optimized analytical method was validated as per ICH Q2 (R1) guidelines for system suitability, specificity, linearity, accuracy, precision, limit of detection, limit of Quantitation and robustness.

2.5 Forced Degradation Studies

The drug was exposed to various stress conditions as mentioned in ICH Q1A (R2) guidelines to determine whether the developed method was stability indicating.

3. RESULTS AND DISCUSSION

3.1 AQbD assisted method development

3.1.1 Screening for the selection of initial chromatographic conditions

A 2^3 Factorial design consisting of three factors at two levels was considered for the experimental plan initially to select the appropriate stationary phase, organic mobile phase and buffer which majorly affect the selectivity. The stationary phases selected were Waters Symmetry C18 and Agilent Zorbax Eclipse XDB C18 since they can be used in the acidic and entire pH range of 2-9 respectively. Methanol and Acetonitrile were chosen as organic solvents since they were most commonly used in RP-HPLC. Since the drug has high solubility at low pH the buffers selected were OPA pH 3 and Phosphate pH 6. The factors and the levels selected for the screening design were given in Table 1.

Table 1. Factors and levels selected for 2^3 factorial design of pgb

Factors	Levels
Column	Waters Symmetry C18 / Agilent Zorbax Eclipse XDB C18
Organic phase	Methanol/Acetonitrile
Buffer pH	OPA pH 3/ Phosphate buffer pH 6

The selected 2^3 factorial design resulted in 8 trial runs suggesting various combinations for the factors chosen were presented in the Table 2. The responses selected were retention time, theoretical plates and tailing factor.

3.2 Statistical Analysis of 2^3 Factorial Design Experimental Data by Design-Expert Software

Analysis of variance (ANOVA) was applied to study the significance of the model shown in the Table 3.

From the table it is seen that the Model F-values of 4525.80, 550.78, 16.45 for RT, TP and TF respectively implies the model is significant. Values of $P<0.05$ indicate model terms are significant. A, B, C and BC are significant model terms for RT and TP with $P< 0.05$. A, B, C are significant model terms for TF with $P< 0.05$. The significance of the terms A, B, C indicates that the initial chromatographic conditions selected have a greater influence on the responses. The responses obtained were feeded back to the Design expert software and the cube plots for retention time, theoretical plates and tailing factor were drawn as shown in the Fig. 4.

From the 2^3 factorial design, based on the cube plots for the responses the initial chromatographic conditions selected for the further study were Zorbax XDB column, acetonitrile and OPA buffer pH 3 at which retention time is less, theoretical plates are more and tailing factor is less.

3.3 Optimization by Response Surface Methodology- BBD

AQbD method involves identifying Critical method parameters (CMP) and Critical quality attributes (CQA) with risk assessment and generating design space. In the present study CMP's selected were flow rate, % organic content in the mobile phase and pH of the buffer. The CQA's selected were retention time,

theoretical plates and tailing factor. So BBD was used to optimize these parameters which were varied over three level (high, mid and low) [10]. Different ranges of three parameters 20-40% Acetonitrile, flow rate of 0.9-1.1 ml/min and pH of the buffer 2-4 were taken. A 3-factor 3-level BBD design was established [11]. This study design of 17 experimental runs was generated and analysed by Design-Expert software as shown in the Table 4.

3.4 Statistical Analysis of Experimental Data by Design-Expert Software

ANOVA was applied to study the significance of the model for the 3 responses [12].

From the ANOVA Table 5 for retention time, the Model F-value of 69.15 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. *P*-values less than 0.0500 indicate model terms are significant. In this case A, B, C are significant model terms. 2D Contour plots were analysed to visualize the effect of factors and their interactions on the responses using the Design Expert® software. The regions shaded in dark blue represents lower values and shaded in dark red represents higher values. The regions shaded in light blue, green and yellow represents intermediate values.

From the above 2D Contour plots of retention time shown in the Fig. 5, it was found that at a higher organic phase content, higher flow rate and lower pH the value of retention time is less.

From the ANOVA Table 6 for theoretical plates, the Model F-value of 2.61 implies the model is not significant and there is a 9.62% chance that an F-value this large could occur due to noise. In this case, only A is a significant model term. In the screening studies, at pH 3 theoretical plates are more when compared to pH 6 and the model was found to be significant. Hence OPA pH 3 buffer was selected for optimization study. In BBD study, pH range of 2-4 was selected. According to the results obtained from the BBD Table 4, from pH 3-4 the number of theoretical plates were less (8456-9896) when compared to pH 2-3(8745-13488). But this 2-4 pH range was found to be statistically insignificant at $p<0.05$. Even though model was statistically insignificant, in optimization around pH 2-3 was selected because more theoretical plates were found in this range. To study the effect of significant term A on TP, 2D contour plot was analysed using Design Expert® software.

From the above 2D Contour plot of theoretical plates shown in the Fig. 6, it was found that at a higher organic phase content and lower pH the value of theoretical plates is more.

From the ANOVA Table 7 for tailing factor, the Model F-value of 5.42 implies the model is significant. There is only a 1.83% chance that an F-value this large could occur due to noise. *P*-values less than 0.0500 indicate model terms are significant. In this case AB and B^2 are significant model terms. To study the effect of significant terms AB and B^2 on TF, 2D contour plot was analysed using Design Expert® software.

From the above 2D Contour plot of tailing factor shown in the Fig. 7, it was found that at a higher organic phase content and at pH range of 2-3 the value of tailing factor is less.

From the fit statistical parameters obtained from ANOVA, it was found that the predicted R^2 value of retention time 0.8909 is in reasonable agreement with the adjusted R^2 0.9274 i.e. the difference is less than 0.2. A negative predicted R^2 value of tailing factor -0.9613 implies that the overall mean may be a better predictor of the response than the current model. Adequate Precision measures the signal to noise ratio. A ratio greater than 4 is desirable and the obtained values for the responses 25.694, 6.393 for RT and TF respectively indicates an adequate signal and these models can be used to navigate the design space.

3.5 Design Validation

From the predicted versus actual plots [13] of RT and TF shown in the Fig. 8, it was observed that the selected models for the respective responses were suitable for the selected design as this plot indicates uniform distribution of the data points around 45^o line. It was further evidenced from the ANOVA tables 4 & 6 that the selected models were significant with $P < 0.05$ and suitable for the design employed in this work.

3.6 Optimization by Desirability Functions Approach [14]

The optimized chromatographic conditions selected based on the desirability functions

approach were mobile phase consisting of Acetonitrile: 0.1% OPA buffer pH 2.5 (40: 60% v/v) pumped at a flow rate of 1.06 mL/min gave the highest desirability of 0.900. In the overlay contour plot shown in the Fig. 9, the flag represents the optimized combination of the three selected independent factors which gives the maximum desirability. To confirm these optimum set of conditions, three replicate injections of 100µg/mL PGB was analyzed to determine if their observed responses were within the predicted range as shown in Table 8 and the corresponding optimized chromatogram was shown in Fig. 10.

3.7 Optimized Chromatographic Condi tions Suggested by DOE

Column: Agilent Zorbax XDB C18 (250×4.6 mm, 5 µm)

Mobile phase: 0.1% OPA (60%): Acetonitrile (40%)

Buffer pH: 2.5

Flow rate: 1.06 mL/min

Wavelength: PDA-UV detection at 262nm

Column temperature: Ambient

Run time: 5 min

3.8 Method Validation [15]

The developed method was linear over the concentration range of 25-150 µg/ml with correlation coefficient of 0.999. For the accuracy studies, at 50, 100 and 150% levels the % recovery of the drug was found to be within 98-102%. Intermediate precision and repeatability were carried out and the % RSD values were found to be less than 2%. LOD & LOQ values were found to be 0.125 µg/ml and 0.375 µg/ml. Robustness of the developed method was checked by making minor changes in the experimental conditions like flow rate, % organic composition and %RSD values for the peak area were found to be less than 2%. From the system suitability tests, the number of theoretical plates were found to be more than 2000 and tailing factor was found to be less than 2. The summary of the method validation parameters were shown in the Table 9.

3.9 Forced Degradation Studies

Forced degradation studies of Pemigatinib in various conditions like acidic, basic, peroxide, thermal, photolytic, hydrolytic and reductive were performed [16]. The drug showed significant degradation in reductive condition with 10% sodium bisulphate represented in Fig. 11. Results of forced degradation studies were presented in Table 10.

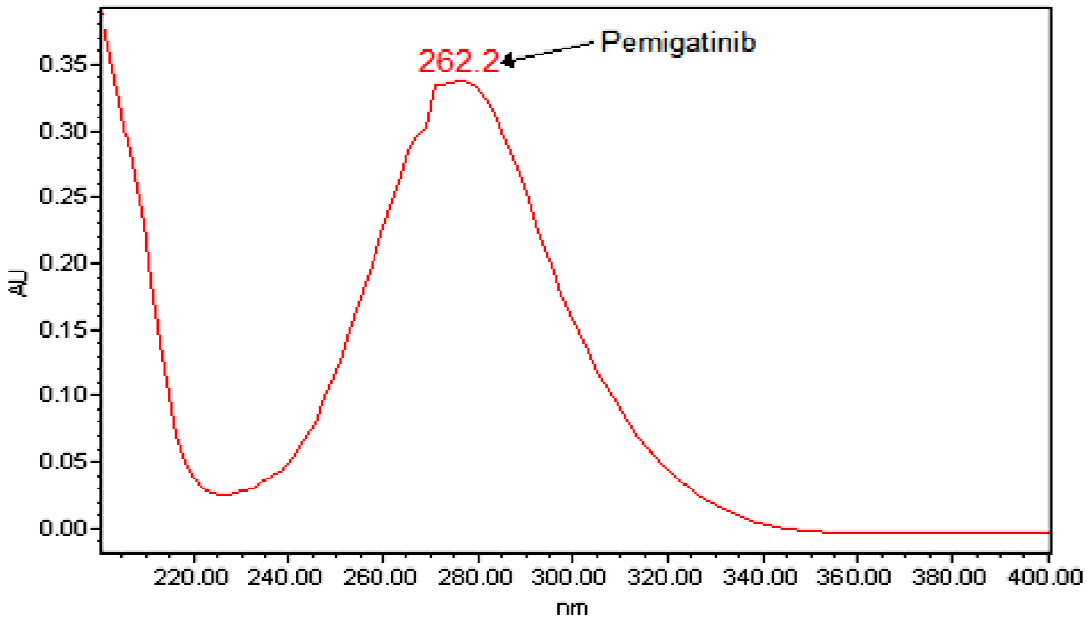


Fig. 2. UV spectrum of Pemigatinib

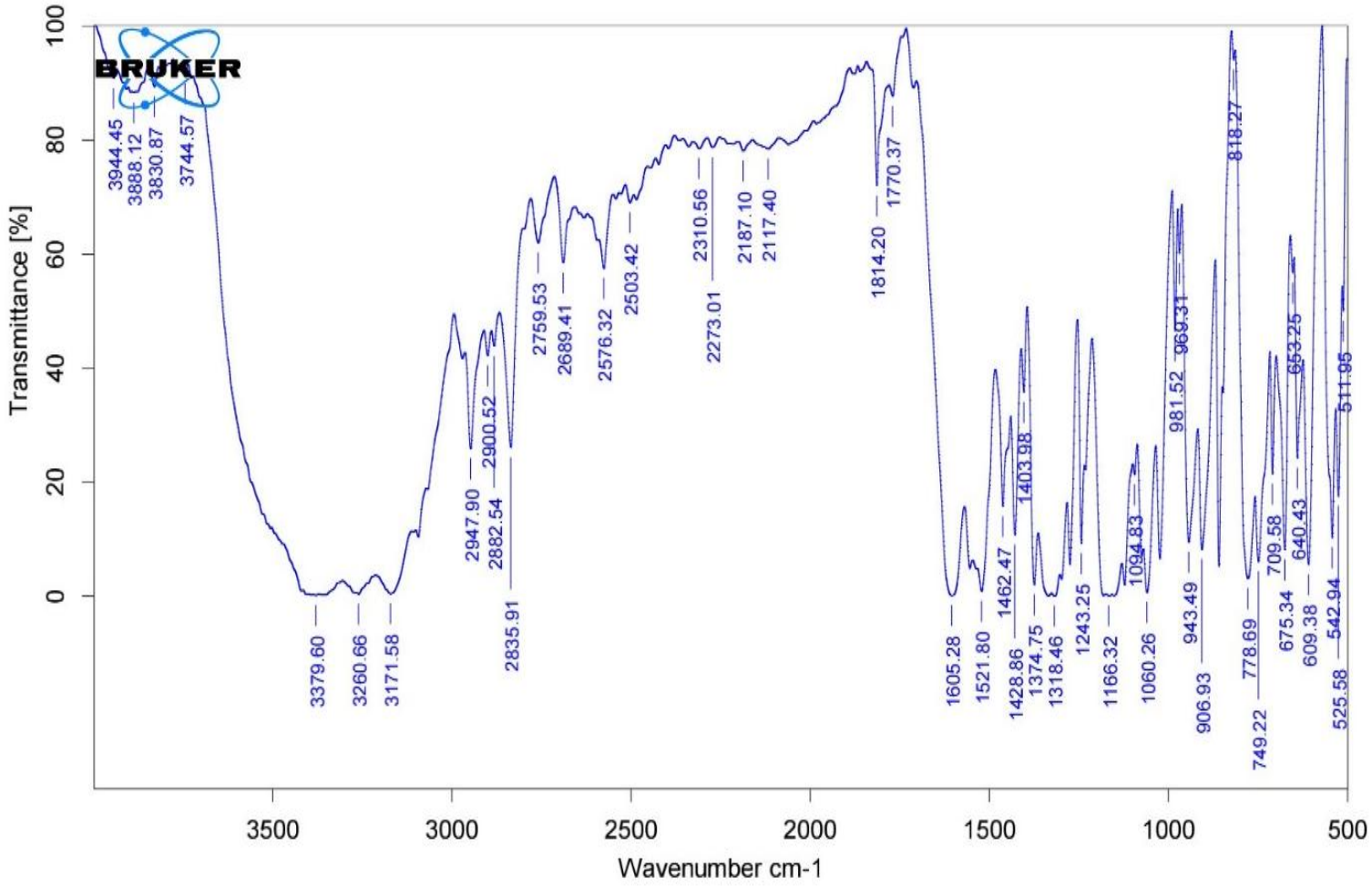
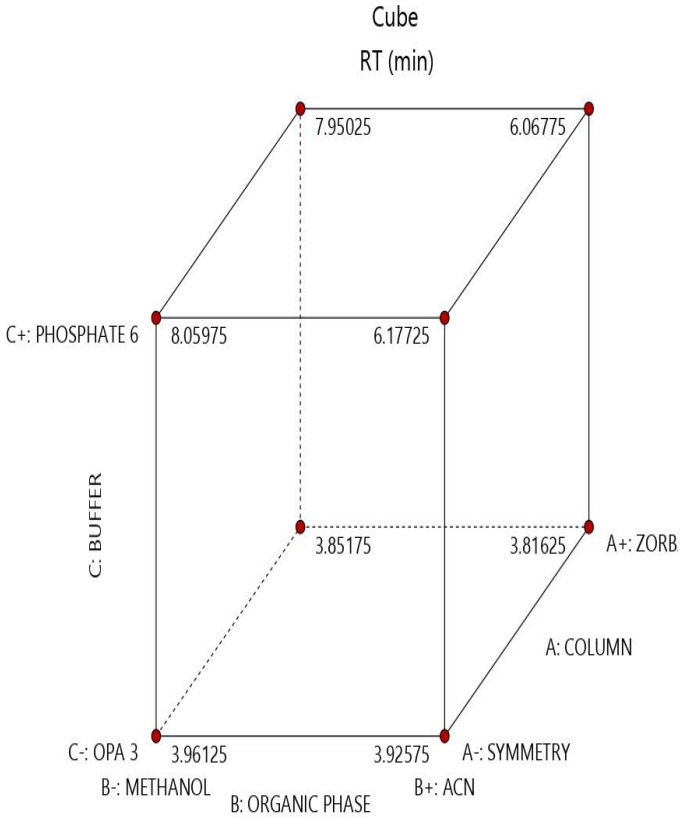


Fig. 3. FT-IR spectrum of Pemigatinib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C: BUFFER
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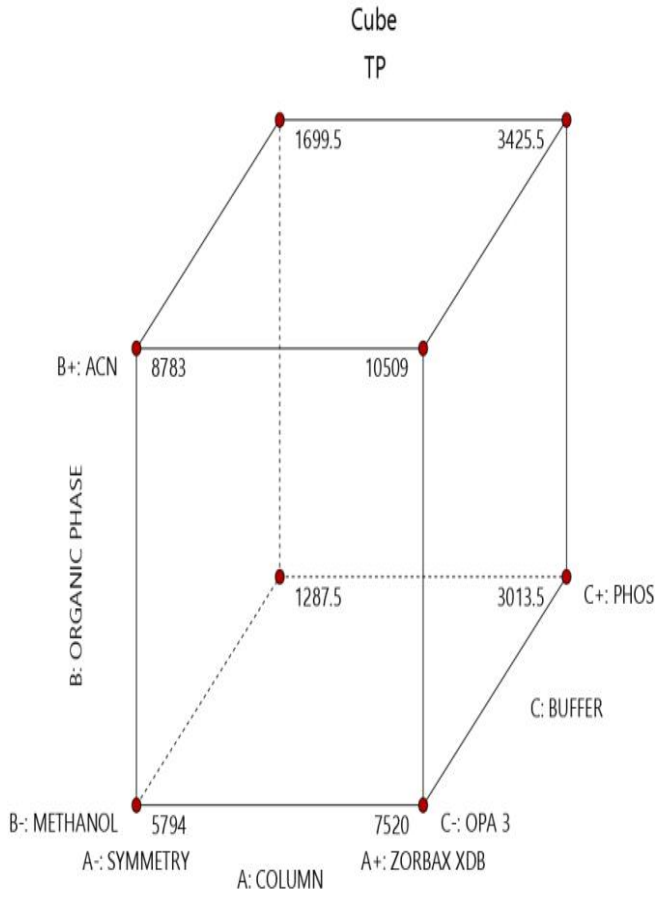
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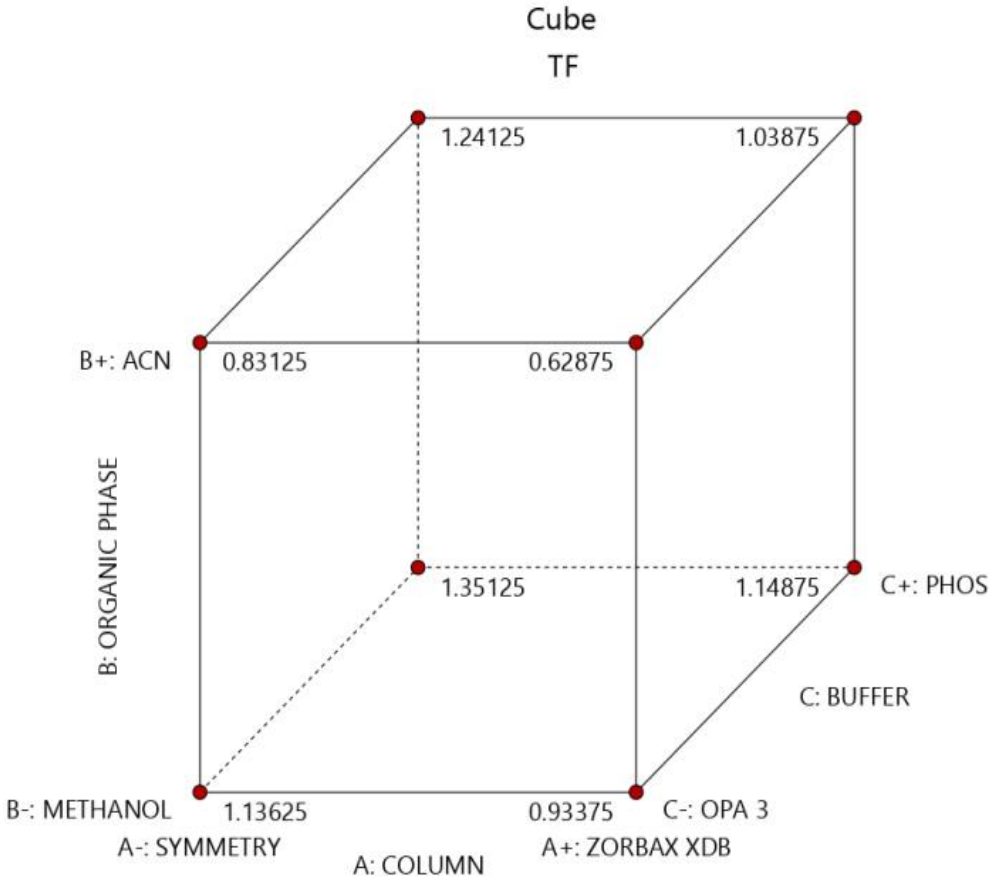


Fig. 4. Cube plots of RT, TP and TF for 2^3 Factorial design

Table 2. Trial runs with responses for 2^3 factorial design

Run order	Column	Organic phase	Buffer pH	Retention time(min)	Theoretical plates	Tailing factor
1	Zorbax XDB	Methanol	OPA pH 3	3.826	7451	0.95
2	Zorbax XDB	Methanol	Phosphate pH 6	7.985	2872	1.11
3	Symmetry	Acetonitrile	OPA pH 3	3.925	8745	0.78
4	Zorbax XDB	Acetonitrile	Phosphate pH 6	6.058	3598	1.01
5	Symmetry	Acetonitrile	Phosphate pH 6	6.187	1527	1.27
6	Symmetry	Methanol	OPA pH 3	3.987	5863	1.12
7	Symmetry	Methanol	Phosphate pH 6	8.025	1429	1.39
8	Zorbax XDB	Acetonitrile	OPA pH 3	3.817	10547	0.68

Table 3. ANOVA for the responses by 2^3 factorial model

ANOVA for selected Factorial model							
Analysis of variance table [Partial sum of squares - Type III]							
Response	**Source**	**Sum of Squares**	**d f**	**Mean Square**	**F Value**	***P*-value**	**Inference**
Retention time	Model	23.73	4	5.93	4525.80	<0.0001	Significant
	A-Column	0.024	1	0.024	18.29	0.023	
	B-Organic phase	1.84	1	1.84	1403.20	< 0.0001	
	C-Buffer	20.16	1	20.16	15380.48	< 0.0001	
	BC	1.71	1	1.71	1301.24	< 0.0001	
	Residual	3.932E-003	3	1.311E-003			
Theoretical plates	Model	8.223E+007	4	2.056E+007	550.78	<0.0001	Significant
	A-Column	5.958E+006	1	5.958E+006	159.64	0.001	
	B-Organic phase	5.783E+006	1	5.783E+006	154.96	0.001	
	C-Buffer	6.716E+007	1	6.716E+007	1799.57	<0.0001	
	BC	3.320E+006	1	3.320E+006	88.97	0.002	
	Residual	1.120E+005	3	37322.3			
Tailing factor	Model	0.36	3	0.12	16.45	0.010	Significant
	A-Column	0.082	1	0.082	11.14	0.028	
	B-Organic phase	0.086	1	0.086	11.70	0.026	
	C-Buffer	0.20	1	0.20	26.53	0.006	
	Residual	0.029	4	7.362E-003			

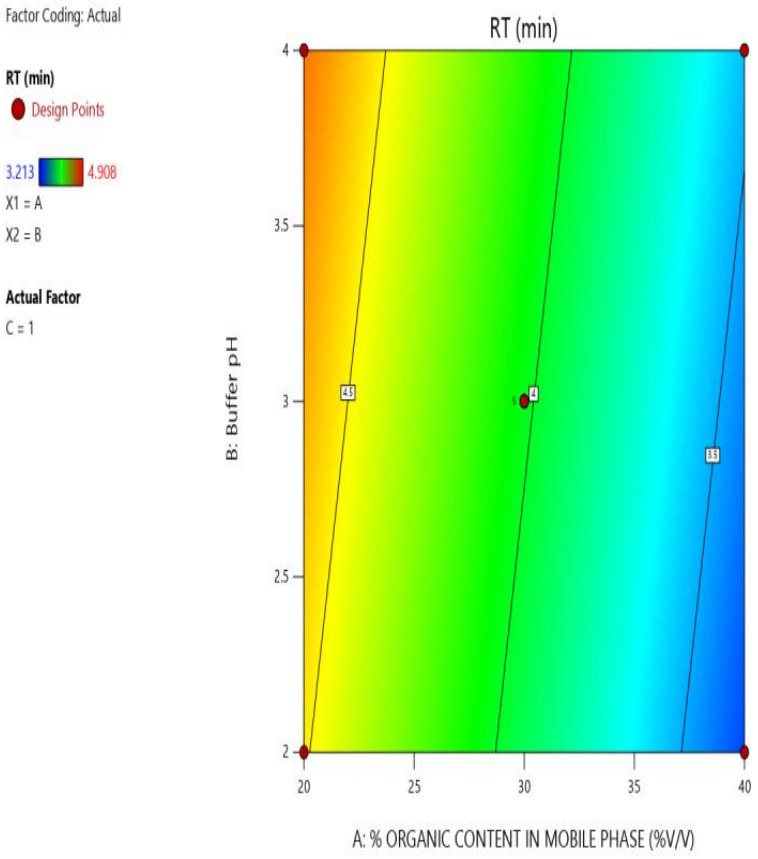
Table 4. Box-Behnken experimental design with responses

Trail no	S.No	%Organic content in mobile phase	Buffer pH	Flow rate (FR) (ml/min)	Retention Time(RT) (min)	USP theoretical plates(TP)	Tailing factor (TF)
5	1	20	3	0.9	4.908	12457	0.54
7	2	20	3	1.1	4.281	7896	1.16
10	3	30	4	0.9	4.334	9896	0.84
17	4	30	3	1	3.927	10457	0.62
4	5	40	4	1	3.483	9847	1.14
16	6	30	3	1	3.924	10521	0.65
12	7	30	4	1.1	4.144	8456	1.02
13	8	30	3	1	3.928	10486	0.68
15	9	30	3	1	3.925	10551	0.63
11	10	30	2	1.1	3.955	13488	0.72
3	11	20	4	1	4.827	8745	1.05
14	12	30	3	1	3.926	10483	0.62
8	13	40	3	1.1	3.213	11478	0.72
9	14	30	2	0.9	4.095	8745	1.05
1	15	20	2	1	4.492	6879	1.17
6	16	40	3	0.9	3.643	14562	0.65
2	17	40	2	1	3.427	12554	0.66

Table 5. ANOVA for retention time using BBD

ANOVA for Response Surface Linear model						
Analysis of variance table [Partial sum of squares - Type III]						
Source	**Sum of Squares**	**d f**	**Mean Square**	**F Value**	***P*-value**	**Inference**
Model	3.14	3	1.05	69.15	< 0.0001	significant
A-% Organic content in mobile phase	2.81	1	2.81	185.98	<0.0001	significant
B- Buffer pH	0.0838	1	0.0838	5.55	0.034	significant
C-FR	0.2405	1	0.2405	15.91	0.001	significant
Residual	0.1965	13	0.0151			

df: degrees of freedom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Design Points
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4.908
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X2 = B
Actual Factor
C = 1
RT (min)
B: Buffer pH
A: % ORGANIC CONTENT IN MOBILE PHASE (%V/V)
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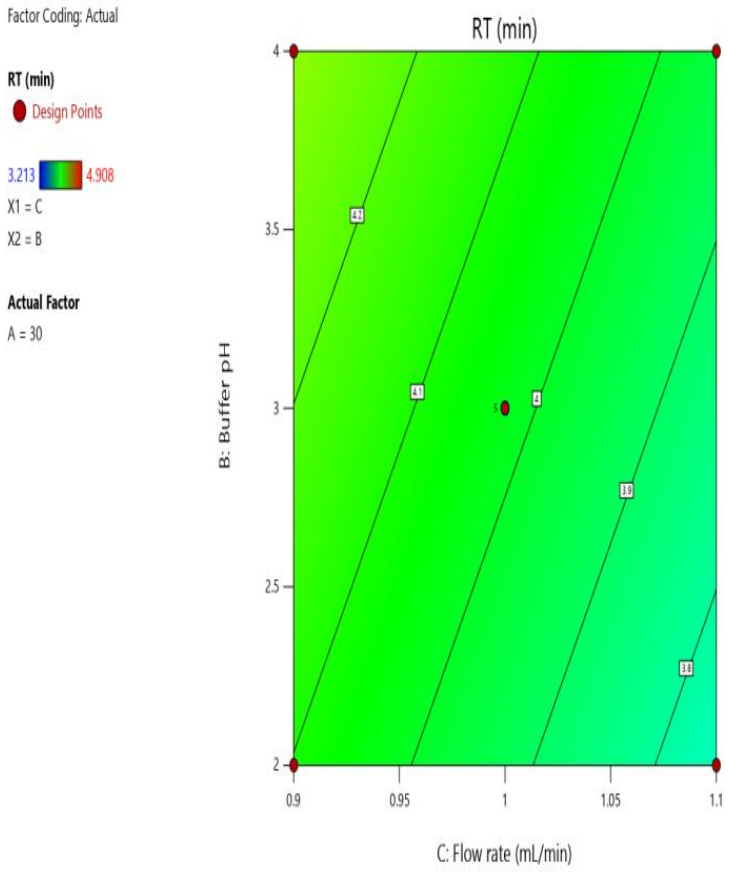
4
3.5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RT (min)
Design Points
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4.908
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X2 = B
Actual Factor
A = 30
RT (min)
B: Buffer pH
C: Flow rate (mL/min)
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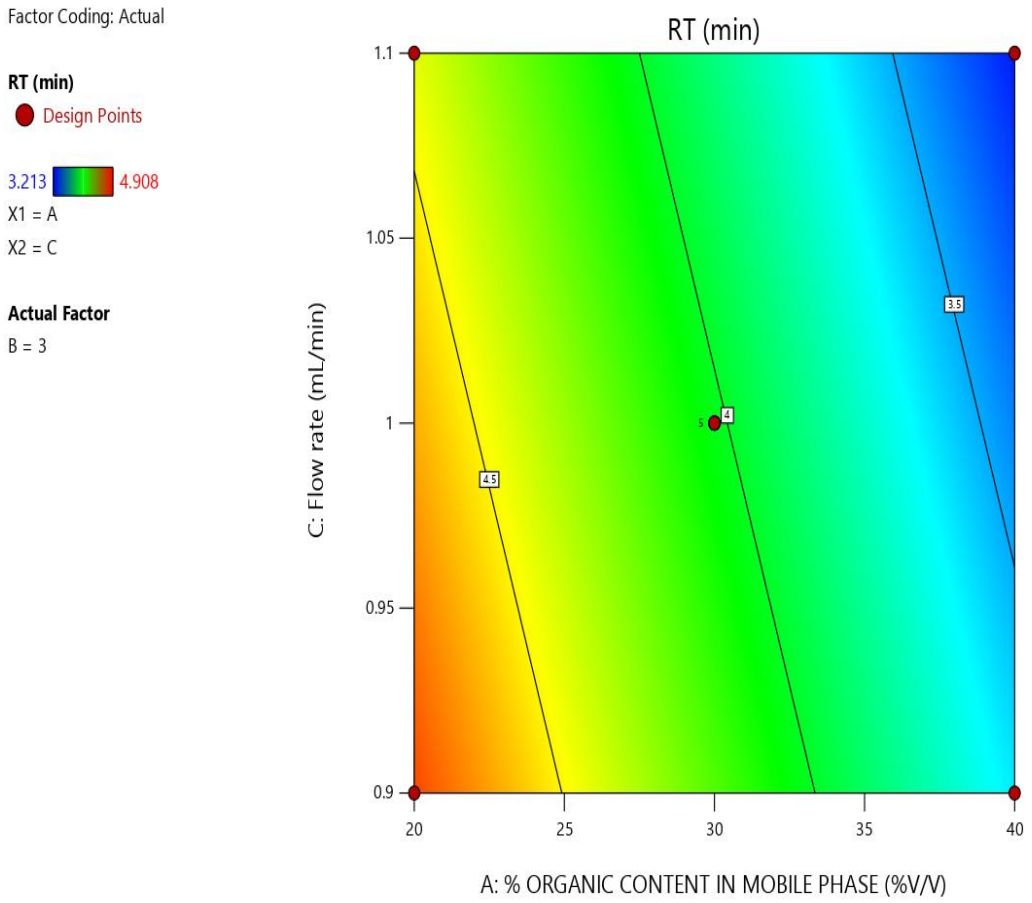

Fig. 5. 2D Contour plots of retention time as a function of % organic content in mobile phase, buffer pH and flow rate

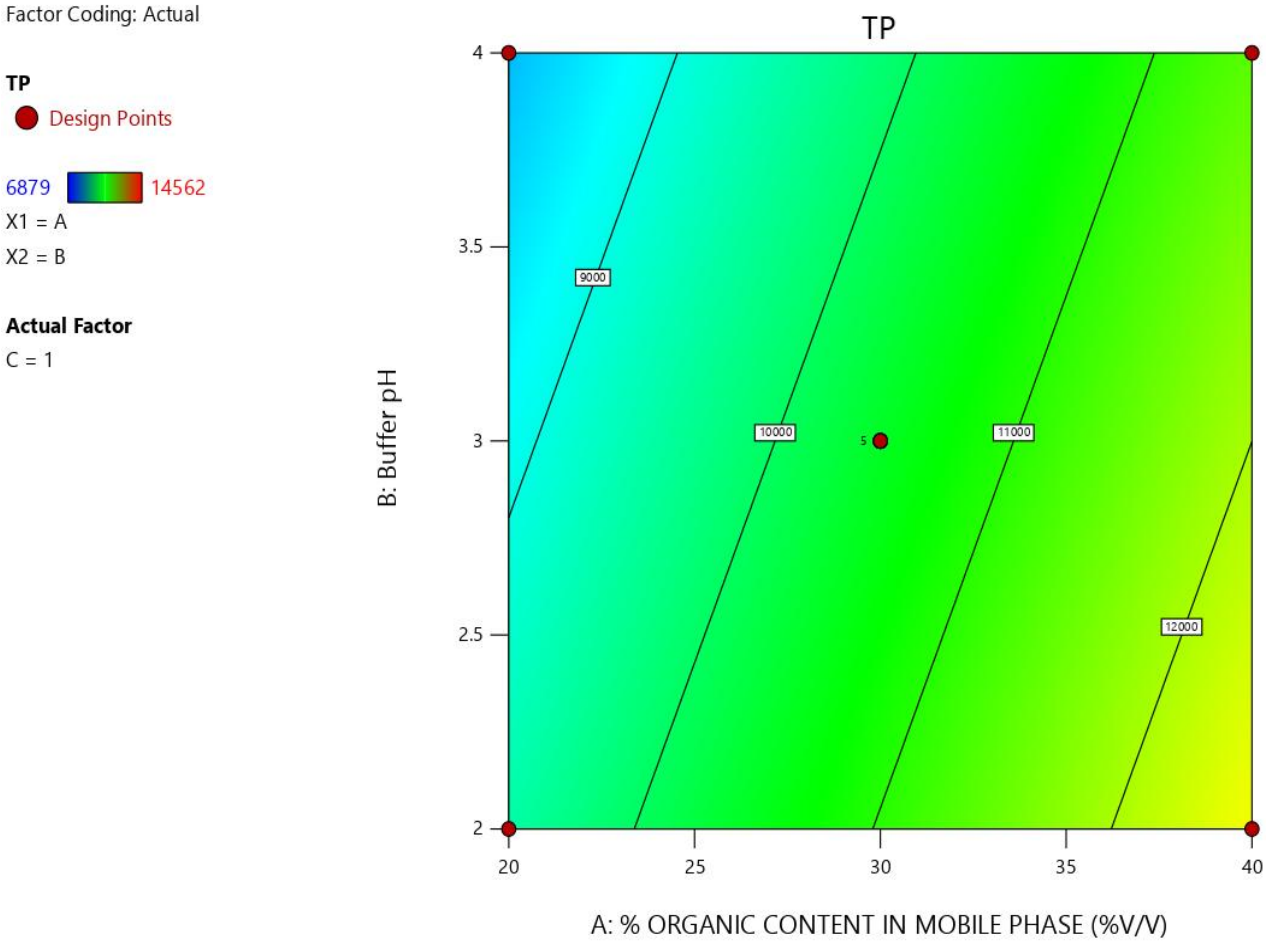

Fig. 6. 2D Contour plot of theoretical plates as a function of % organic content in mobile phase versus buffer pH

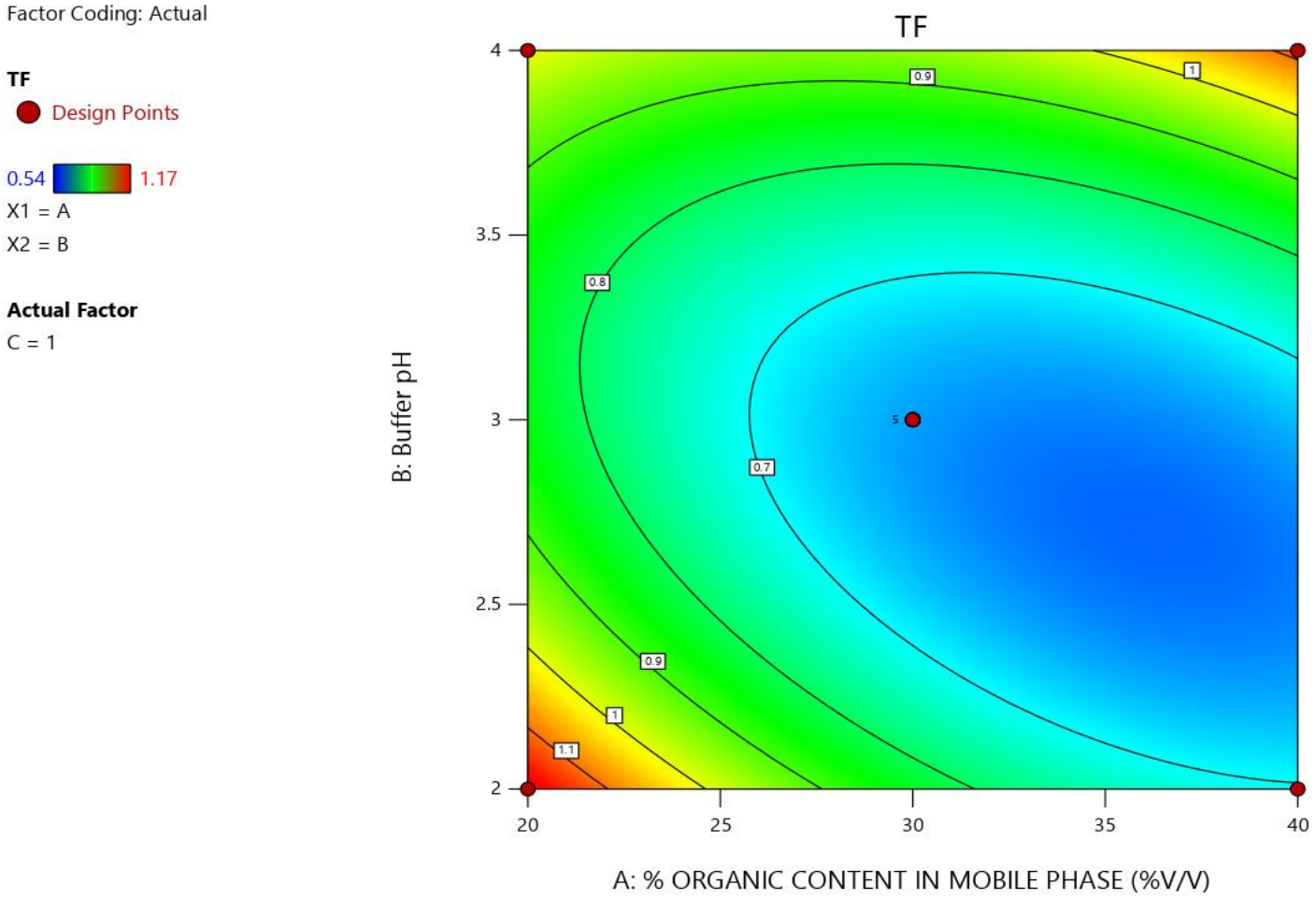

Fig. 7. 2D contour plot of tailing factor as a function of % organic content in mobile phase versus buffer pH

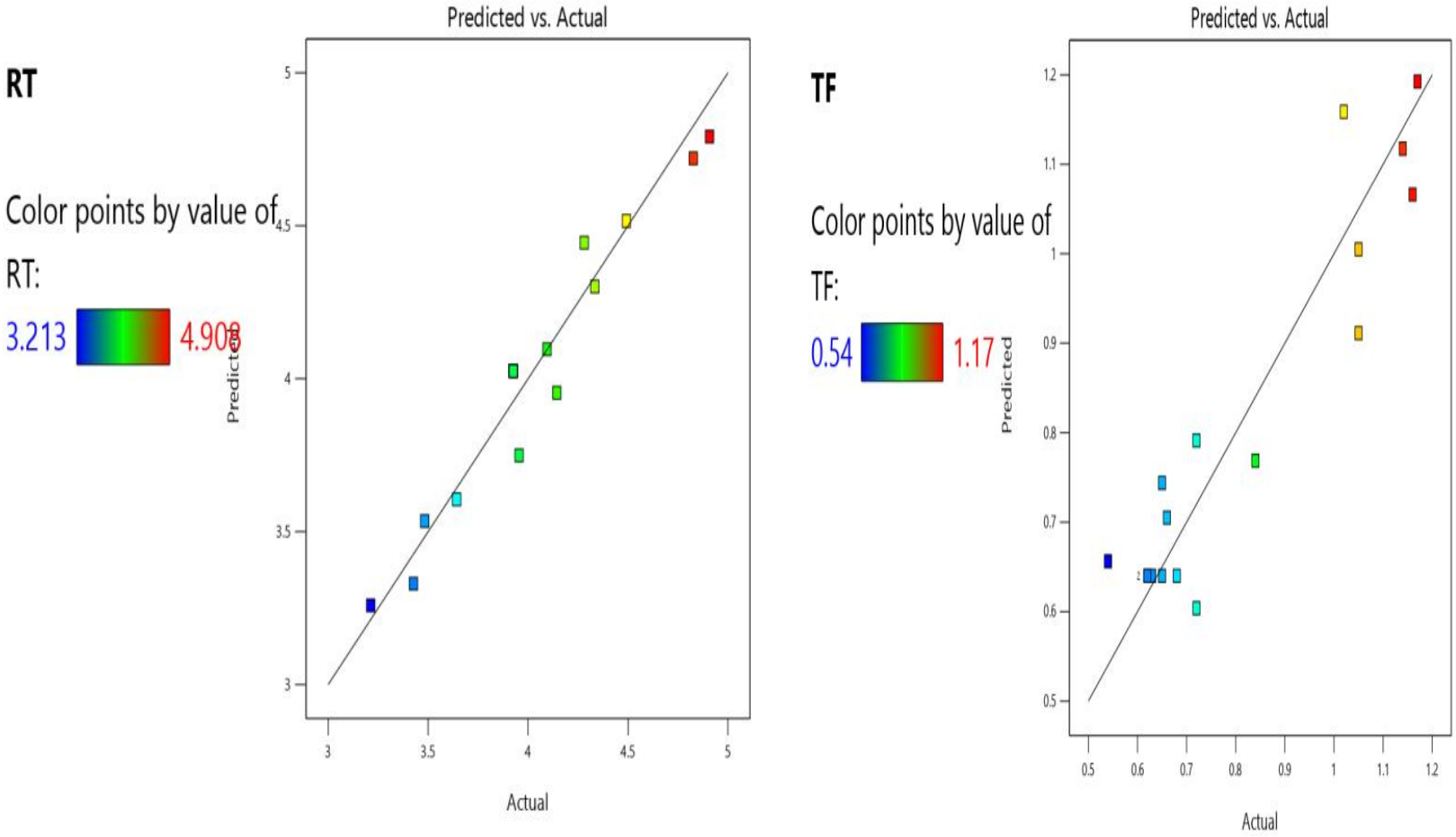

Fig. 8. Predicted versus actual plots of RT & TF

Factor Coding: Actual

Overlay Plot

RT

TP

TF

X1 = A

X2 = B

Actual Factor

C = 1.06504

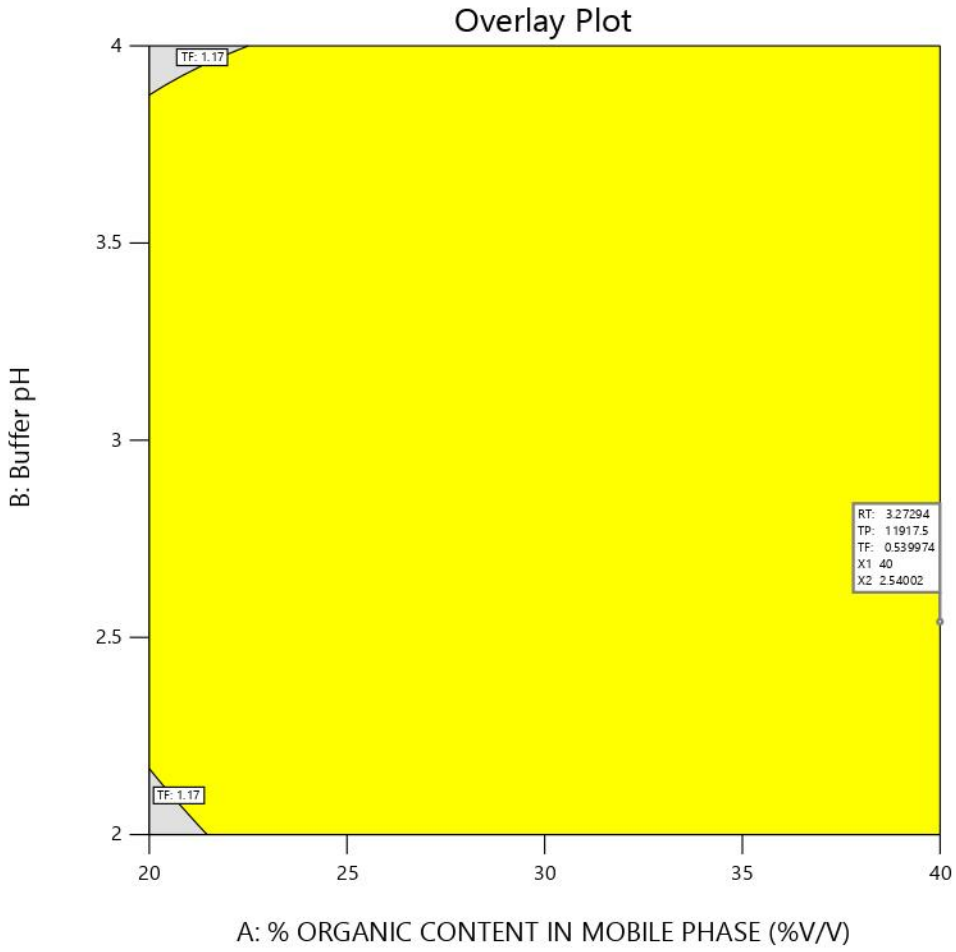

Fig. 9. Overlay contour plot supported by responses

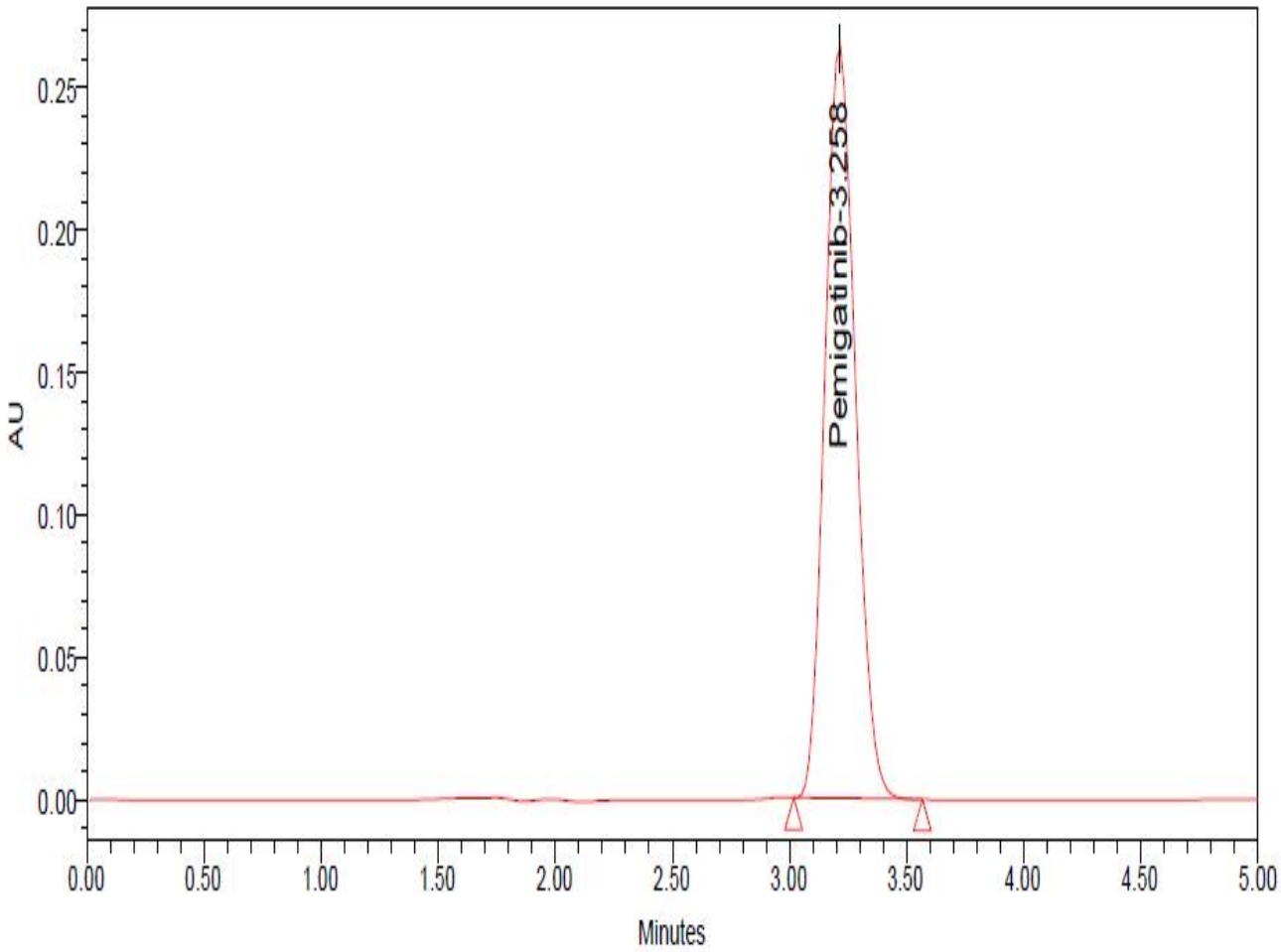

Fig. 10. Chromatogram of the optimized method

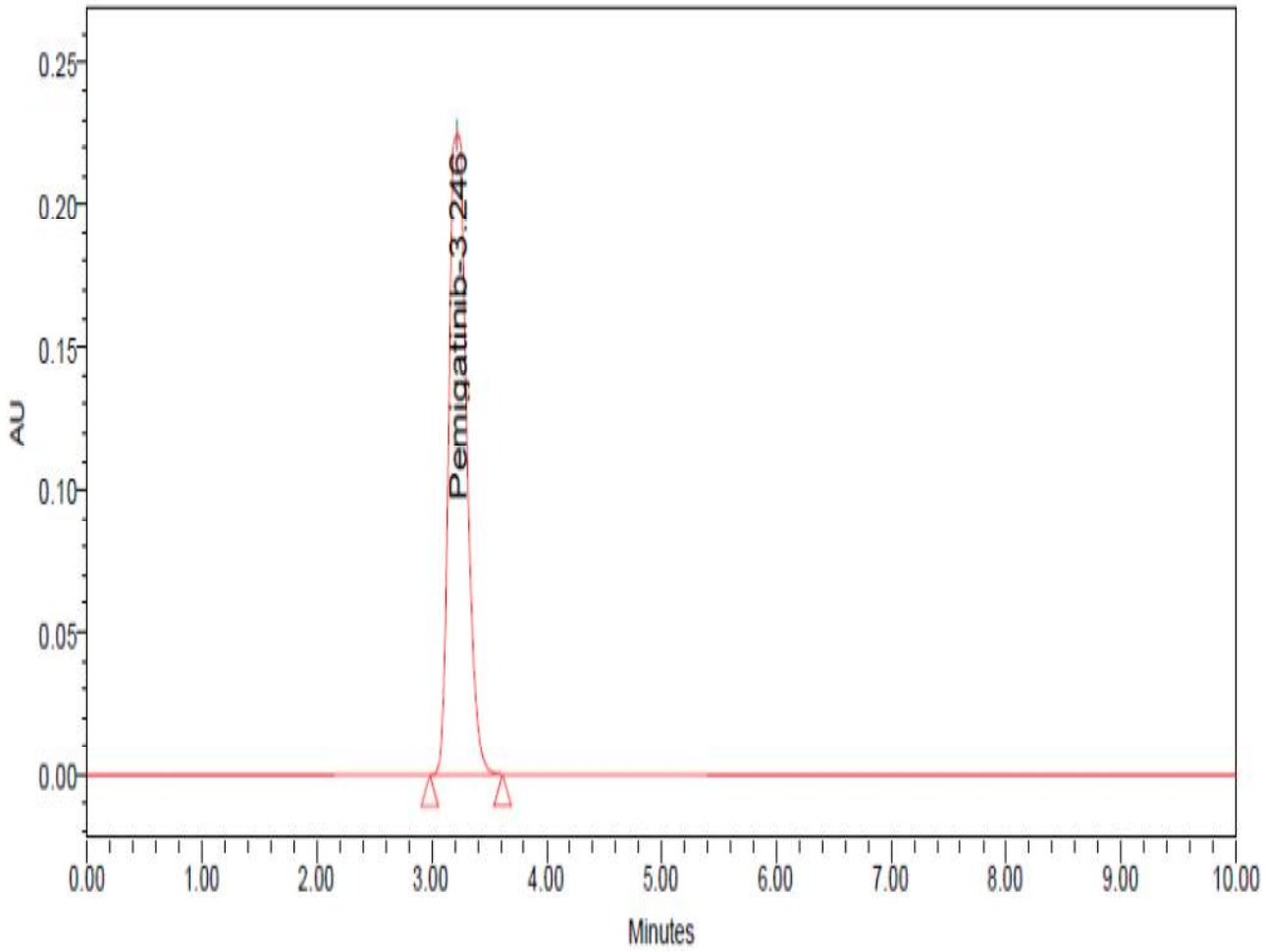

Fig. 11. Chromatogram of reductive (10% sodium bisulphate) degradation

Table 6. ANOVA for theoretical plates using BBD

ANOVA for Response Surface Linear model						
Analysis of variance table [Partial sum of squares - Type III]						
Source	**Sum of Squares**	**d f**	**Mean Square**	**F Value**	***P*-value**	**Inference**
Model	2.456E+07	3	8.188E+06	2.61	.096	Not significant
A-% Organic content in mobile phase	1.942E+07	1	1.942E+07	6.18	.027	significant
B- Buffer pH	2.787E+06	1	2.787E+06	0.8872	.363	
C-FR	2.357E+06	1	2.357E+06	0.7502	.402	
Residual	4.084E+07	13	3.141E+06			

Table 7. ANOVA table for tailing factor using BBD

ANOVA for Response Surface Quadratic model						
Analysis of variance table [Partial sum of squares - Type III]						
Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	***P*-value**	**Inference**
Model	0.7033	9	0.0781	5.42	.018	Significant
A-%Organic content in mobile phase	0.0703	1	0.0703	4.88	.063	
B- Buffer pH	0.0253	1	0.0253	1.76	.226	
C-FR	0.0365	1	0.0365	2.53	.155	
AB	0.0900	1	0.0900	6.24	.041	Significant
AC	0.0756	1	0.0756	5.25	.055	
BC	0.0650	1	0.0650	4.51	.071	
A^2	0.0533	1	0.0533	3.70	.096	
B^2	0.2684	1	0.2684	18.62	.003	Significant
C^2	0.0009	1	0.0009	0.0657	.805	
Residual	0.1009	7	0.0144			

Table 8. Responses of the optimized method for PGB

S.No.	Response variables	Predicted value	Actual value	Desirable Range
1	Retention time(min)	3.272	3.258	2.974 - 3.571
2	Theoretical plates	11917	11579	7614.27 - 16221.2
3	Tailing factor	0.539	0.81	0.188 - 0.891

Table 9. Results of the validation parameters

S.No.	Parameter		Results
1	Linearity	Linearity Range(µg/ml)	25-150
		Correlation Coefficient	0.999
		Regression equation	y = 24721x + 6728.8
2	Accuracy (% recovery)	50%, 100%,150% levels	Between 99.75-100.39%.
3	Precision(% RSD of peak area)	Intermediate precision	0.04
		Repeatability	0.07
4	Sensitivity	LOD(µg/ml)	0.125
		LOQ(µg/ml)	0.375
5	Robustness(% RSD of peak area)	Flow rate (±0.05 ml/min)	0.08
		Organic phase (±5%)	0.07
6	System suitability	Retention time(min)	3.257
		Tailing factor	0.85
		Plate count	11602

Table 10. Results of forced degradation studies

Stress condition	% Drug recovered	% Drug degraded	Retention time of degradant (min)
Control	100.2	-0.2	-
Acidic(1N HCl, 60^0C, 30 min)	89.9	10.1	1.07, 2.26
Alkali(1N NaOH,60^0C, 30 min)	89.88	10.12	1.06, 2.25
Neutral(H_2O, 60^0C, 30 min)	99.54	0.46	-
Oxidative(20% H_2O_2, RT, 30 min)	93.15	6.85	1.475
Reduction(10% Sodium bisulphate, 60^0C, 30 min)	83.17	16.83	-
UV light(24 hrs)	89.9	10.1	-
Thermal(70^0C, 24 hrs)	89.15	10.85	1.847

4. CONCLUSION

A simple, accurate and robust RP-HPLC method was developed for the determination of Pemigatinib by using Design of Experiments approach. Box-Behnken Design consisting of three factors at three levels was selected as optimization design for the present study. The critical method parameters selected for optimization were % organic content in the mobile phase, flow rate and pH of the buffer. The critical quality attributes are retention time, theoretical plates and tailing factor. Optimized chromatographic conditions suggested by desirability functions approach consisted of mobile phase 0.1% OPA (60%): Acetonitrile (40%), buffer pH 2.5 pumped at a flow rate of 1.06ml /min gave the highest desirability of 0.9. The retention time of the drug was found to be 3.258min. Theoretical plates and tailing factor were found to be within the limits. The developed method was validated as per ICH Q2 (R1) guidelines. Utilization of RSM provides a better insight for method development and robustness testing. Degradation studies were performed in various stress conditions and the drug was found to be degraded more in reductive condition.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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