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(RESEARCH ARTICLE)

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QbD-Driven Optimization and Validation of an HPTLC Method for Quantitative Determination of Bosutinib

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Abstract

A Quality by Design (QbD) driven HPTLC method was developed and validated for quantitative determination of Bosutinib. Three Factor I – Optimal Design was employed to optimize mobile phase composition, saturation time and detection wavelength. The optimized chromatographic condition using acetonitrile 7: water 3: glacial acetic acid 0.5 with densitometric detection at 267 nm produced compact and well resolved bands with Rf value around 0.6. The method demonstrated excellent linearity over 200–1200 ng/spot (R^2 = 0.9956) with regression equation y = 3.3397x + 45.207.

Precision studies showed repeatability of 0.29% RSD with intra- and inter-day precision below 2%. Accuracy 99.08 %. LOD and LOQ were 12.17 and 36.88 ng/spot respectively. Robustness evaluation confirmed minimal influence of deliberate variations and assay results showed 101.80 ± 1.08 %. Greenness assessment revealed AGREE score of 0.57 indicating environmentally acceptable analytical performance.

Keywords: AQbD Method; Bosutinib; HPTLC method; Method Validation; Greenness assessment

1. Introduction

Bosutinib is a second-generation tyrosine kinase inhibitor used in the management of chronic myeloid leukemia. Reliable analytical methods are essential for quality control and formulation development. High-performance thin layer chromatography offers advantages including minimal solvent consumption, simultaneous sample analysis and low operational cost. Integration of Quality by Design principles ensures systematic optimization, improved robustness and regulatory flexibility. Therefore, the present work focuses on development and validation of a QbD-based HPTLC method with greenness assessment for Bosutinib quantification.

2. Materials and Methods

Chromatographic separation was performed on silica gel 60 F254 plates using CAMAG HPTLC system. The mobile phase consisting of acetonitrile 7: water 3: glacial acetic acid 0.5 was optimized using Three Factor I – Optimal Design. Sample application was performed using automated applicator followed by chamber saturation and densitometric scanning at 267 nm. Experimental design evaluated the influence of mobile phase ratio, saturation time and wavelength on Rf and peak area responses.

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3. Results and Discussion

Experimental design confirmed significant influence of mobile phase composition and saturation time on chromatographic responses. Quadratic models adequately described the behavior of Rf and peak area enabling establishment of a robust design space. Optimization predicted desirability close to unity producing compact bands with acceptable symmetry and high peak area. Compared to HPLC, the developed HPTLC method demonstrated reduced solvent consumption, improved throughput and better environmental compatibility.

3.1. Validation

The method exhibited linear response within 200–1200 ng/spot with correlation coefficient 0.9956. Precision studies demonstrated %RSD values below 2% confirming repeatability and intermediate precision. Accuracy studies showed recoveries between 99–104 %. Sensitivity evaluation indicated LOD 12.17 ng/spot and LOQ 36.88 ng/spot. Robustness confirmed reliability under small deliberate variations while assay results validated applicability for marketed formulation analysis.

3.2. Greenness Assessment

Greenness evaluation using AGREE tool produced a score of 0.57 while Eco-scale assessment indicated acceptable environmental impact. Lower solvent consumption, absence of complex derivatization and reduced energy requirement contributed to the eco-friendly nature of the method. The developed HPTLC approach demonstrated improved sustainability compared to conventional chromatographic techniques.

3.3. Conclusion

The developed QbD-assisted HPTLC method is precise, accurate, robust and environmentally responsible. Statistical optimization ensured reliable chromatographic performance and validation confirmed suitability for routine pharmaceutical quality control applications.

3.4. Development of HPTLC method

HPTLC separates compounds based on their different adsorption and partition behavior between stationary and mobile phases, followed by densitometric detection. HPTLC is a rapid, sensitive, cost-effective, and reliable technique that allows simultaneous qualitative and quantitative analysis with minimal solvent and sample consumption.

3.4.1. Selection of Wavelength

The analytical wavelength that gave good absorbance value was selected for estimation of drug i.e., 267 nm for Bosutinib was selected.

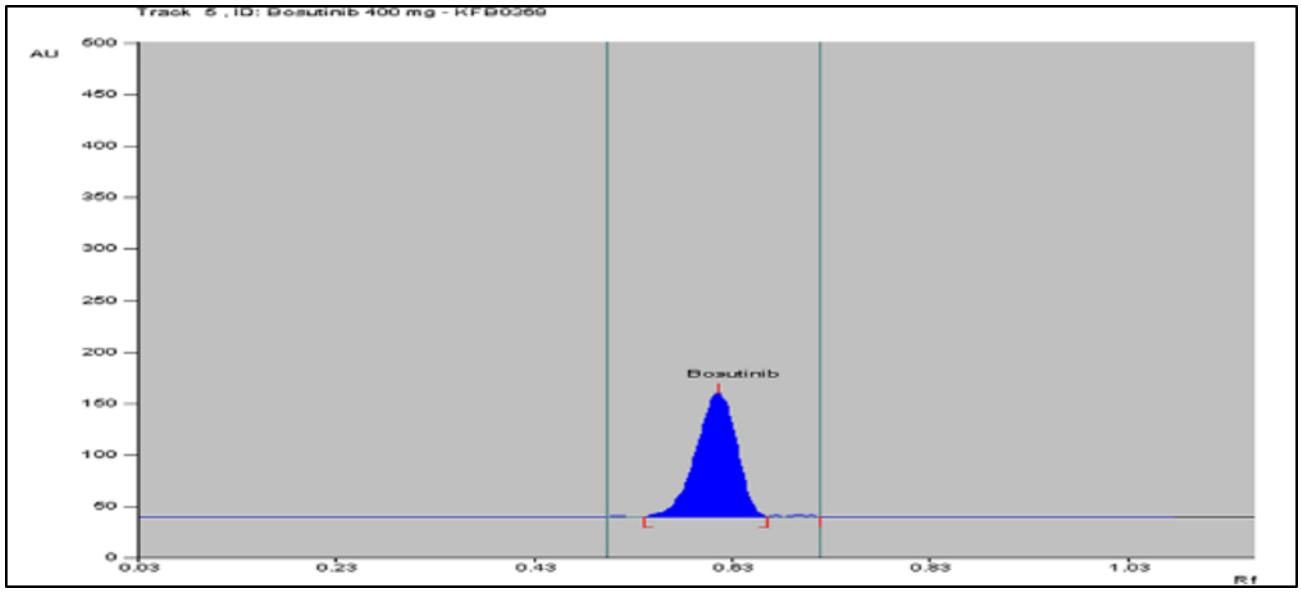


Figure 1 Chromatogram of Bosutinib Sample 100 ng/µL Set-1

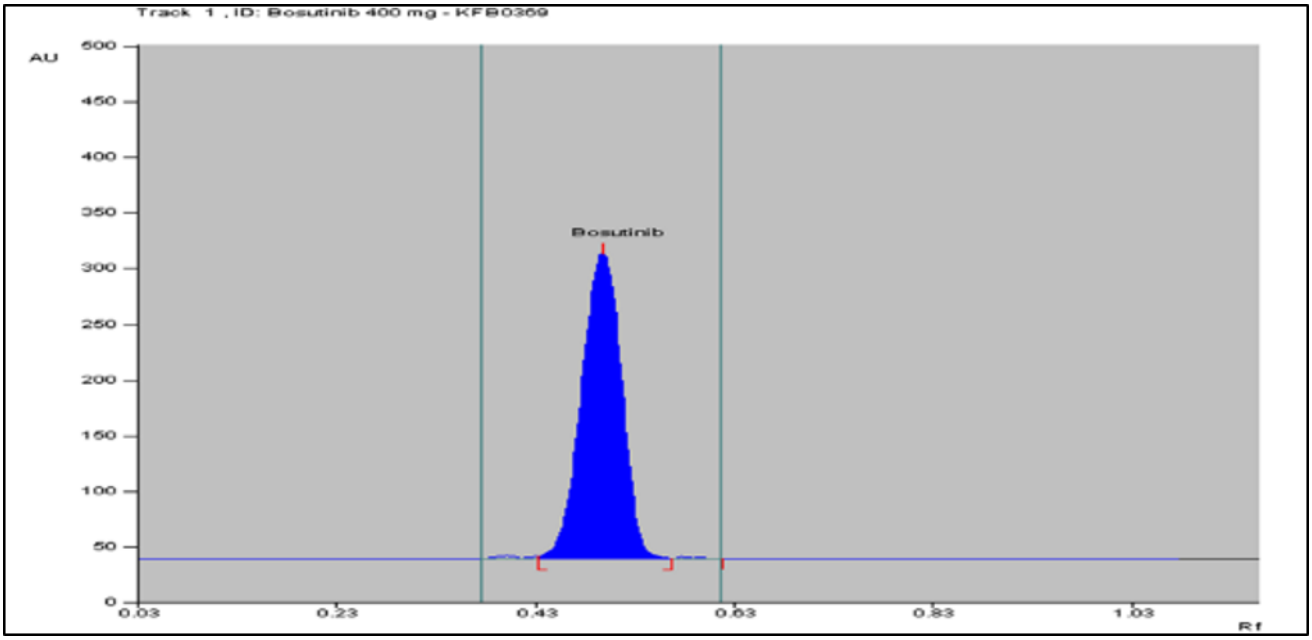


Figure 2 Chromatogram of Bosutinib Sample 100 ng/µL Set-2

3.5. HPTLC Method Development by QbD Approach

HPTLC method development using QbD approach was done.

3.5.1. Stage 1: Quality Target Product Profile (QTPP):

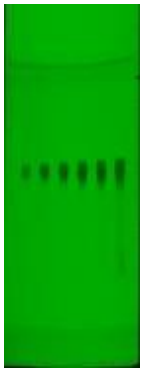
The Quality Target Product Profile chosen was Rf Value, Peak Area respectively.

For mobile phase optimization following some TLC trials were taken:

Trials	Mobile Phase Composition (v/v)	Saturation Time (min)	Representative TLC Chromatogram	Visual Diagnostic Features & Chromatographic Identification	Final Status
1	Methanol (10)	Nil		The analyte spot remained confined to the lower baseline, migrating below the 30% mark with poor vertical resolution.	Rejected

2	Chloroform (10)	Nil		No migration detected; the compound completely failed to move from the application origin.	Rejected
11	Methanol: Chloroform (7:3)	Nil		Inadequate elution power. The spot remained low on the plate (Rf = 0.23), yielding weak separation.	Rejected
19	Methanol: Acetone (7:3)	30		Improved migration profile (Rf = 0.26), but the spot failed to break past the 30% thresholds required for stable development.	Rejected
35	Sodium Benzoate: 20% Phenol	30		Achieved mid-range migration (Rf = 0.53), but the run required specialized chemical indicators (Methyl Orange).	Rejected

44	10% Camphor: Methanol (8:2)	30		Excessive elution. The analyte co-migrated near the solvent front (Rf = 0.94), risking out-of-range errors.	Rejected
52	Ethanol: Acetone: TEA (85:15:2 drops)	30		Achieved mid-range migration (Rf = 0.76) using a basic modifier, but peak symmetry was sub-optimal.	Rejected
54	Butanol: Mono Ethyl Amine: Water: 1,4 Dioxane (50:5:5:10)	30		Clear, distinct spot obtained in the mid-range (Rf = 0.64), but excluded due to hazardous, non-green solvent metrics.	Rejected
59	Acetonitrile: Water: Formic Acid	30		Good spot migration above 70%, but plagued by prominent, asymmetrical peak tailing.	Rejected

62	Acetonitrile: Water: Glacial Acetic Acid (70:30:0.5)	30		Highly compact, symmetric, and well-resolved spot (Rf = 0.60) with zero baseline tracking or tailing.	OPTIMIZED

3.5.2. Stage 2: Determine Critical Quality Attributes (CQAS):

Critical Quality Attributes selected were Mobile Phase Ratio Acetonitrile 7: Water 3: Glacial Acetic Acid 0.5 for HPTLC method development.

3.5.3. Stage 3: Develop A Design Space and Design of Experiment:

Perform Experimental Design

Experimental design chosen for HPTLC method was Three Factor I – Optimal Design.

Table 1 Experiments Model and Condition

Study Type	**Response Surface**	**Subtype**	**Randomized**
Design Type	Three Factor I – Optimal Design	Runs	11
Design Model	Quadratic	Blocks	No Blocks
Build Time (ms)	1.0000		

3.6. Factorial Design

Table 2 Fractional Design Experiment

Run	**Factor-A Acetonitrile (ml)**	**Factor-B Saturation Time (min)**	**Factor-C Wavelength Detection (nm)**
BO1	6.5	25mins	265nm
BO2	6.5	30mins	267nm
BO3	6.5	35mins	269nm
BO4	7	25mins	265nm
BO5	7	30mins	267nm
BO6	7	30mins	267nm
BO7	7	30mins	267nm
BO8	7	35mins	269nm
BO9	7.5	25mins	265nm
BO10	7.5	30mins	267nm
BO11	7.5	35mins	269nm

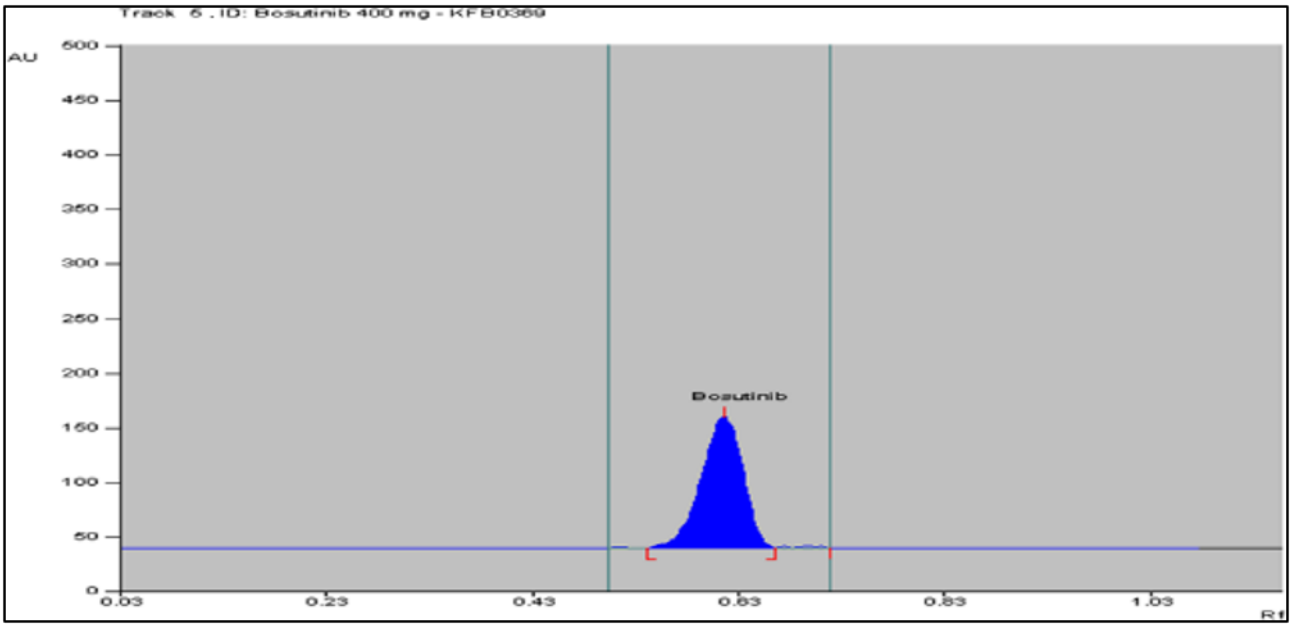


Figure 3 Batch BO 1 Chromatogram (6.5) Sat. Time – 25 mins detection at 265 nm

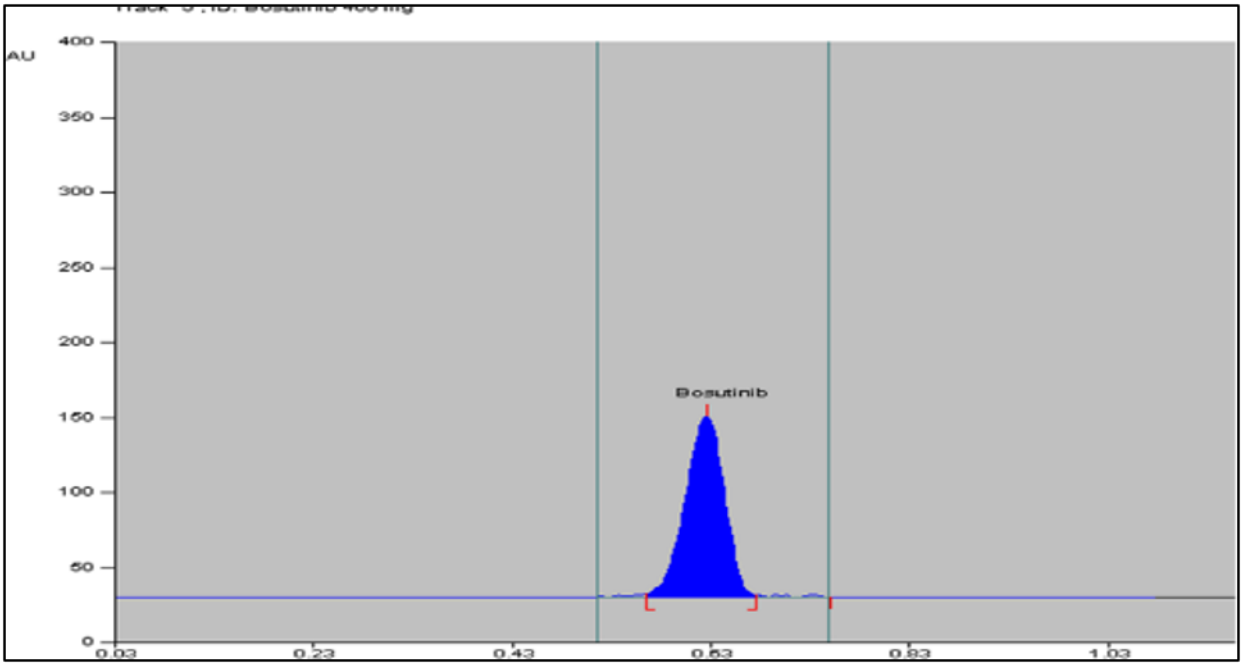


Figure 4 Batch BO 2 Chromatogram (6.5) Sat. Time – 30 mins detection at 267 nm

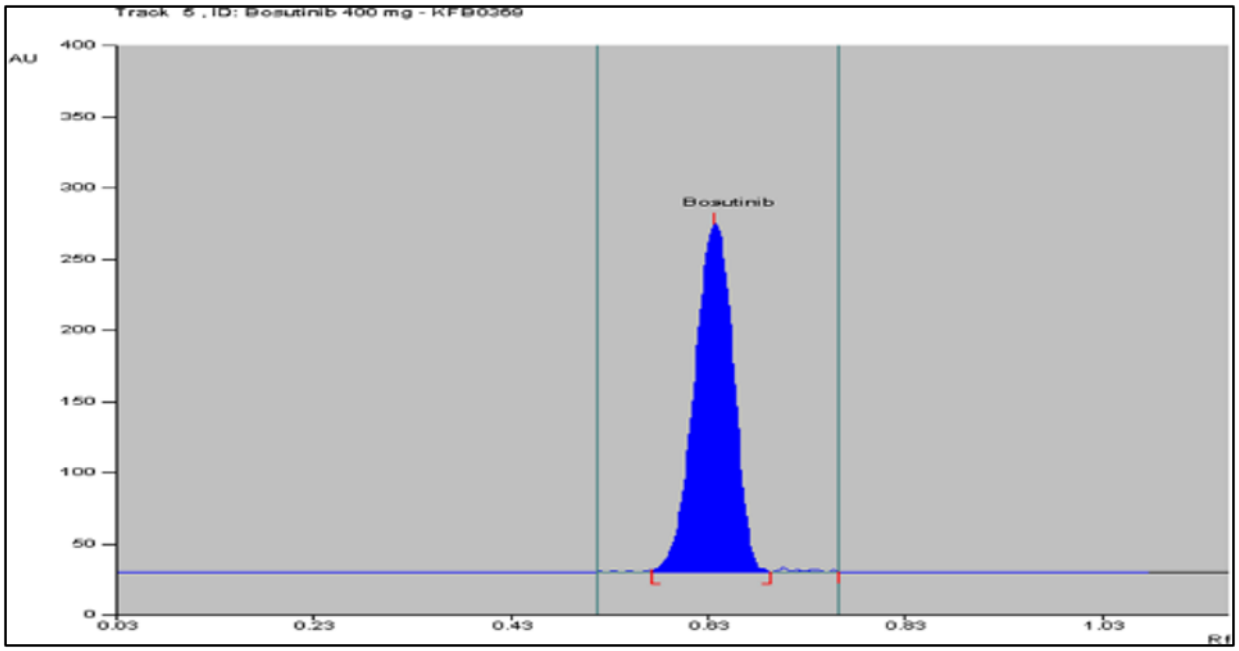


Figure 5 Batch BO 3 Chromatogram (6.5) Sat. Time – 35 mins detection at 269 nm

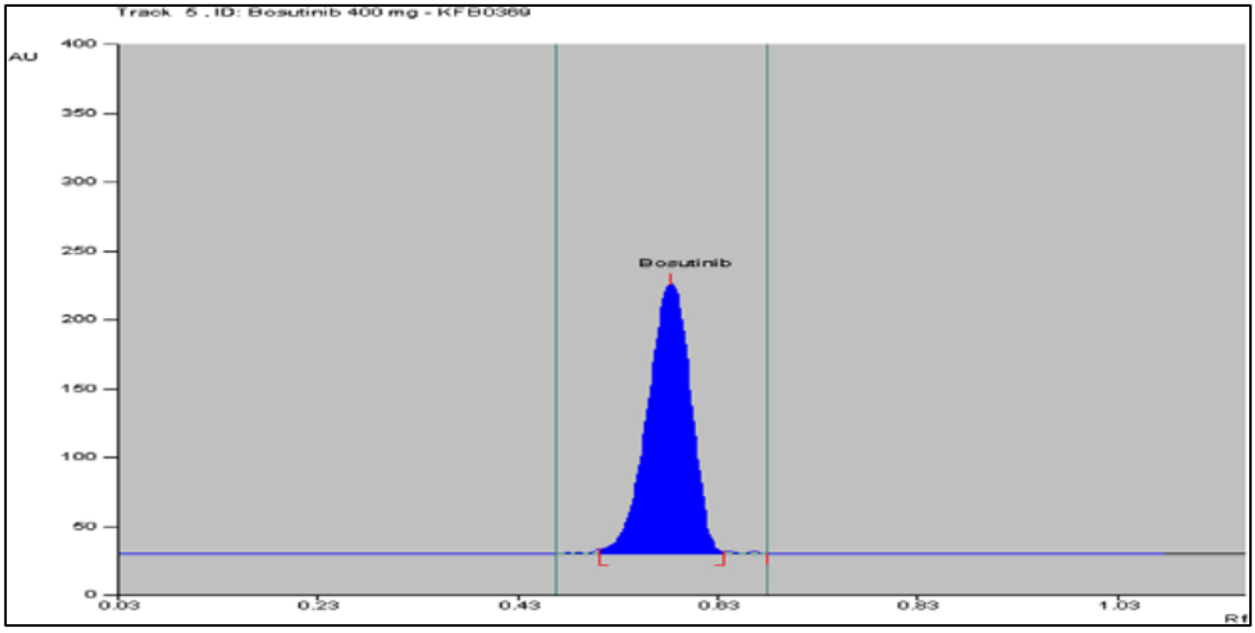


Figure 6 Batch BO 4 Chromatogram (7) Sat. Time – 25 mins detection at 265 nm

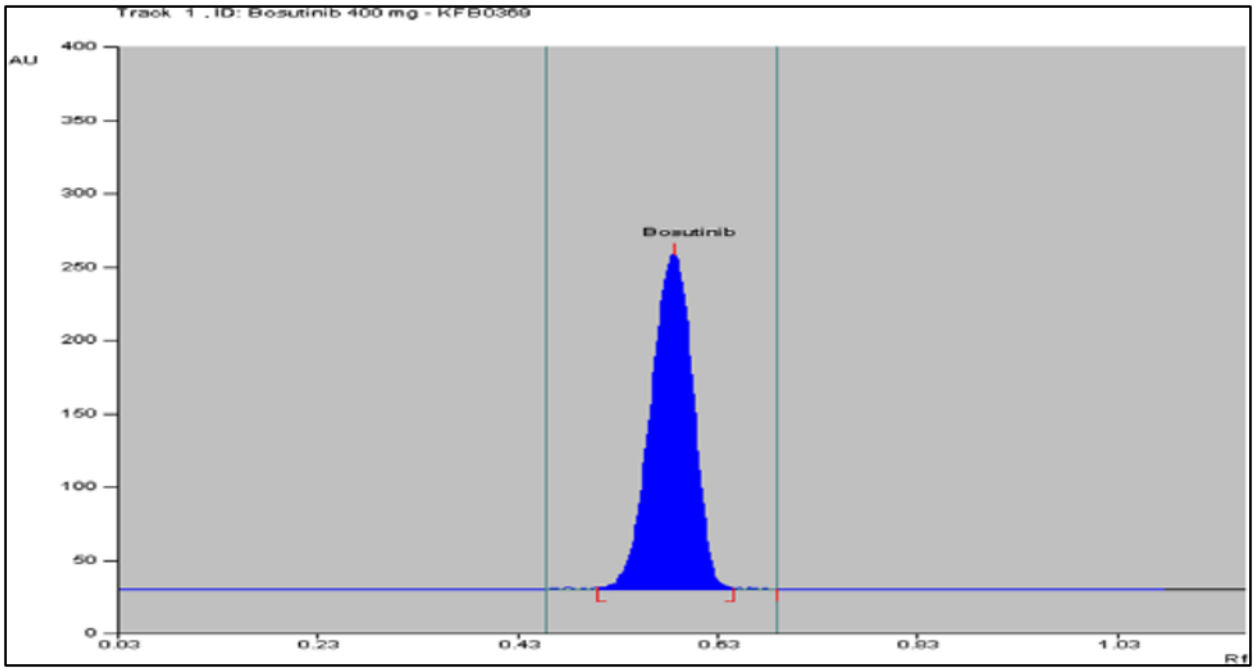


Figure 7 Batch BO 5 Chromatogram (7) Sat. Time – 30 mins detection at 267 nm

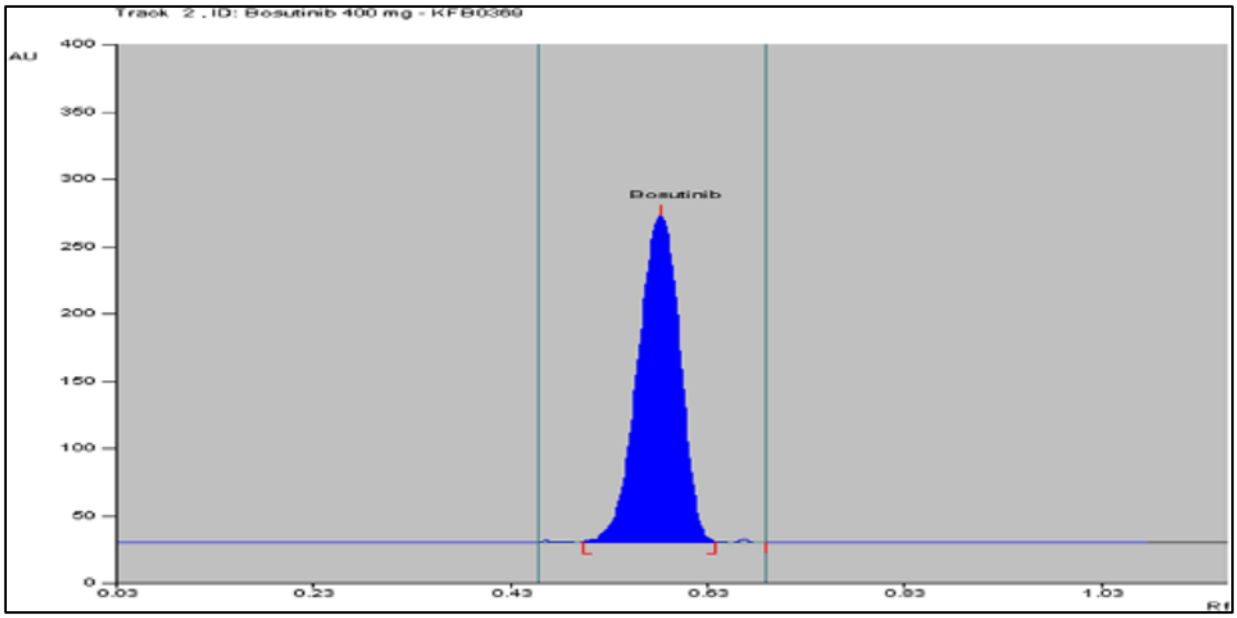


Figure 8 Batch BO 6 Chromatogram (7) Sat. Time – 30 mins detection at 267 nm

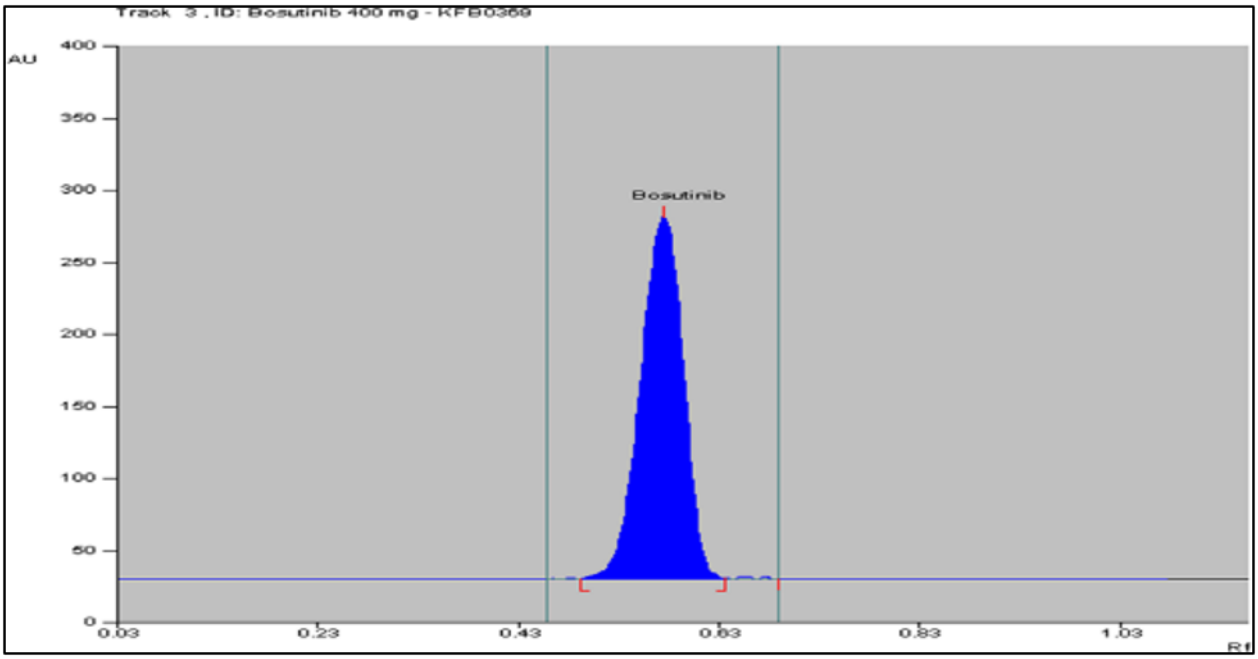


Figure 9 Batch BO 7 Chromatogram (7) Sat. Time – 30 mins detection at 267 nm

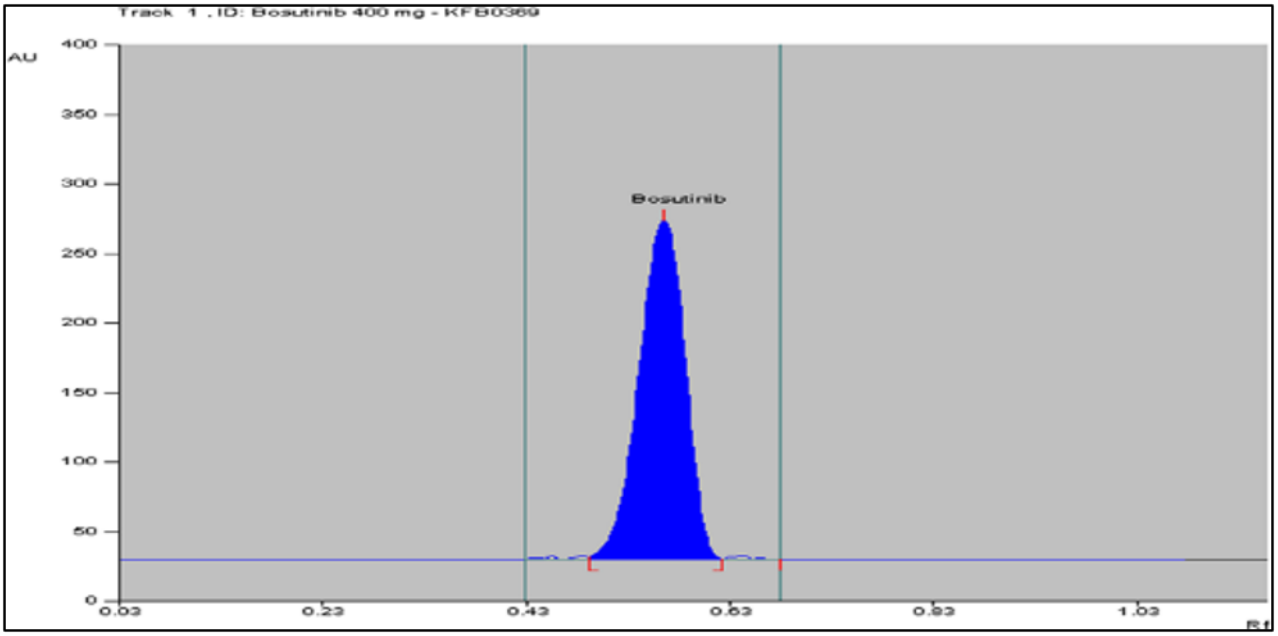


Figure 10 Batch BO 8 Chromatogram (7) Sat. Time – 35 mins detection at 269 nm

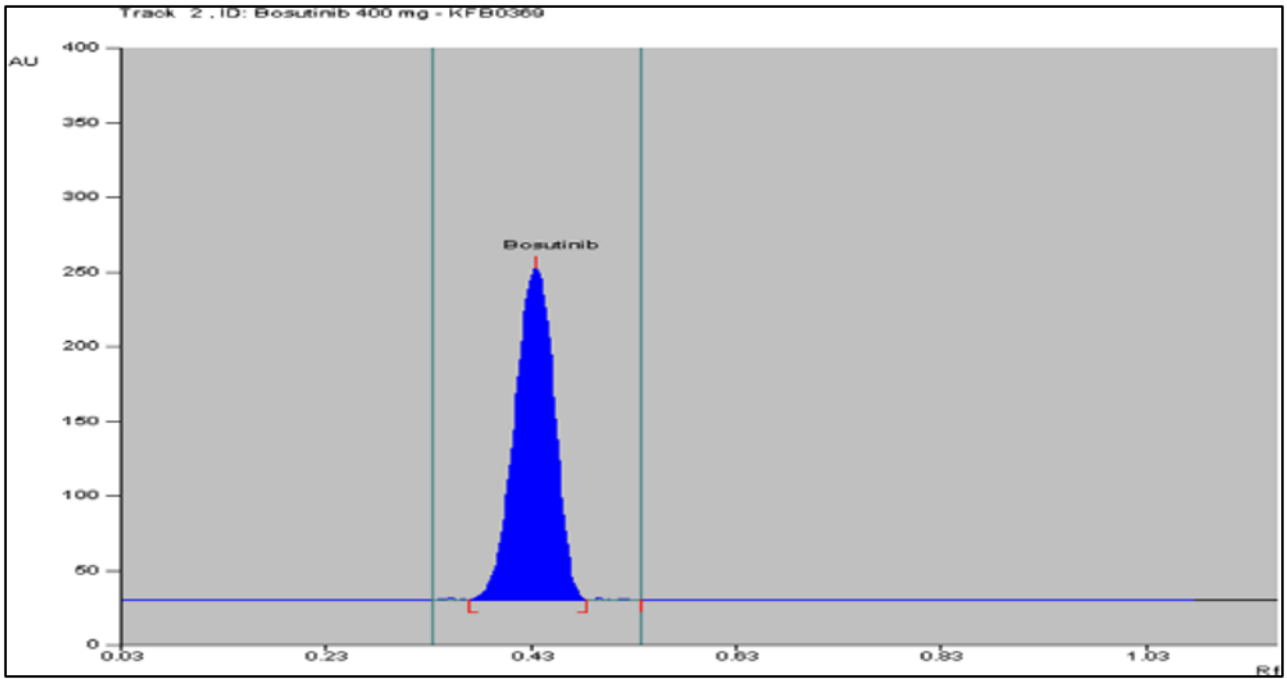


Figure 11 Batch BO 9 Chromatogram (7.5) Sat. Time – 25 mins detection at 265 nm

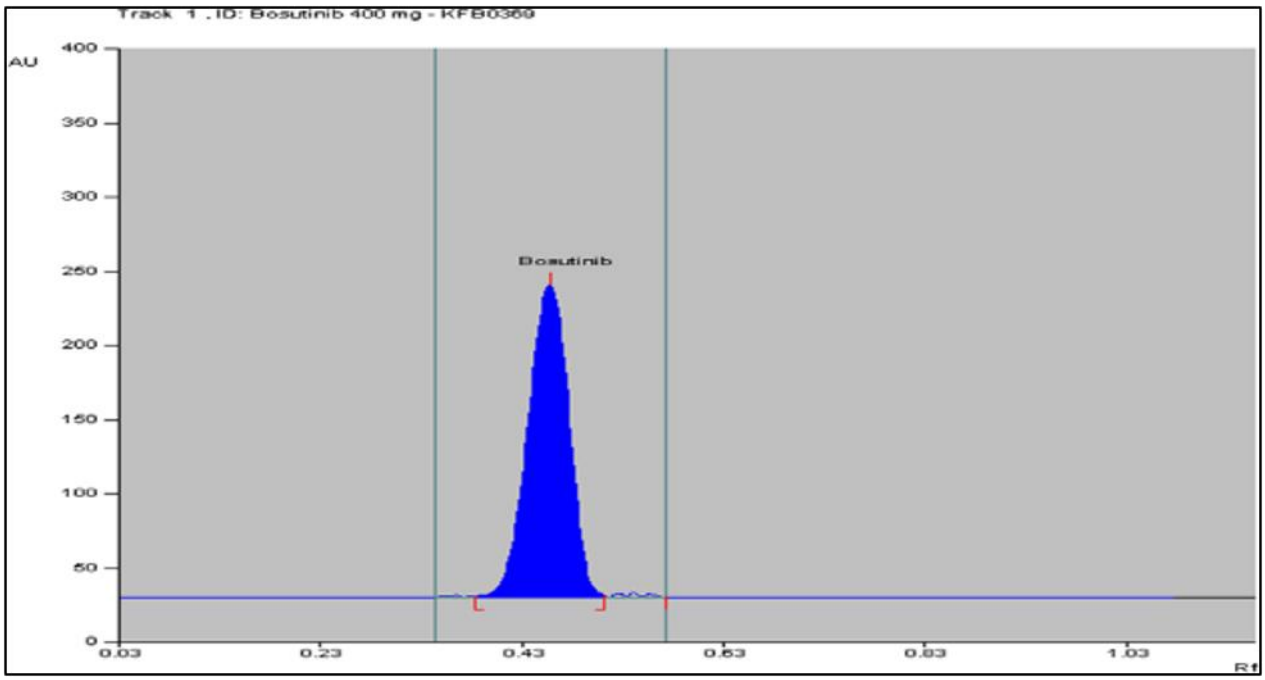


Figure 12 Batch BO 10 Chromatogram (7.5) Sat. Time – 30 mins detection at 267 nm

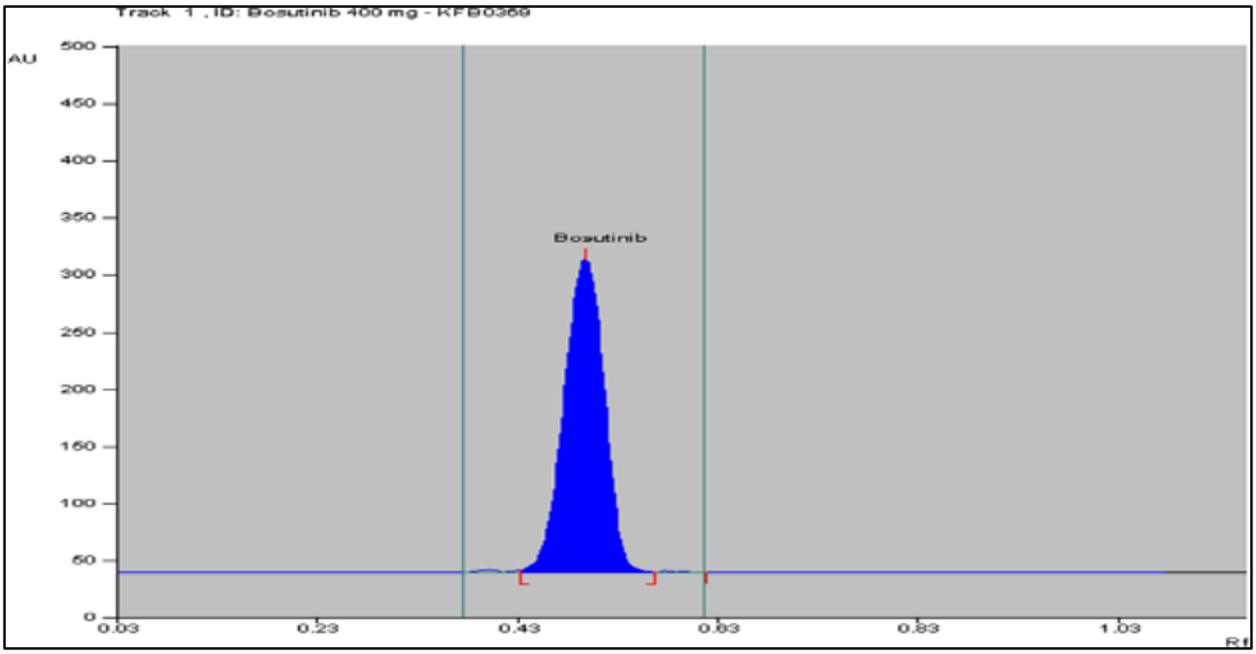


Figure 13 Batch BO 11 Chromatogram (7.5) Sat. Time – 35 mins detection at 269 nm

Table 3 Chromatographic Data Obtain from Experiment Design

Run	Factor-A Acetonitrile (ml)	Factor-B Saturation Time (min)	Factor-C Wavelength Detection (nm)	Response-1 Rf Value	Response-2 Peak Area
BO1	6.5	25	265	0.62	3482.1
BO2	6.5	30	267	0.63	3389.6
BO3	6.5	35	269	0.64	3782.8
BO4	7	25	265	0.59	5986.8
BO5	7	30	267	0.59	6882.0
BO6	7	30	267	0.59	7334.0
BO7	7	30	267	0.59	7664.5
BO8	7	35	269	0.57	7861.4
BO9	7.5	25	265	0.44	6068.9
BO10	7.5	30	267	0.46	6103.4
BO11	7.5	35	269	0.50	7853.9

Table 4 Evaluated Data for Design Expert Software

		Factor-A	Factor-B	Factor-C	Response 1	Response 2
Std	**Run**	**Acetonitrile (ml)**	**B: Saturation Time (min)**	**C: Wavelength Detection (nm)**	**Rf Value**	**Peak Area**
7	1	7	30	267	0.59	7664.5
2	2	6.5	30	267	0.63	3389.6

8	3	7	35	269	0.57	7861.4
4	4	7	25	265	0.59	5986.8
10	5	7.5	30	267	0.46	6103.4
1	6	6.5	25	265	0.62	3482.1
9	7	7.5	25	265	0.44	6068.9
11	8	7.5	35	269	0.5	7853.9
3	9	6.5	35	269	0.64	3782.8
5	10	7	30	267	0.59	6882
6	11	7	30	267	0.59	7334

3.7. Establishment of design space

Optimization of Various Parameters for Analysis of Bosutinib Using HPTLC (By Three Factor I – Optimal Design):

Table 5 Design Summary for Optimization

Study Type	**Response Surface**	**Subtype**	**Face Centered**
Design Type	Three Factor I – Optimal Design	Runs	11
Design Model	Quadratic	Blocks	No Blocks
Build Time (ms)	1.0000		

Table 6 Design Summary for Optimization

Factor Code	**Name**	**Units**	**Type**	**Minimum**	**Maximum**
A	Acetonitrile	ml	Numeric	6.5	7.5
B	Saturation Time	min	Numeric	25	35
C	Wavelength Detection	nm	Numeric	265	269

Table 7 Evaluation Degrees of Freedom of Design for optimization of analysis of Bosutinib by HPTLC

Response	**Name**	**Unit**	**Analysis**	**Minimum**	**Maximum**	**Ratio**	**Model**
R1	Rf Value	-	Polynomial	0.44	0.63	0.69	Quadratic
R2	Peak Area	-	Polynomial	3389.6	7861.4	4.31	Quadratic

3.8. Analysis of r1: rf value data analysis:

Table 8 Selection of Design Model by Analysis of (Rf Value)

Sequential Model Sum of Squares [Type I]						
Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob > F**	
Mean vs Total	3.52	1	3.52			Suggested
Linear vs Mean	0.041	2	0.020	28.72	0.0002	Aliased
2FI vs Linear	7.927E-004	2	3.964E-004	0.49	0.6357	Aliased
Residual	4.863E-003	6	8.106E-004			
Total	3.56	11	0.32			

3.8.1. "Sequential Model Sum of Squares [Type I]"

Select the highest order polynomial where the additional terms are significant and the model is not aliased.

3.8.2. "Model Summary Statistics"

Focus on the model maximizing the "Adjusted R-Squared" and the "Predicted R-Squared".

On the basis of Sequential Model Sum of Squares [Type I] and Model Summary Statistics, Quadratic model was selected for analysis of Rf Value of Bosutinib.

Table 9 Regression Statistics for different Rf Value

ANOVA for Quadratic Three Factor I-Optimal Design Model (Aliased)						
Analysis of variance table [Partial sum of squares - Type III]						
Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	0.045	5	8.979E-003	32.64	0.0008	significant
A-Acetonitrile	*0.040*	*1*	*0.040*	*145.47*	*< 0.0001*	
B-Saturation Time	*6.000E-004*	*1*	*6.000E-004*	*2.18*	*0.1997*	
C-Wavelength Detection	*0.000*	*0*				
AB	*4.000E-004*	*1*	*4.000E-004*	*1.45*	*0.2818*	
AC	*0.000*	*0*				
BC	*1.123E-005*	*1*	*1.123E-005*	*0.041*	*0.8479*	
A^2	*3.488E-003*	*1*	*3.488E-003*	*12.68*	*0.0162*	
B^2	*0.000*	*0*				
C^2	*0.000*	*0*				
Residual	1.375E-003	5	2.751E-004			
Lack of Fit	*1.375E-003*	*3*	*4.585E-004*			*not significant*
Pure Error	*0.000*	*2*	*0.000*			
Cor Total	0.046	10				

Factor coding is **Actual**.

Sum of squares is Type III – Partial.

The Model F-value of 32.64 implies the model is significant. There is only a 0.08% chance that an F-value this large could occur due to noise.

P-values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, A2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

A negative "Pred R-Squared" implies that the overall mean may be a better predictor of your response than the current model.

"Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 15.139 indicates an adequate signal. This model can be used to navigate the design space.

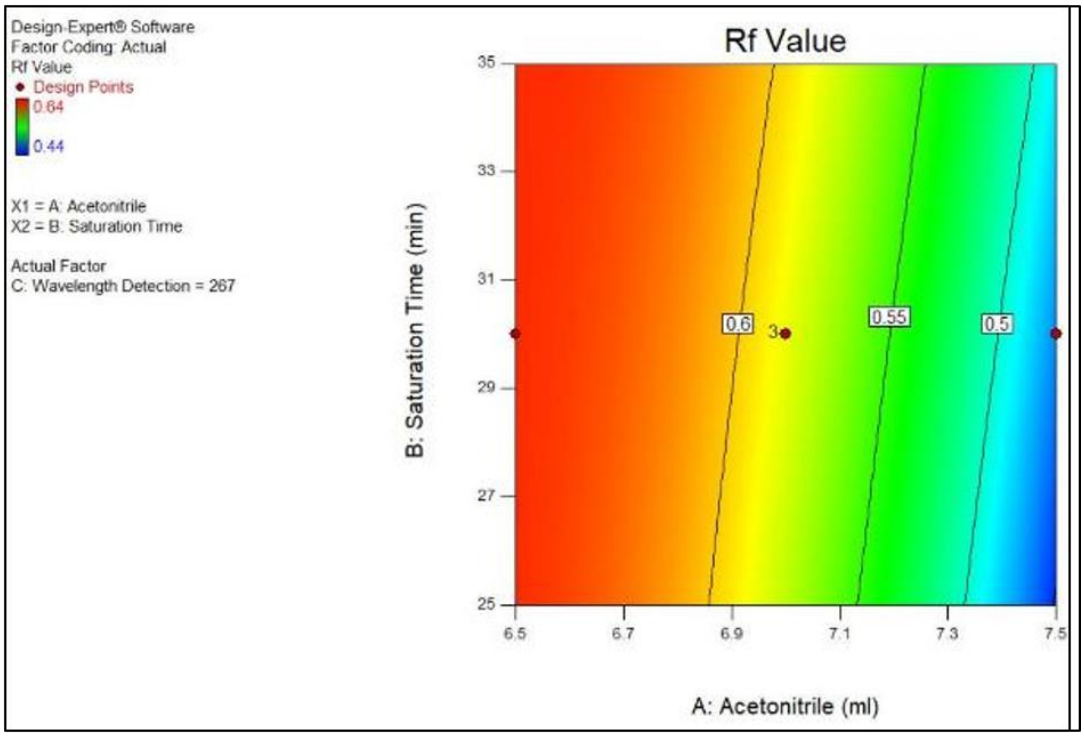


Figure 14 (A) Contour Plot of Rf Value

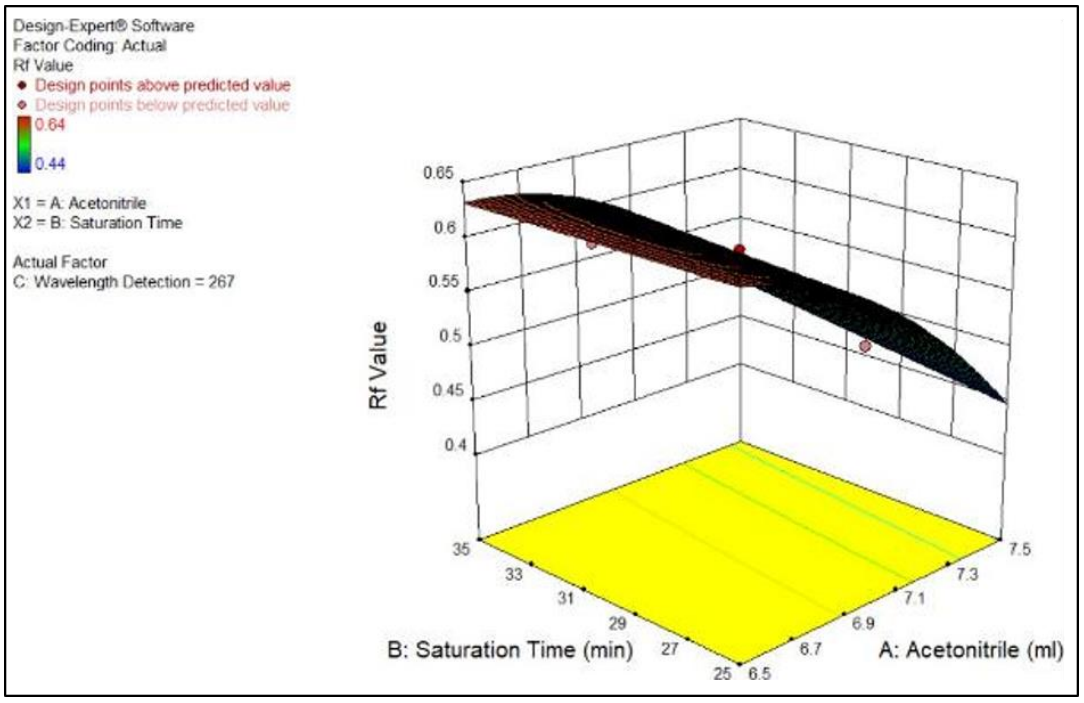


Figure 14 (B) 3D surface plot for Effect of Combination of factors on Rf Value of Bosutinib by using Three Factor I-Optimal Design

3.9. Analysis of r2: peak area data analysis

Table 10 Selection of Design Model by Analysis of R2 (Peak Area)

Sequential Model Sum of Squares [Type I]						
Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob > F**	
Mean vs Total	4.009E+008	1	4.009E+008			Suggested
Linear vs Mean	1.725E+007	2	8.626E+006	5.29	0.0344	Aliased
2FI vs Linear	1.068E+006	2	5.339E+005	0.27	0.7740	Aliased
Residual	1.198E+007	6	1.997E+006			
Total	4.312E+008	11	3.920E+007			

3.9.1. "Sequential Model Sum of Squares [Type I]"

Select the highest order polynomial where the additional terms are significant and the model is not aliased.

3.9.2. "Model Summary Statistics"

Focus on the model maximizing the "Adjusted R-Squared" and the "Predicted R-Squared".

On the basis of Sequential Model Sum of Squares [Type I] and Model Summary Statistics, Quadratic model was selected for analysis of Peak Area of Bosutinib.

Table 11 Regression Statistics for different Peak Area

ANOVA for Quadratic Three Factor I-Optimal Design Model (Aliased)						
Analysis of variance table [Partial sum of squares - Type III]						
Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob > F**	
Model	2.910E+007	5	5.820E+006	24.26	0.0016	significant
A-Acetonitrile	*1.464E+007*	*1*	*1.464E+007*	*61.01*	*0.0006*	
B-Saturation Time	*2.614E+006*	*1*	*2.614E+006*	*10.89*	*0.0215*	
C-Wavelength Detection	*0.000*	*0*				
AB	*5.508E+005*	*1*	*5.508E+005*	*2.30*	*0.1902*	
AC	*0.000*	*0*				
BC	*33339.47*	*1*	*33339.47*	*0.14*	*0.7246*	
A^2	*1.078E+007*	*1*	*1.078E+007*	*44.93*	*0.0011*	
B^2	*0.000*	*0*				
C^2	*0.000*	*0*				
Residual	1.200E+006	5	2.399E+005			
Lack of Fit	*8.911E+005*	*3*	*2.970E+005*	*1.92*	*0.3599*	*not significant*
Pure Error	*3.086E+005*	*2*	*1.543E+005*			
Cor Total	3.030E+007	10				

Factor coding is Actual;

Sum of squares is Type III – Partial.

The Model F-value of 24.26 implies the model is significant. There is only a 0.16% chance that an F-value this large could occur due to noise.

P-values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, A2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The "Pred R-Squared" of 0.7378 is in reasonable agreement with the "Adj R-Squared" of 0.9208; i.e. the difference is less than 0.2.

"Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 12.643 indicates an adequate signal. This model can be used to navigate the design space.

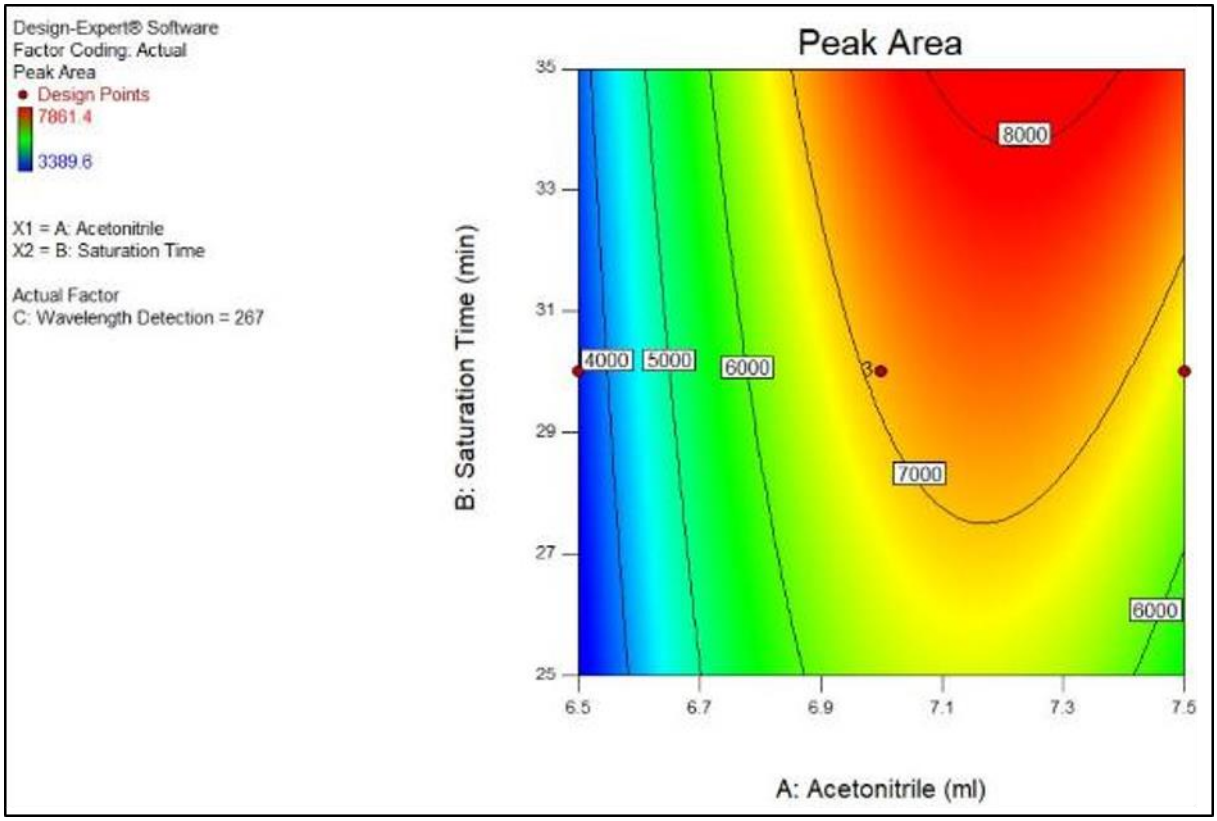


Figure 15 (A) Contour Plot of Peak Area

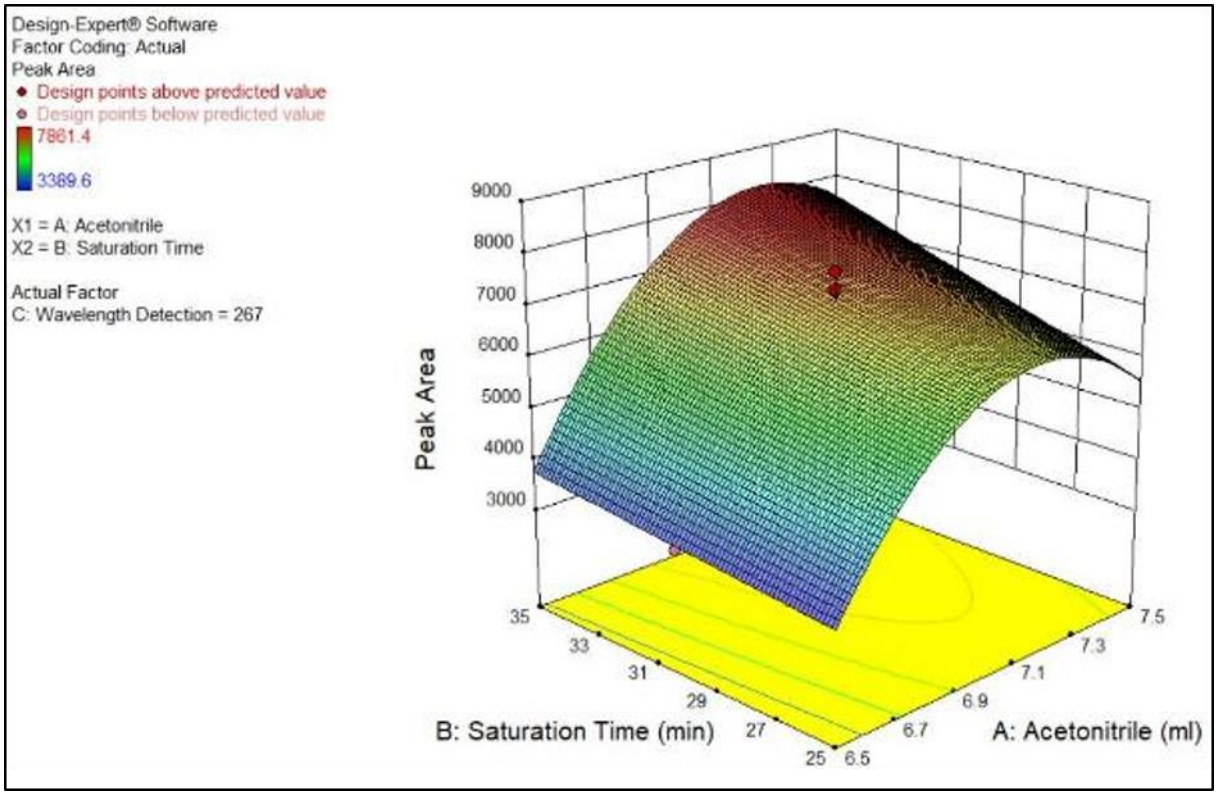


Figure 15 (B) 3D surface plot for Effect of Combination of factors on Peak Area of Bosutinib by using Three Factor I-Optimal Design

3.10. Prediction of optimized formulation

Table 12 Constraints for obtaining optimized formulation

Name	Goal	Lower Limit	Upper Limit
A: Acetonitrile (ml)	is in range	6.8	7.5
B: Saturation Time (min)	is in range	30	35
C: Wavelength Detection (nm)	is in range	266	268
Rf Value	is in range	0.44	0.64
Peak Area	maximize	7000	8000

Table 13 Obtained solution for optimized formulation

No.	Optimization	Acetonitrile (ml)	Saturation Time (min)	Wavelength Detection (nm)	Rf Value	Peak Area	Desirability
1	Actual	7.149	34.939	267.768	0.571	8187.490	1.000
2	Adjust. (≈)	7	35	268	0.57	8187	1.00

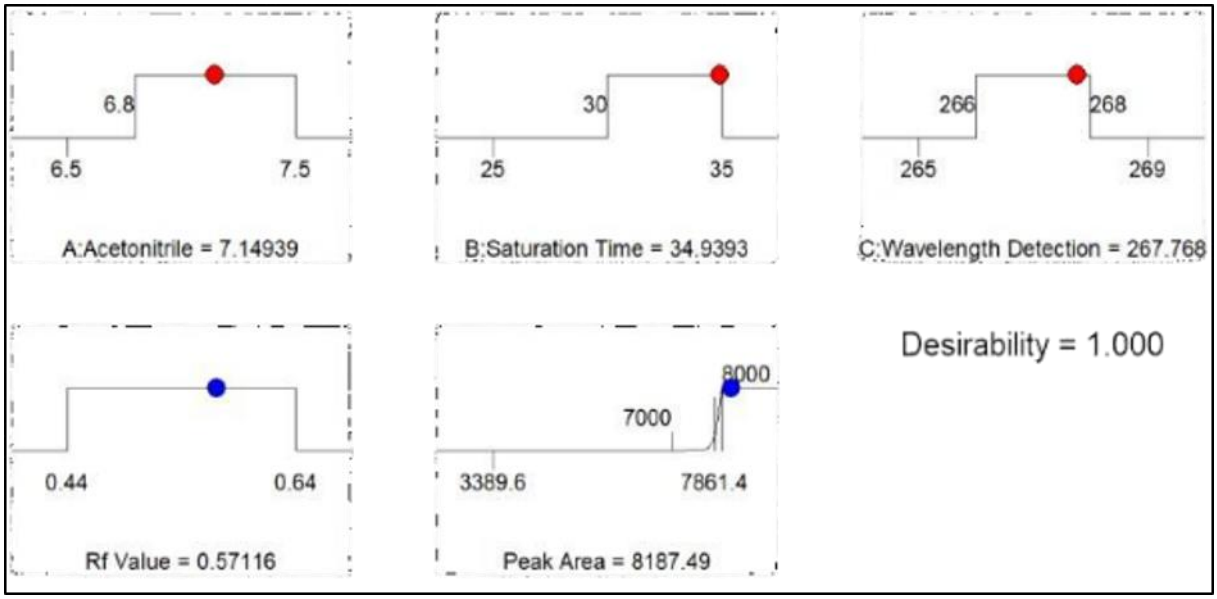


Figure 16 Solution Ramp According to Individual Responses

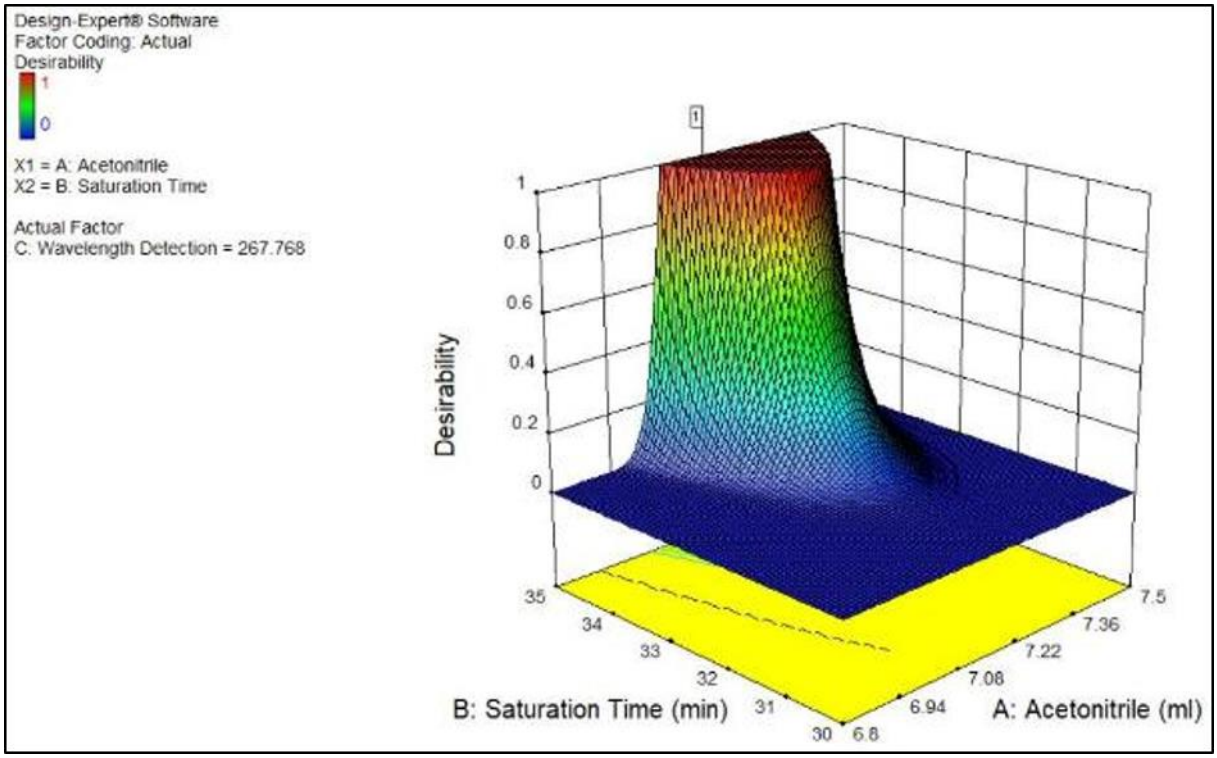


Figure 17 3D surface plot of Desirability for Obtaining Optimized Formulation

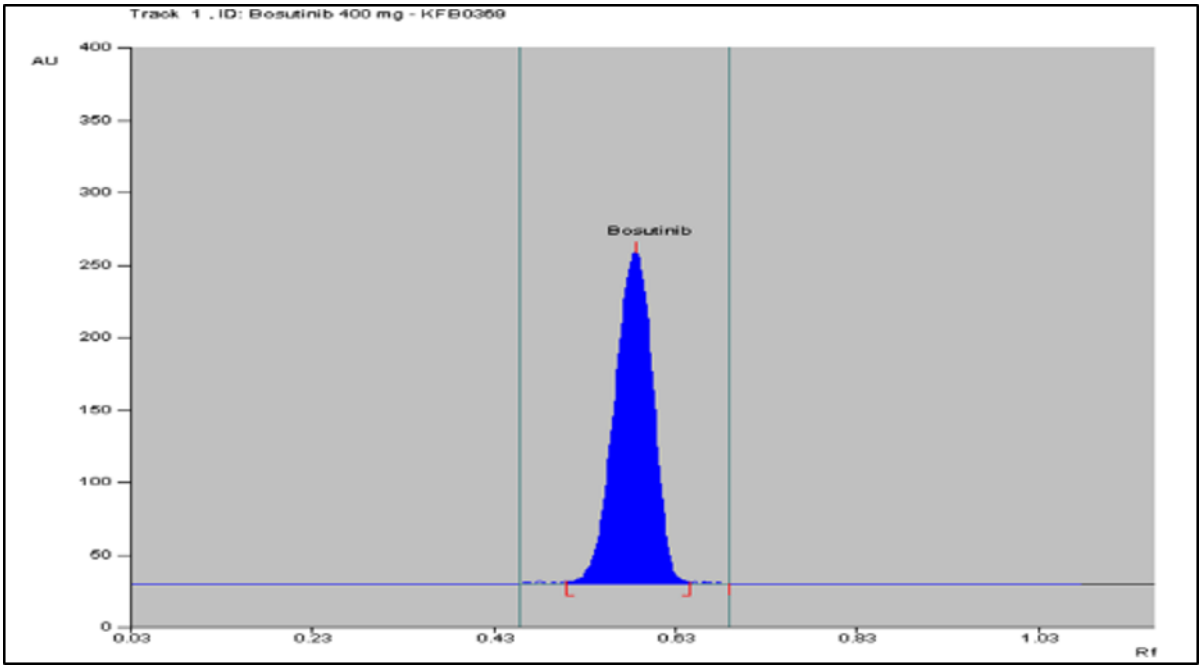


Figure 18 Chromatogram Obtained from the Optimized Formula (Acetonitrile 7: Water 3: Glacial Acetic Acid 0.5; Saturation Time – 35 mins; Conc. – 10 ng/spot)

Table 14 Data Comparison after Analysis

Response	Predicted value	Observed value	% Prediction Error
Rf Value	0.53 ± 5	0.57	7.55 %
Peak Area	7100 - 9600	8187	1.95 %

Table 15 Final Optimized Method Condition

Sr. No	Parameters	Results
1.	Equipment	HPTLC CAMAG, Switzerland-ATS 04-Vision CATS Software (Auto Sampler)
2.	Mobile phase	Acetonitrile 7: Water 3: Glacial Acetic Acid 0.5
3.	Detected wavelength	268 nm
4.	Saturation Time	35 mins

3.11. Validation of proposed hptlc method

After satisfactory development of method. The method was validated to demonstrate that it is suitable for its intended purpose by the standard procedure to evaluate adequate validation characteristics.

3.11.1. Linearity

Linearity was performed by diluting stock solution. To made 200-1200 ng/spot of Bosutinib. Each sample injected for 5 times for each concentration level and calibration curve was constructed by plotting the Peak area versus the Concentration.

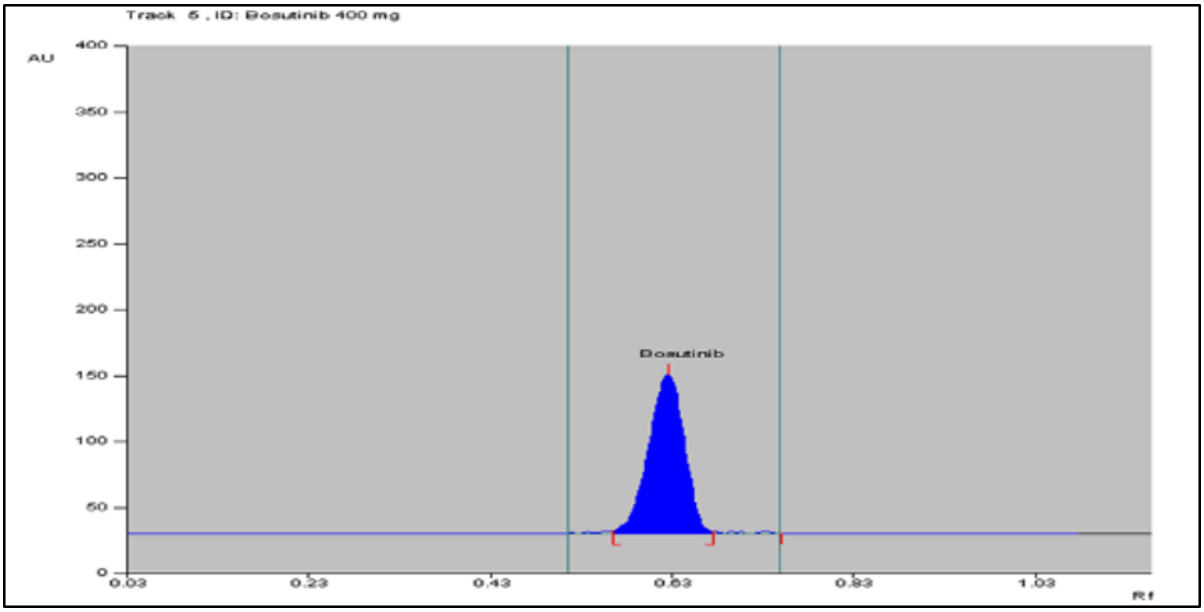


Figure 19 Chromatogram of 200 ng/spot of Bosutinib

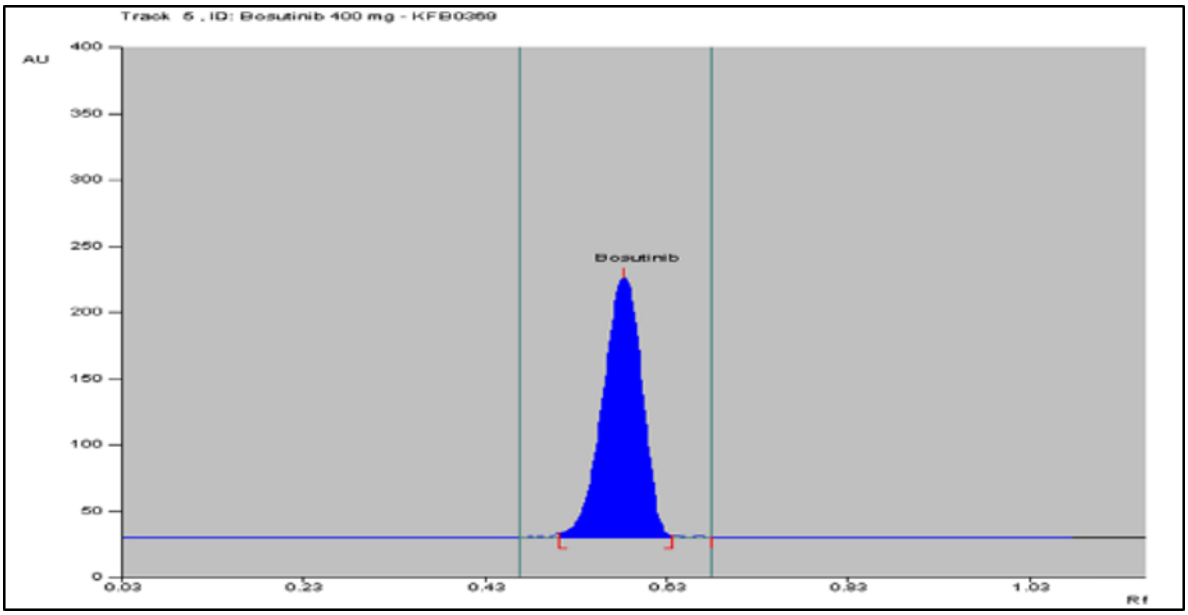


Figure 20 Chromatogram of 400 ng/spot of Bosutinib

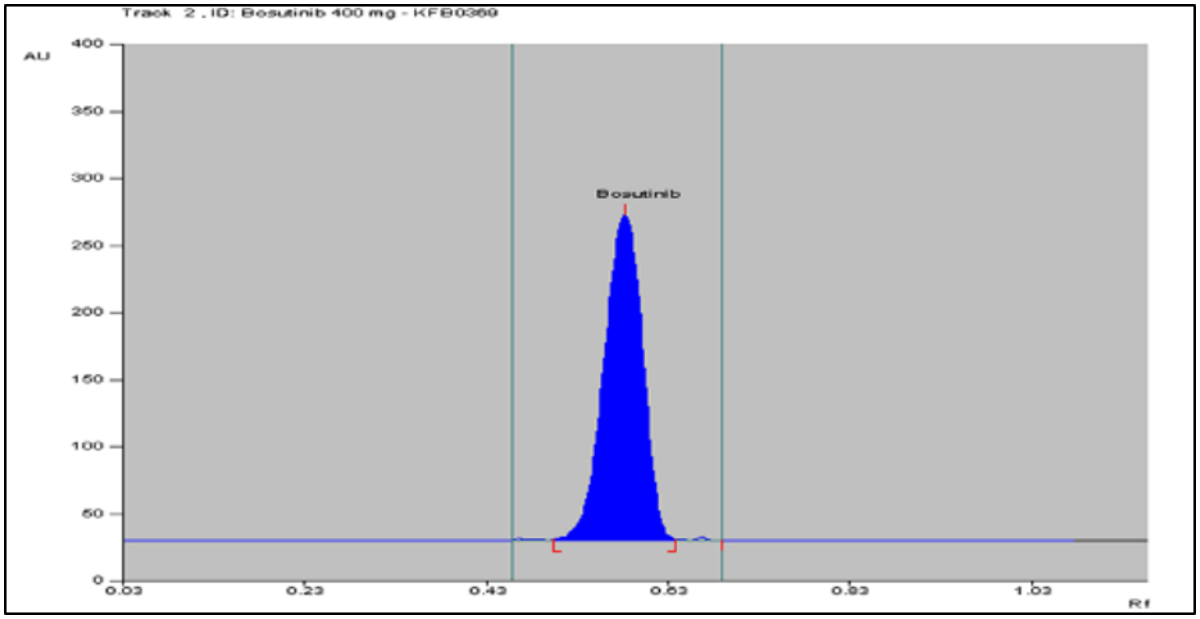


Figure 21 Chromatogram of 600 ng/spot of Bosutinib

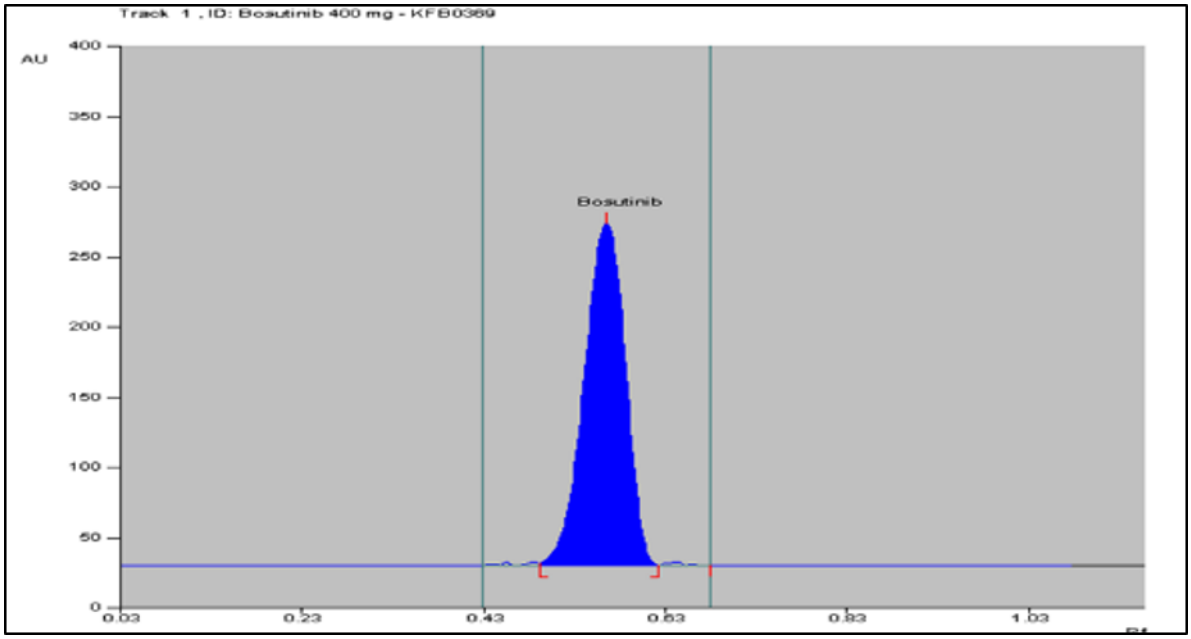


Figure 22 Chromatogram of 800 ng/spot of Bosutinib

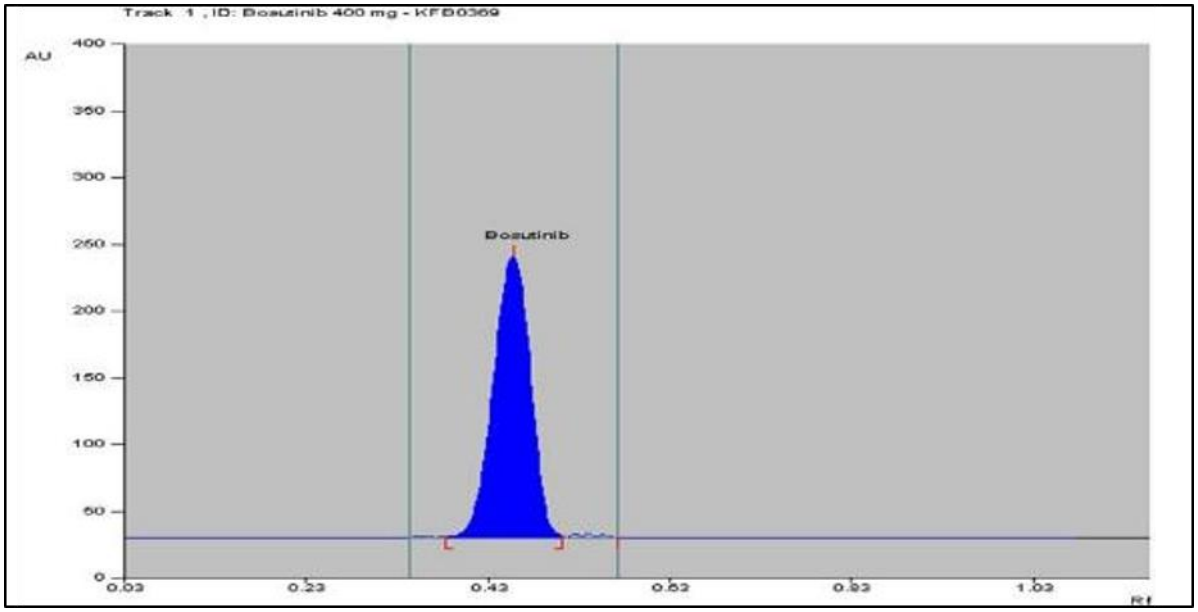


Figure 23 Chromatogram of 1000 ng/spot of Bosutinib

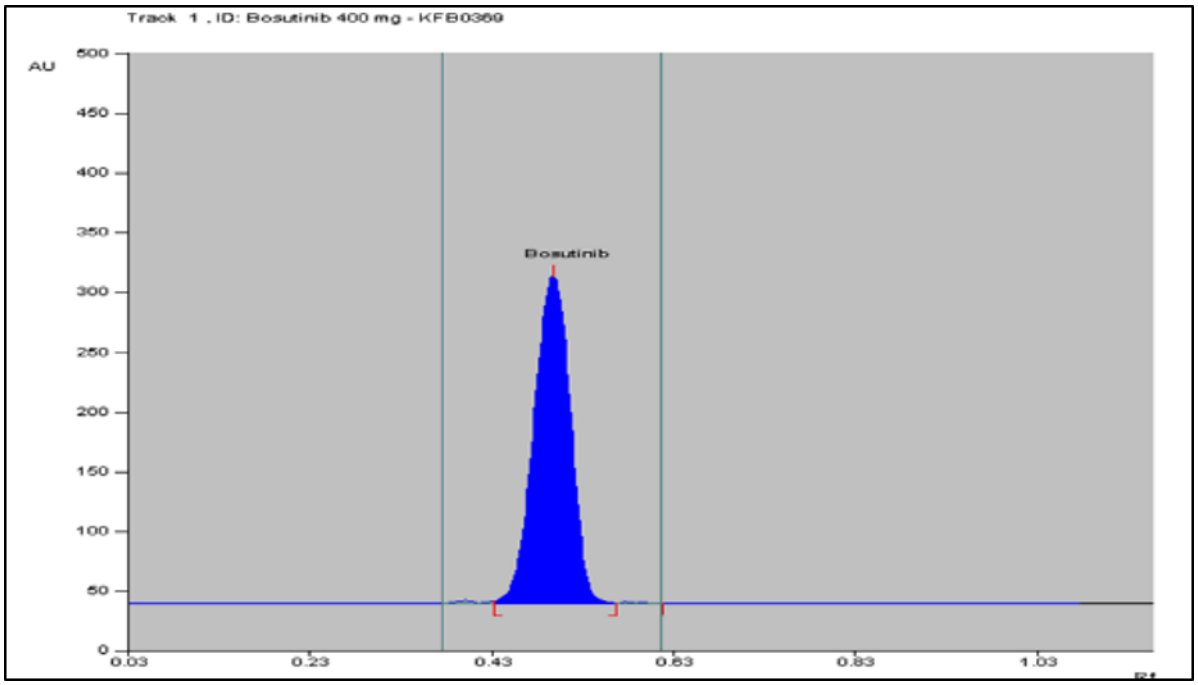


Figure 24 Chromatogram of 1200 ng/spot of Bosutinib

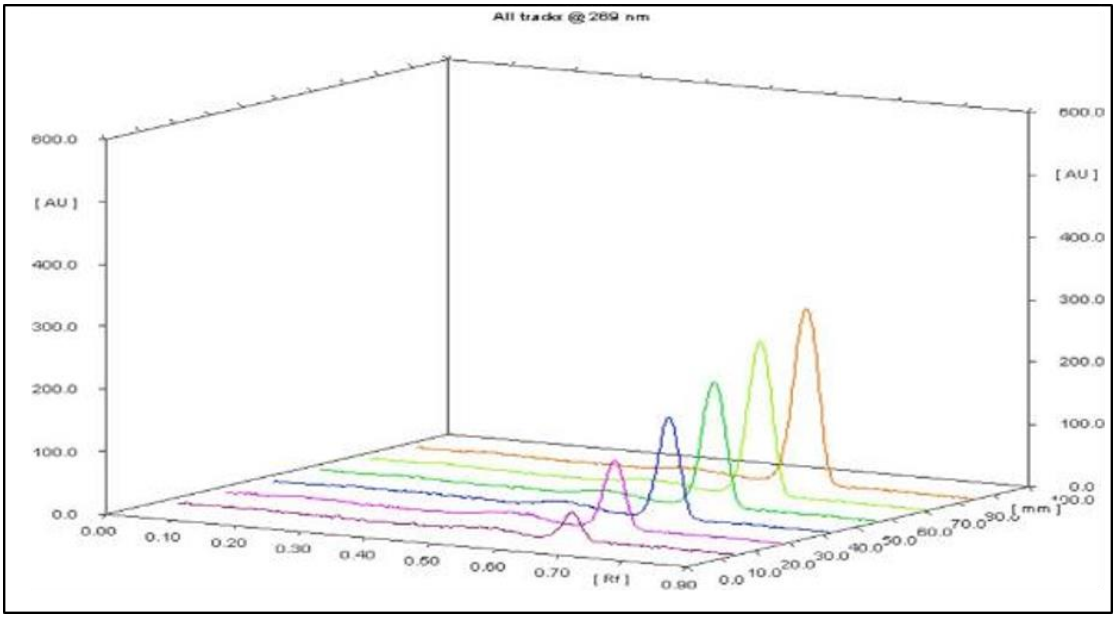


Figure 25 Overlay Chromatogram of Linearity of Bosutinib in 200-1200 ng/spot

Table 16 Linearity for Bosutinib

Sr. No.	Concentration (ng/spot)	Peak Area
1	200	644.8
2	400	1380.1
3	600	2105.5
4	800	2756.6
5	1000	3482.1
6	1200	3929.0

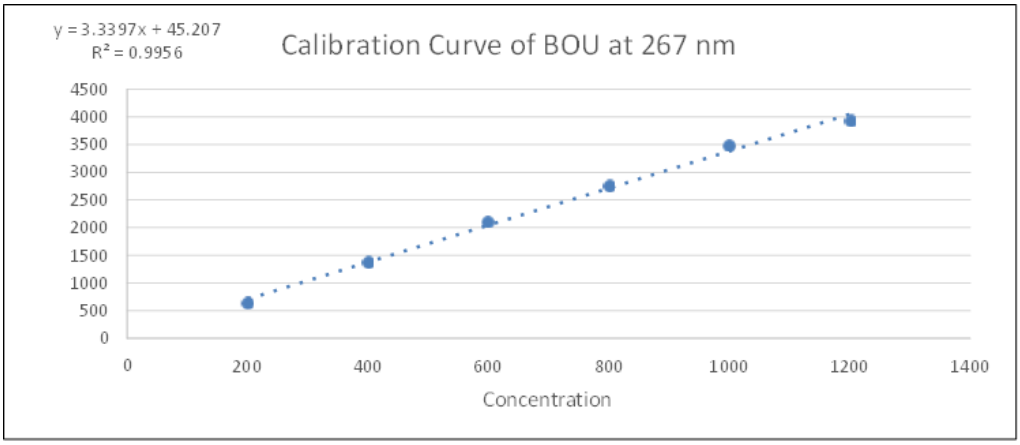


Figure 26 Calibration Curve for Bosutinib (200-1200 ng/spot)

3.12. Precision

The injection (system) precision was evaluated by performing six replicate injections for Repeatability and three replicates for intermediate of the standard Bosutinib. The procedure for Repeatability and interday precision and intra-day (Intermediate) was established by performing replicate assays.

3.13. Repeatability

The data for repeatability of peak area measurement for Bosutinib of 1200 ng/spot at 267 nm based on six-time measurement.

Limit: %RSD for area NMT 2.0%

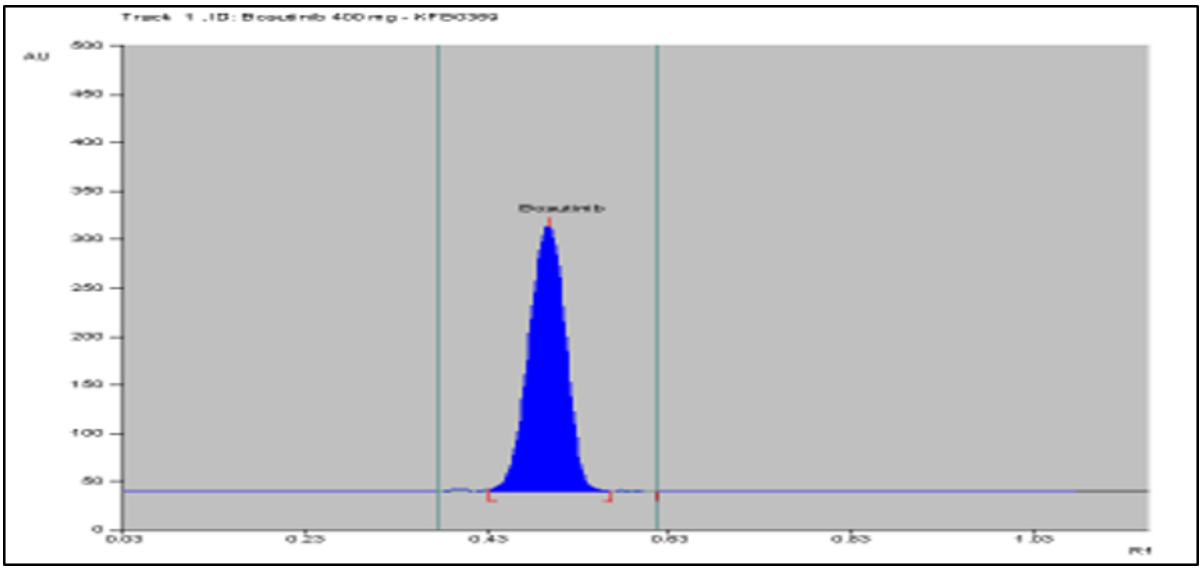


Figure 27 Chromatogram of Repeatability of 1200 ng/spot of Bosutinib

Table 17 Data for Repeatability of Bosutinib

Sr. No.	Concentration (ng/spot)	Area	Mean	SD	%RSD
1	1200	3929.0	3928.78	11.59	0.29
2	1200	3918.3			
3	1200	3940.6			
4	1200	3925.7			
5	1200	3943.9			
6	1200	3915.2			

3.13.1. Interday precision

- The results for interday precision for Bosutinib are presented in Table.
- The % RSD for interday Bosutinib was found to be 0.18 – 1.02 for Bosutinib.
- The % RSD for drug was found to be less than 2, which indicates the method is precise.

3.13.2. Intraday precision:

- The results for intraday precision for Bosutinib are presented in Table.
- The % RSD for intraday precision was found to be 0.18 – 1.03 for Bosutinib.
- The % RSD for drug was found to be less than 2, which indicates the method is precise.

Table 18 Precision for Bosutinib

Sr. No.	Precision Period	Concentration (ng/spot)	Mean	SD	%RSD
1	Interday Precision	200	644.87	6.60	1.02
		600	2105.33	5.15	0.24
		1200	3930.60	6.94	0.18
2	Intraday Precision	200	644.17	6.65	1.03
		600	2105.70	5.62	0.27
		1200	3929.10	7.11	0.18

3.14. Accuracy

It was done by recovery study. Sample solutions were prepared by spiking at about 50%, 100 % and 150 % of specification limit to Placebo and analyzed by the proposed HPTLC method.

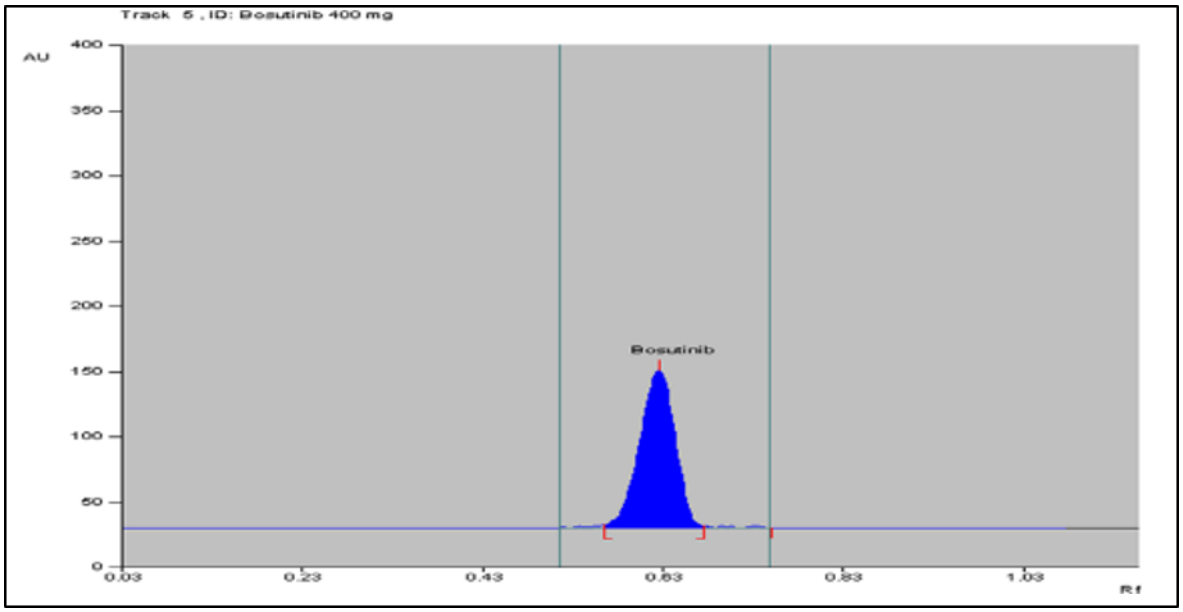


Figure 28 Chromatogram of Recovery 50%

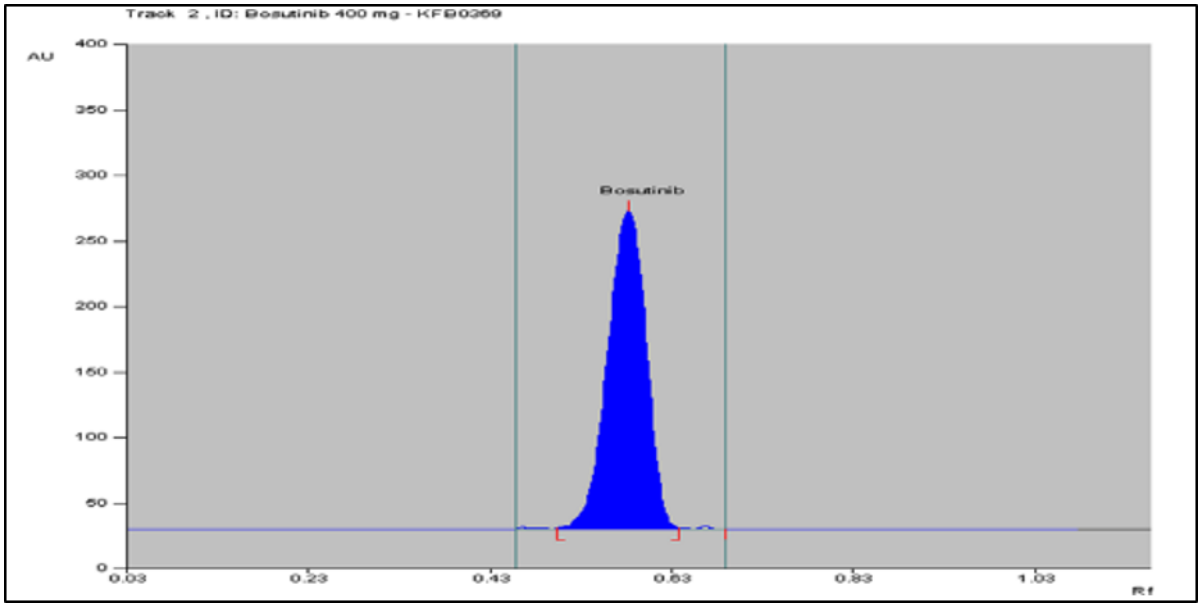


Figure 29 Chromatogram of Recovery 100%

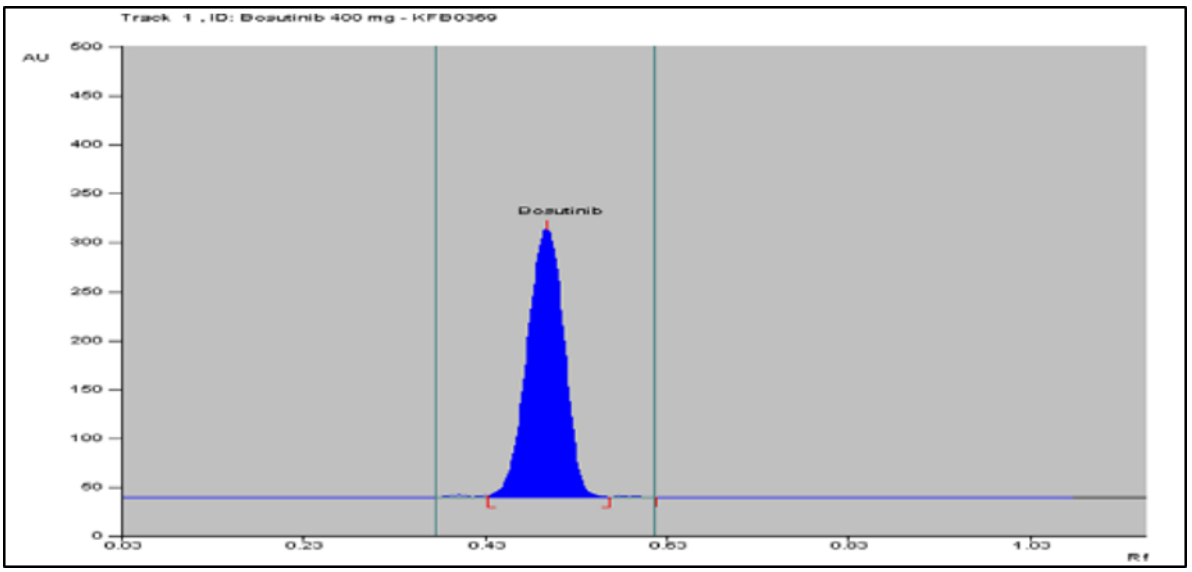


Figure 30 Chromatogram of Recovery 150%

Table 19 % Recovery of Bosutinib

Amt from Drug (ng/spot)	Amt from API (ng/spot)	TOTAL CONC (ng/spot)	Area	CONC (ng/spot)	Mean (ng/spot)	SD	%Assay
400	0	400	1380.1	396	396.33	1.53	99.08%
400	0	400	1392.5	398			
400	0	400	1368	395			

Table 20 Spiking of Bosutinib

Spike Level (%)	Base Conc (ng/spot)	Conc Spiked (ng/spot)	Total Theoretical (ng/spot)	Total Conc Found (ng/spot)	Amt of API Recovered (ng/spot)	Mean (ng/spot)	SD	% Recovery
50	400	200	600	607	207	209.33	2.52	104.67
				612	212			
				609	209			
100	400	400	800	814	414	414	2.00	103.5
				816	416			
				812	412			
150	400	600	1000	1029	629	626	3.00	104.33
				1026	626			
				1023	623			

3.15. LOD and LOQ:

3.15.1. LOD:

Analyte must reliably differentiate from background noise. It is the lowest concentration of analyte in a sample that can be detected but not necessarily quantified.

3.15.2. LOQ:

The limit of Quantitation is the minimum injected amount that gives precise measurements, in chromatography typically requiring peak heights 10 times higher than baseline noise.

Table 21 LOD and LOQ of Bosutinib

Parameters	Results
	BOU
Mean slope of the calibration curves	3.34
Standard deviation of the Y-intercepts of the calibration; (n=3) S	12.31
LOD (ng/spot)	12.17
LOQ (ng/spot)	36.88

3.16. Robustness

The robustness of the method was evaluated using 1200 ng/spot of Bosutinib;

- By Change in Mobile Phase Ratio (ACN 7: Water 3: GAA 0.5) (ACN= 7%V ± 0.5%V):

6.5: 3.5: 0.5,

7.5: 2.5: 0.5

- By Change in Saturation Time (30 min ± 5 min):

25 min

35 min

- By Change in Wavelength (267 nm ± 2 nm):

265 nm

269 nm

Table 22 Robustness for Bosutinib

Sr. No.	Parameter	Mean	SD	%RSD
Change in Mobile Phase Ratio (ACN 7: Water 3: GAA 0.5) (ACN= 7%V ± 0.5%V)				
1	6.5:3.5:0.5 (V/V/V)	2104.83	5.73	0.27
2	7.5:2.5:0.5 (V/V/V)	2112.03	3.76	0.18
Change in Saturation Time (30 min ± 5 min)				
1	25 min	2104.27	4.2	0.20
2	35 min	2107.13	4.11	0.20
Change in Wavelength (267 nm ± 2 nm)				
1	265 nm	2045	6.2	0.30
2	269 nm	2155.03	5.64	0.26

3.16.1. SPECIFICITY and SELECTIVITY

Placebo formulation samples yielded clean chromatograms with no interference from the tablet excipients. The chromatograms of Standard and Sample were same identical with almost same retention time.

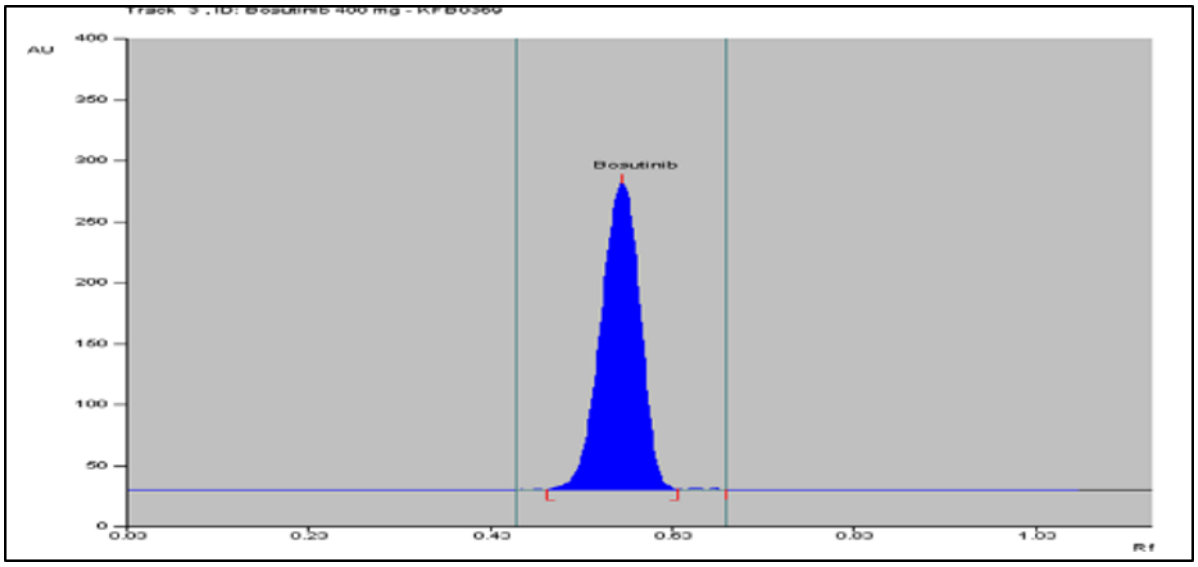


Figure 31 Chromatogram of Standard for Specificity and Selectivity

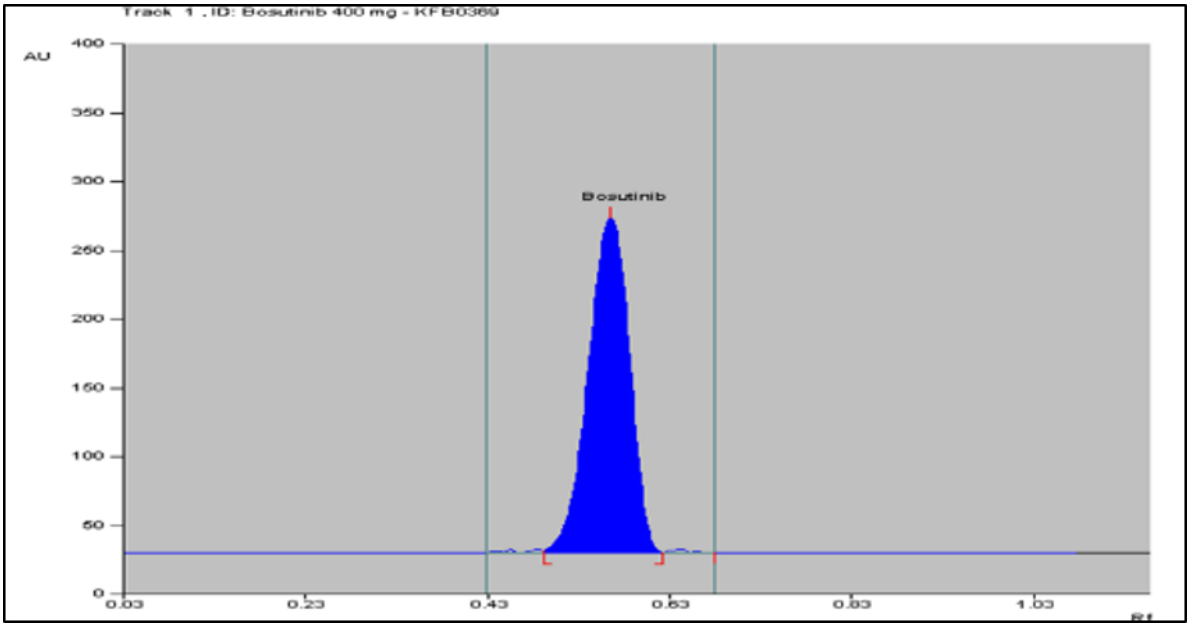


Figure 32 Chromatogram of Sample for Specificity and Selectivity

3.17. Assay

The proposed method was successfully applied to the marketed tablet dosage form. Results are shown in Table. The corresponding chromatograms for standard and Marketed Formulation are shown in Figure respectively.

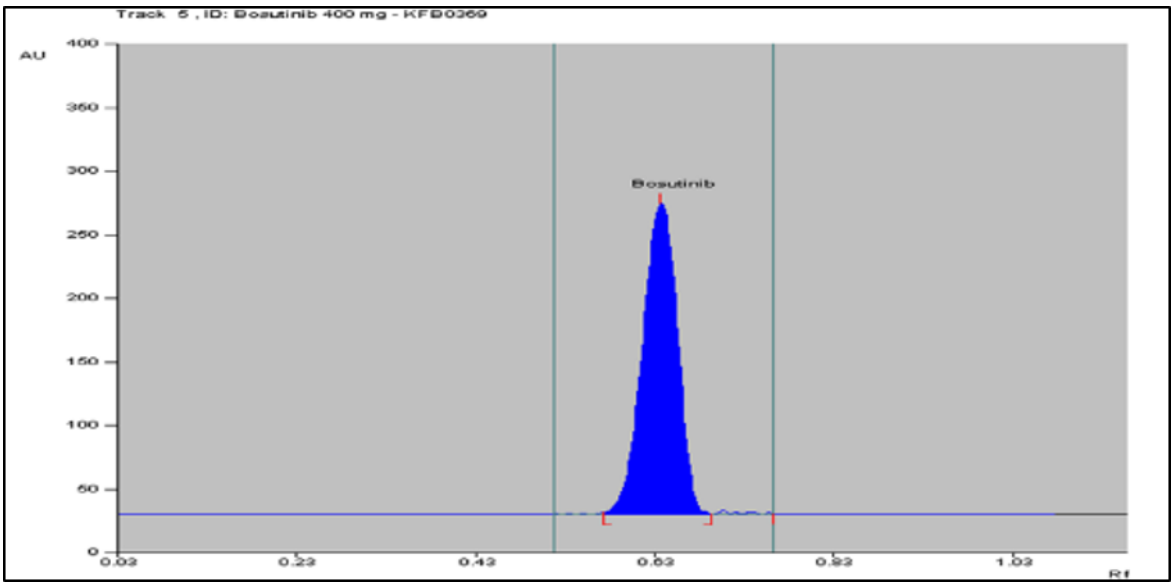


Figure 33 Chromatogram of standard of 200 ng/spot of Bosutinib

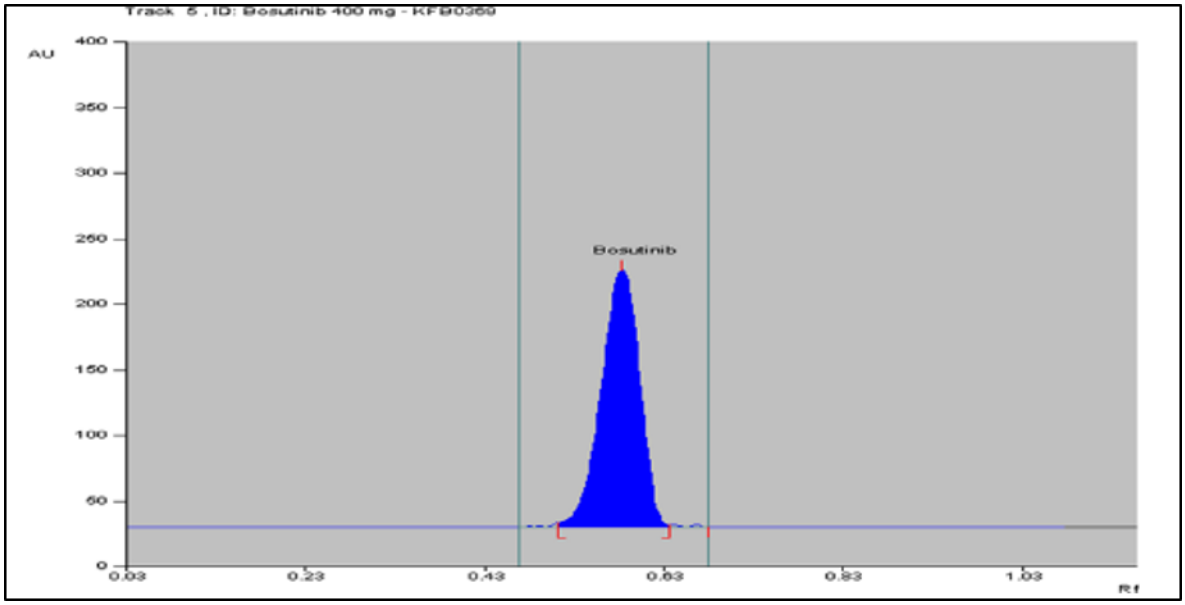


Figure 34 Chromatogram of marketed formulation of 200 ng/spot of Bosutinib

Table 23 Assay of Bosutinib

Sr. No.	Label Claim (mg)	Found (mg)	Mean (mg)	SD	%Assay	%RSD
1	400.00	408.50	407.20	4.40	101.80	1.08
2		402.30				
3		410.80				

3.18. Summary of validation parameters for bosutinib:

Table 24 Summary of Validation Parameter

PARAMETER	RESULT
Linearity and Range (ng/spot)	200 – 1200
Correlation Coefficient R^2	0.99
Regression Equation	Y= 3.3397x + 45.207
Repeatability (%RSD)	0.29
Inter-Day Precision (%RSD)	0.18 - 1.02
Intra-Day Precision (%RSD)	0.18 – 1.03
Accuracy (%RSD)	99.08 %
LOD (ng/spot)	12.17
LOQ (ng/spot)	36.88
Robustness (%RSD)	0.18 – 0.30
% Assay	101.80 ± 1.08 %

3.19. Greenness Assessment of the Developed HPTLC Method: (AGREE Score = 0.57)

Table 25 Analytical Eco-Scale penalty point assessment for the developed HPTLC method

Parameter	Observation in method	Penalty points
Acetonitrile as major solvent	Toxic, flammable organic solvent	8
Glacial acetic acid	Corrosive reagent	4
Solvent consumption (20 mL/run)	Between 10–20 mL	4
Waste generated (20 mL/run)	Organic solvent waste	4
Chamber saturation time (35 min)	Moderate energy/time usage	2
Occupational exposure	Organic vapors and acidic fumes	3
Total penalty points		25

3.20. Eco-Scale calculation

Eco-Scale score = 100 − 25 = **75**

3.20.1. Interpretation

An Eco-Scale score of 75 indicates that the developed HPTLC method can be considered a green analytical method, showing better environmental compatibility compared to the corresponding HPLC method due to lower solvent and energy requirements [13-17].

The greenness of the developed HPTLC method was evaluated using the AGREE based on the 12 principles of Green Analytical Chemistry. The method achieved an AGREE score of 0.57, indicating a reasonable degree of compliance for a chromatographic technique employing organic solvents [13-17].

Higher compliance was observed for principles related to minimal sample size, absence of derivatization, simple sample preparation, and low energy requirement due to UV-based detection at 267 nm. The HPTLC technique also benefits from lower instrumentation energy demand compared to HPLC.

The score is influenced by the use of acetonitrile as the primary mobile phase component, generation of approximately 20 mL of solvent waste per run, and the 35-minute chamber saturation time, which contribute to the environmental impact. The assessment highlights that, although the method is analytically effective, further improvement in greenness can be achieved by reducing solvent volume or replacing acetonitrile with greener alternatives [13-17].

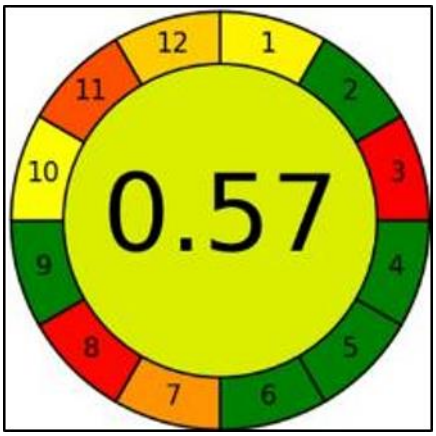


Figure 35 AGREE greenness evaluation chart for the developed HPTLC method

4. Conclusion

The present study successfully developed and validated a Quality by Design (QbD) assisted HPTLC method for the quantitative estimation of Bosutinib in pharmaceutical dosage form. Application of Three Factor I-Optimal Design enabled systematic optimization of critical chromatographic variables, including mobile phase composition, saturation time, and detection wavelength, resulting in a robust analytical design space.

The optimized chromatographic condition using acetonitrile 7: water 3: glacial acetic acid 0.5 with densitometric detection at 267 nm produced well-resolved, compact bands with an Rf value around 0.6. The method exhibited excellent linearity over 200–1200 ng/spot (R^2 = 0.9956) with satisfactory sensitivity (LOD 12.17 ng/spot and LOQ 36.88 ng/spot).

Validation studies confirmed that the method is precise, accurate, robust, and specific, with %RSD values below 2%, recovery within 99–104%, and assay results of 101.80 ± 1.08 % for marketed formulation. Robustness evaluation demonstrated minimal effect of small deliberate variations, supporting the reliability of the method for routine analysis.

Greenness assessment revealed an AGREE score of 0.57 and Eco-scale score of 75, indicating acceptable environmental sustainability with advantages of reduced solvent consumption, minimal sample preparation, and lower energy requirement compared to conventional chromatographic techniques.

Overall, the developed HPTLC method is simple, economical, environmentally acceptable, and suitable for routine quality control analysis of Bosutinib, while the QbD approach ensures enhanced method understanding, robustness, and regulatory flexibility.

Compliance with ethical standards

Disclosure of conflict of interest

We all authors declare that we have no conflicts of interest or competing interests to disclose regarding the publication of this manuscript or any institution, product, or entity mentioned within. Furthermore, we have no affiliations or financial interests in products or organizations that could influence the study outcomes presented or compete with those discussed in the manuscript.

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