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VALIDATED RP-HPLC ASSAY METHOD FOR DETERMINATION OF ACALABRUTINIB IN PHARMACEUTICAL FORMULATION BY QBD APPROACH

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Acalabrutinib, QbD Approach, Method development, RP-HPLC

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ABSTRACT: A method by using a quality-by-design approach for the development and validation of an RP-HPLC method for determination of Acalabrutinib in API and marketed formulation. Chromatogram was run through SunFire C18 Column, 100Å, 10 µm, 4.6 mm X 150 mm. Mobile phase containing 0.1% OPA: Ethanol and water (50:50) taken in the ratio 60: 40 (%v/v) was pumped through the column at a flow rate of 1.0 ml/min. Temperature was maintained at 30°C. The optimized wavelength selected was 220nm. The retention time of Acalabrutinib was found to be 2.343 min %RSD of the Acalabrutinib was found to be 1.0%. %RSD of Method precision of Acalabrutinib was found to be 0.3%. %Recovery was obtained as 100.47% for Acalabrutinib. LOD, LOQ values obtained from the regression equation of Acalabrutinib were 0.28, 0.86. The regression equation of Acalabrutinib is y = 17109x + 5534.8. The method was easy to use and cost-effective, making it suitable for frequent quality control testing in industries. Both retention times and run times were reduced. Results which were obtained from the validation of the developed analytical method were within the limit as per ICH guidelines.

INTRODUCTION: The chemical name of Acalabrutinib is 4-[8 – amino – 3 - [(2S) – 1 - but-2-ynoylpyrrolidin-2yl] imidazo[1,5-a] pyrazin-1-yl]-N- pyridin - 2 - ylbenzamide. The molecular formula of Acalabrutinib is $C_{26}H_{23}N_7O_2$ and its molecular weight is 465.517g/mol. Acalabrutinib is a white to yellow powder with pH-dependent solubility. It is essentially insoluble at pH levels above 6 and freely soluble in water at pH values below 3 [1]. Acalabrutinib is a highly selective Bruton's tyrosine kinase inhibitor, is associated with high overall response rates, and is used for treating chronic lymphocytic leukemia and mantle cell lymphoma (MCL).

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One uncommon subtype of B-cell non-Hodgkin lymphomas (NHLs) is mantle cell lymphoma (MCL). This medicine is also used to treat chronic lymphocytic leukemia (CLL) or small lymphocytic lymphoma (SLL) [2-3]. Analytical Quality by Design (AQbD) is a systematic approach to design the methods that start with defining the separation goals and target method profile. The main AQbD focus areas are comprehension of method parameters and controls, founded on reliable science and quality risk management.

Along with other elements including process parameters, material attributes, equipment operating conditions, in-process controls, and finished product specifications, a QbD is also a crucial component of the product development control plan. Regulatory agencies do not define any specific process of AQbD, however, a parallel approach can be drawn based on product QbD e.g., Quality target product profile (QTPP) can be

inferred as Quality target method profile (QTMP), CQA can be interpreted as critical quality attributes such as tailing factor, the resolution between adjacent peaks, and plate count, *etc*. Design space can be called method operable design range (MODR) [4-8].

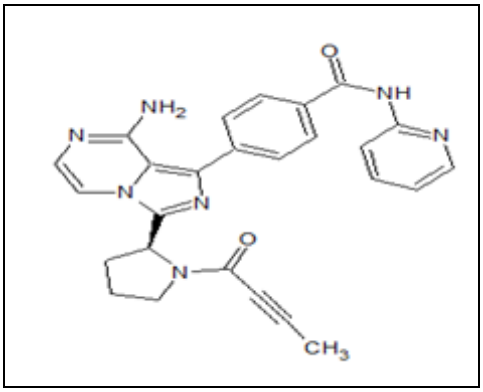

FIG. 1: CHEMICAL STRUCTURE OF ACALABRUTINIB

A literature survey reveals that various methods have been reported for Acalabrutinib individually or in combination with other drugs are UV [9], HPLC [10-12], stability-indicating [13, 14], LC-MS [15] but no one has developed RP-HPLC by Quality by Design method of this drug, which is fast, simple, and sensitive with less run time and good peak symmetry. Finally, in accordance with ICH requirements, the established method was verified in terms of specificity, linearity, precision, accuracy, robustness, LOD, and LOQ.

MATERIALS AND METHOD:

Materials: Acalabrutinib, a pure drug was gifted by Acrivis Laboratories Private Limited, Balanagar, Hyderabad. The solvents used in this experiment were of HPLC grade.

Methods: The analysis was performed using High-Performance Liquid Chromatography (Waters 2695 separation system) with a PDA detector having Empower software, UV-VIS Spectrophotometer Model Microprocessor UV vis-294 single Beam, pH meter BVK enterprises, India, electronic balance (WENSAR High-Resolution Balance), Ultra sonicator. The column used is SunFire C18 Column, 100Å, 10 µm, 4.6 mm X 150 mm (as Stationary phase) with a flow rate of 1 ml/min.

Standard Stock Preparation for the Analysis: 10mg of Acalabrutinib was transferred to 50ml volumetric flask 3/4th of the diluents was added to the flask and sonicated for 10 minutes. The flask was made up with diluents and labeled as a Standard stock solution. (200µg/ml of Acalabrutinib).

Sample Stock Preparation for the Analysis: Take ten capsules (Acalabrutinib Capsule, 100mg cap−1 strength) into 100 ml volumetric flask and dissolve in 50 ml of water then sonicated to disintegrate the capsules. Further the solution was diluted with 100 ml of ethanol and water, subsequently sonicated for about 20 min with intermittent shaking. Allowed to cool to room temperature and made up the volume with ethanol and water and filtered through 0.2 µm nylon membrane filter. (1000µg/ml of Acalabrutinib).

Determination of Detection Wavelength: Between 200 to 400 nm, the standard solution was scanned as shown in **Fig. 2.** The wavelength of maximum absorption for the drug was determined to be 220 nm.

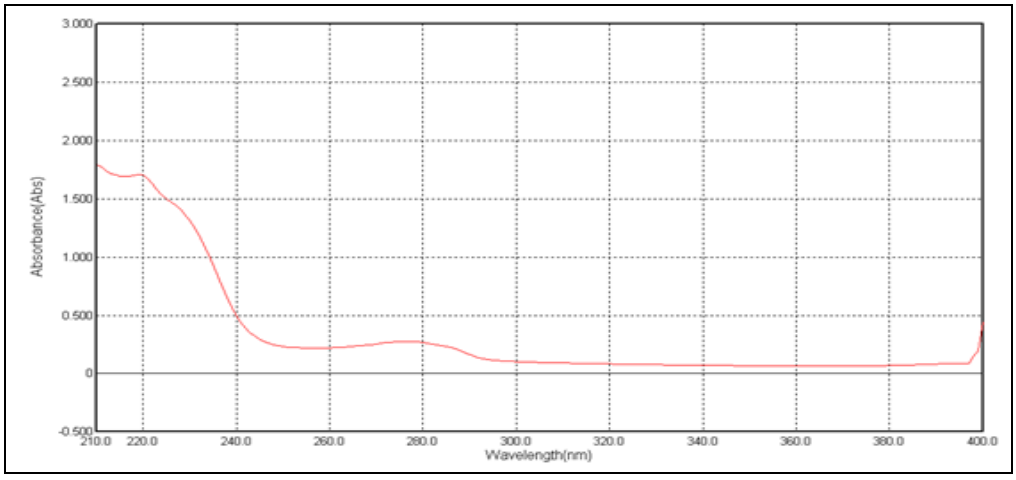

FIG. 2: UV SPECTRUM OF ACALABRUTINIB

Chromatographic Condition: Column C-18 (10 µm, 4.6 mm X 150 mm particle size equilibrated with mobile phase consisting of 0.1% OPA: Ethanol and Water (50:50%V/V) taken in a ratio of 60:40% V/V) was used. The flow rate was kept at 1 ml/min, and the column temperature was at 30°C. Eluents were supervised using a PDA detector at 220 nm.

Initial Method Development by QbD Approach: A Quality by Design with Design of Experiments approach to the development of an analytical method mainly involves two phases as follows:

(a) Screening Phase

(b) Statistical Analysis and Final Optimization

Screening Phase: The experimental design was constructed using design expert software version 11 for the study of different variables (% organic phase, flow rate) and to verify method performances. The levels of these variables are given in **Table 1.** The retention time, peak area, theoretical plates, and tailing factor were used as a response in experimental design as a controlling response, which is expected to affect and control method responses. A 2^4 factorial design consisting of two factors at four responses were considered for the experimental plan. Initially and after confirming that the process is non-linear, Central Composite Design was used. The experimental observations along with the Design (DOE) plan are shown in **Table 2.**

TABLE 1: CENTRAL COMPOSITE DESIGN AND RESPONSES

		Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3
Std	Run	A:FR	B:MP	C: Temperature	RT	NTP	TF
		ml/min	%	0 C	min	num	num
1	8	0.9	35	27	3.485	4035.7	1.3
2	1	1.1	35	27	2.607	4348.3	1.2
3	13	0.9	45	27	2.525	4506.4	1.2
4	11	1.1	45	27	1.65	5244.2	1.2
5	18	0.9	35	33	3.415	4523.4	1.3
6	3	1.1	35	33	2.611	3530.3	1.3
7	14	0.9	45	33	2.334	3649.3	1.3
8	7	1.1	45	33	1.684	4049.3	1.2
9	17	0.831821	40	30	3.093	3888.9	1.3
10	6	1.16818	40	30	1.911	4538.7	1.2
11	16	1	31.591	30	3.722	3833.4	1.4
12	5	1	48.409	30	1.719	4633.4	1.2
13	9	1	40	24.9546	2.443	4849.9	1.1
14	15	1	40	35.0454	2.344	3684.6	1.2
15	10	1	40	30	2.403	4224.2	1.18
16	4	1	40	30	2.392	4286.3	1.2
17	12	1	40	30	2.388	4238.8	1.2
18	20	1	40	30	2.395	4248.1	1.2
19	2	1	40	30	2.385	4258.1	1.2
20	19	1	40	30	2.384	4275.5	1.2

Statistical Analysis and Final Optimization: The responses obtained after carrying out the above trial runs were fed back to Design Expert software and plots like 3D-response surface plots and Graph plots were plotted.

These graphs demonstrated how important procedure factors affected the chosen quality criteria. The analysis of these plots was used to estimate which method parameter gave the most acceptable responses. Thus, the method's final critical method parameters and optimal chromatographic conditions were established in light of these observations. Additionally, the significance of each method parameter chosen for the study was determined using a statistical analysis tool like ANOVA for each response using the p-value (probability).

Validation of the Optimized Method: Validation of analytical procedures was performed for Acalabrutinib using the following parameters.

Specificity: The following solutions will be made and injected (peak purity double-checked) to show the accuracy of the procedure.

1. Blank (methanol 100% as a diluent)

2. Standard solution

3. Sample solution

4. Placebo treatments

Linearity: The method's linearity was examined at six distinct Acalabrutinib concentrations ranging from 5 to 30 µg/ml. To produce the calibration curve, peak area was plotted on the x axis against concentration. The regression line equation and correlation coefficient values were computed.

Accuracy (% Recovery): The accuracy of the method was confirmed by a recovery study from marketed formulation at 3 levels of standard addition. The percentage recovery of Acalabrutinib was found out.

Precision: The precision is reported in terms of Relative standard deviation (RSD). There are three levels of precision: repeatability, reproducibility and intermediate precision. It takes place using a sample API.

- Repeatability (Intraday precision)
- Intermediate precision (Interday precision)

Limits of Detection and Quantitation: Limits of detection (LOD) and limit of quantitation (LOQ) were determined from the signal-to-noise ratio. The detection limit was referred to as the lowest level of concentration resulting in a peak area of three times the baseline noise. The quantitation limit was referred to as the lowest possible concentration that provided a peak area with a signal-to-noise ratio higher than ten.

$$LOD = 3.3 \times \delta/S$$

$$LOQ = 10\times \delta/S$$

Robustness: To demonstrate the robustness of the procedure, the following optimized conditions were slightly varied.

- (60 %) 0.1% OPA: ethanol and Water (50:50%v/v) (40 %) ratio of mobile phase.
- 1 ml/min of flow rate.
- 30°C of temperature.

RESULTS AND DISCUSSION:

Statistical Analysis of Experimental Data by Design-expert Software: Analysis of variance (ANOVA) was applied to study the significance of the model generated for the five responses shown in **Table 3-6**. 2D Contour and 3D Surface plots were analyzed to visualize the effect of factors and their interactions on the Design Expert® software's responses. The regions shaded in dark blue represent lower values, and shaded in dark red represent higher values. The regions shaded in light blue, green and yellow represent intermediate values.

TABLE 2: ANOVA TABLE FOR RETENTION TIME USING CCD

Source	Sum of Squares	df	Mean Square	F-value	P-value	
Model	6.10	9	0.6778	141.47	<0.0001	significant
A-FR	1.98	1	1.98	412.47	<0.0001	
B-MP	3.90	1	3.90	813.07	<0.0001	
C-TEMP	0.0111	1	0.0111	2.32	0.1588	
AB	0.0031	1	0.0031	0.6431	0.4412	
AC	0.0112	1	0.0112	2.33	0.1577	
BC	0.0010	1	0.0010	0.2161	0.6520	
A^2	0.0201	1	0.0201	4.19	0.0677	
B^2	0.1892	1	0.1892	39.50	<0.0001	
C^2	0.0000	1	0.0000	0.0031	o. 9565	
Residual	0.0479	10	0.0048			
Lack of Fit	0.0477	5	0.0095	187.00	<0.0001	significant
Pure Error	0.0003	5	0.0001			
Cor Total	6.15	19				

The Model F-value of 141.47 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 Model terms are significant when the value is less than 0.0500. In this case A, B, B^2 are significant model terms.

TABLE 3: ANOVA TABLE FOR THEORETICAL PLATES USING CCD

Source	Sum of squares	df	Mean square	F-value	P-value	
Model	3.085E+06	6	5.141E+05	28.51	< 0.0001	significant
A-FR	1.759E+05	1	1.759E+05	9.76	0.0081	
B_MP	4.068E+05	1	4.068E+05	22.56	0.0004	
C-Temp	1.381E+	1	1.381E+06	76.56	< 0.0001	
AB	4.133E+05	1	4.133E+05	22.92	0.0004	
AC	3.376E+05	1	3.376E+05	18.72	0.0008	
BC	3.705E+05	1	3.705E+05	20.55	0.0006	
Residual	2.344E+05	13	18031.41			
Lack of Fit	2.317E+05	8	28967.53	54.29	0.0002	significant
Pure Error	2668.07	5	533.61			
Cor Total	3.319E+06	19				

The Model F-value of 28.51 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, AB, AC, BC are significant model terms.

TABLE 4: ANOVA TABLE FOR TAILING FACTOR USING CCD

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0735	9	0.0082	8.10	0.0015	significant
A-FR	0.0099	1	0.0099	9.84	0.0106	
B-MP	0.0211	1	0.0211	20.89	0.0010	
C-Temp	0.0099	1	0.0099	9.84	0.0106	
AB	1.388E-17	1	1.388E-17	1.376E-14	1.0000	
AC	0.0000	1	0.0000	0.0000	1.0000	
BC	1.388E-17	1	1.388E-17	1.376E-14	1.0000	
A^2	0.0069	1	0.0069	6.86	0.0257	
B^2	0.0226	1	0.0226	22.39	0.0008	
C^2	0.0026	1	0.0026	2.59	0.1389	
Residual	0.0101	10	0.0010			
Lack of Fit	0.0097	5	0.0019	29.25	0.0010	significant
Pure Error	0.0003	5	0.0001			
Cor Total	0.0836	19				

The Model F-value of 8.10 implies the model is significant. There is only a 0.15% chance that an F-value this large could occur due to noise. Model terms are considered significant when the P-value is less than 0.0500. A, B, C, A^2, B^2 are important model terms in this instance.

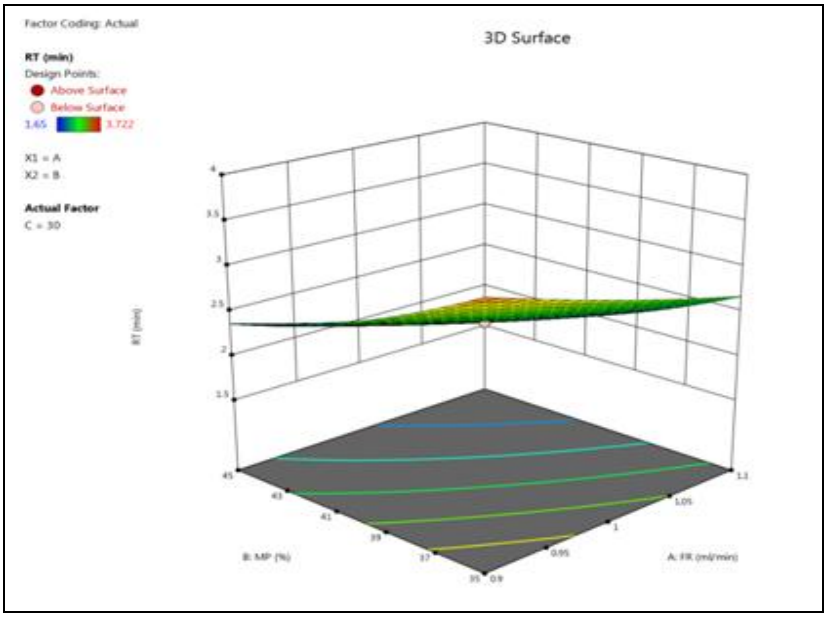

FIG. 3: CONTOUR PLOT FOR RTOF ACALABRUTINIB AGAINST MOBILE PHASE AND FLOW RATE

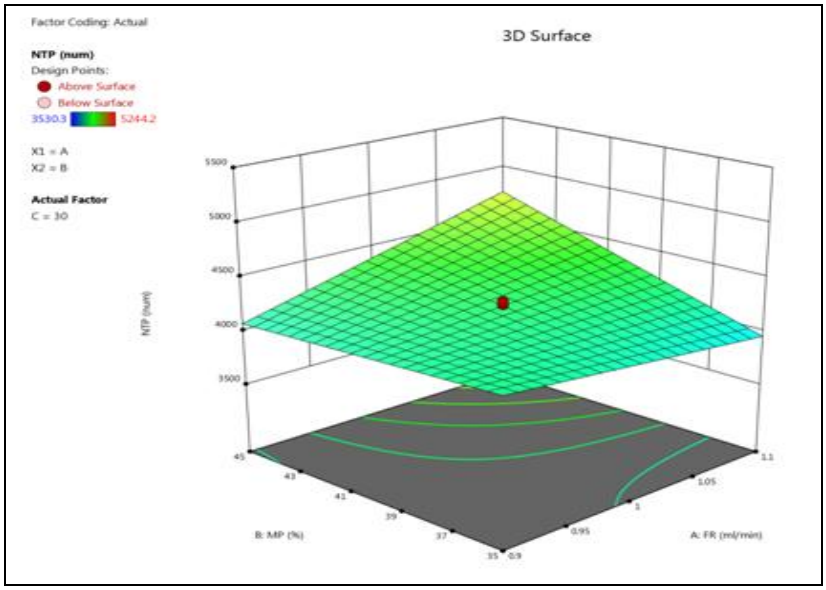

FIG. 4: CONTOUR PLOT FOR THEORETICAL PLATES OF ACALABRUTINIB AGAINST MOBILE PHASE AND FLOW RATE

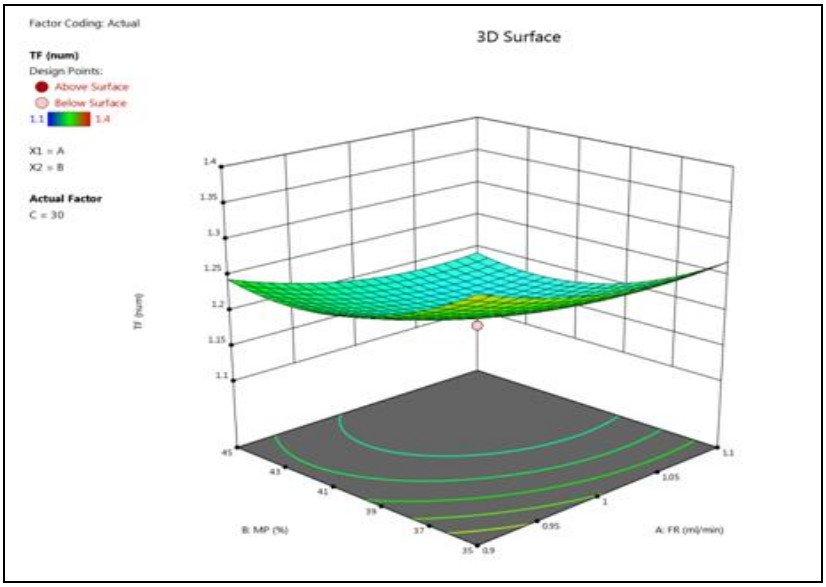

FIG. 5: CONTOUR PLOT FOR TAILING FACTORS OF ACALABRUTINIB AGAINST MOBILE PHASE AND FLOW RATE

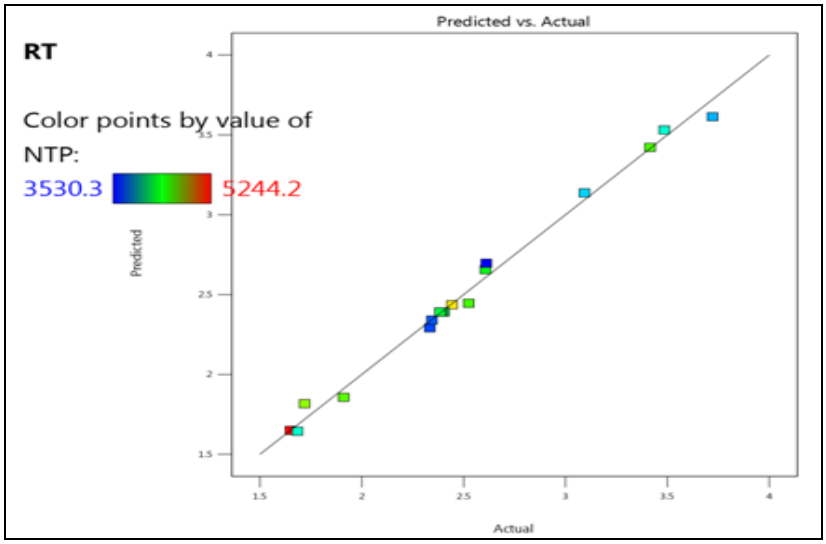

FIG. 6: COLOUR POINT BY VALUE OF RETENTION TIME PREDICTED VS ACTUAL

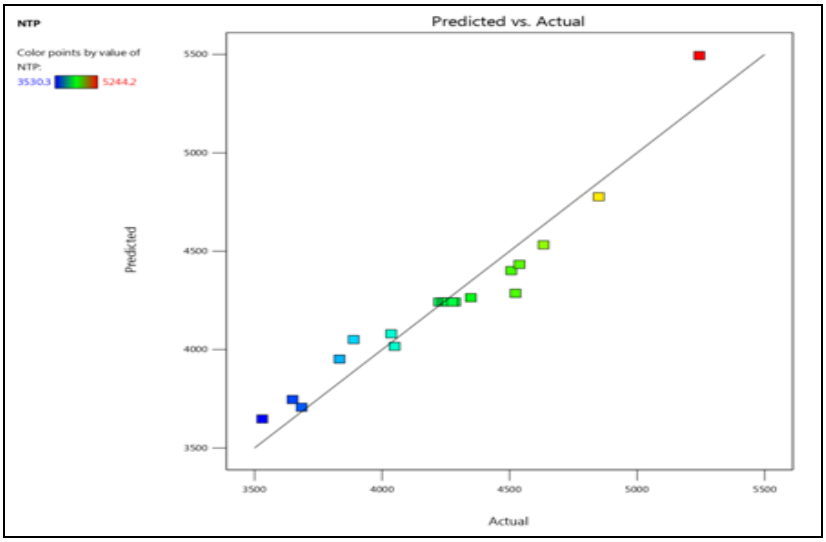

FIG. 7: COLOUR POINT BY VALUE OF THEORETICAL PLATES PREDICTED VS ACTUAL

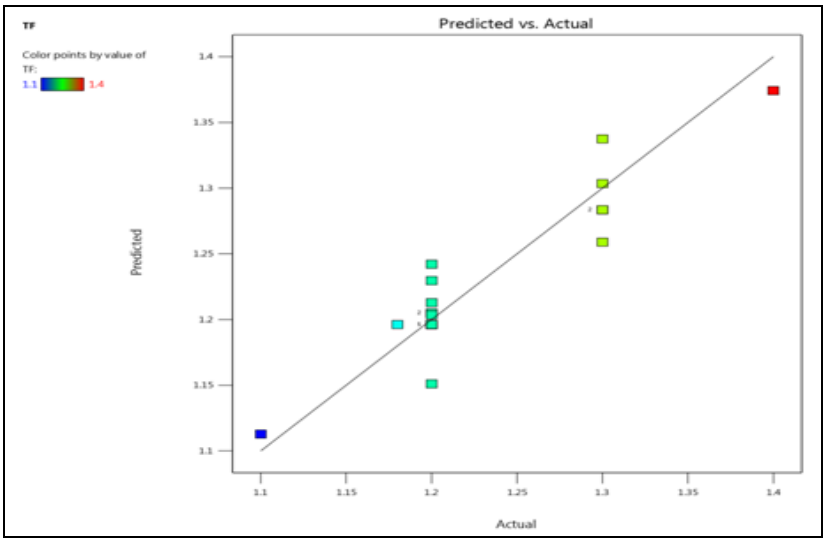

FIG. 8: COLOUR POINT BY VALUE OF TAILING FACTORS PREDICTED VS ACTUAL

TABLE 5: FINAL OPTIMIZED HPLC CHROMATOGRAPHIC CONDITIONS

Property	Value
Mobile phase	0.1% OPA (60%): Ethanol and Water (50:50v/v) (40%)
Flow Rate	1 ml/min

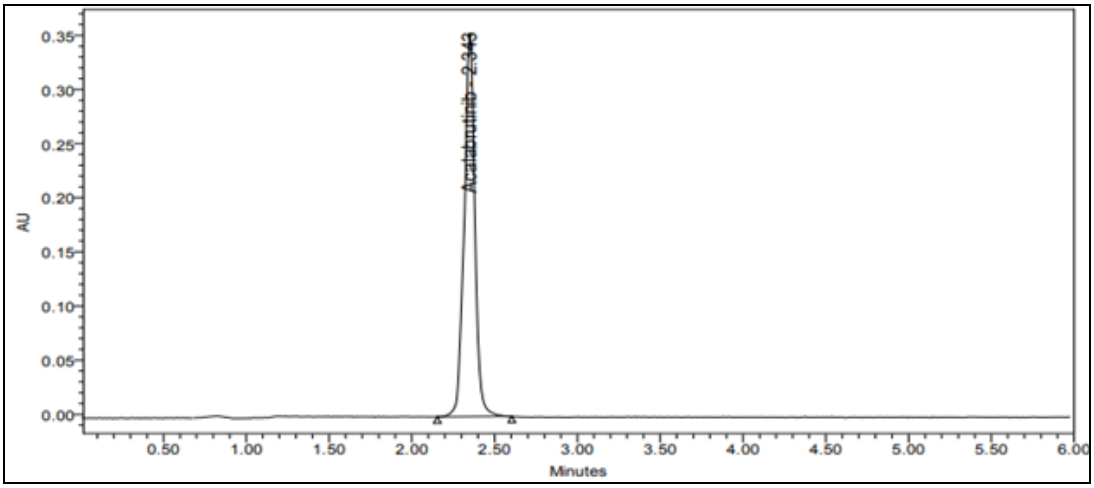

FIG. 9: CHROMATOGRAM OF FINAL OPTIMIZED METHOD

Method Validation: The suggested approach had a linear correlation coefficient (R^2) of 0.999 for concentrations ranging from 5 to 30 µg/ml. The drug recovery percentage had to fall between 98 and 110% for the accuracy experiments conducted at the 50, 100, and 150% levels. After conducting tests for intermediate accuracy, reproducibility and repeatability, it was discovered that the percentage RSD values were less than 2%. The LOD value for Acalabrutinib was discovered to be 0.28 µg/ml. The LOQ value for Acalabrutinib was determined to be 0.86µg/ml. By making small adjustments to the flow rate, wavelength, and percentage organic component, the suggested method's robustness was examined. It was discovered that the RSD values for the peak area were less than 2%. Table 6 displayed an overview of the method validation parameters.

TABLE 6: RESULTS OF THE VALIDATION PARAMETERS

Parameters		Acalabrutinib	Limit
Linearity Range (µg/ml)		5-30µg/ml	R^2< 1
Regression coefficient		0.999	
Slope(m)		17109	
Intercept(c)		5534.8	
Regression equation (Y=mx+c)		y =17109x + 5534.8	
Assay (% mean assay)		99.06%	90-110%
Specificity		Specific	No interference of any peak
System precision %RSD		1.0	NMT 2.0%
Method precision %RSD		0.3	NMT 2.0%
Accuracy % recovery		100.47%	98-102%
LOD		0.28	NMT 3µg/ml.
LOQ		0.86	NMT 10 µg/ml.
Robustness % RSD	FM (0.9)	0.9	%RSD NMT 2 %
	FP (1.1)	1.2	
	MM (30B)	0.9	
	MP (50B)	0.6	
	TM (25°C)	0.8	
	TP (37°C)	1.1	

CONCLUSION: A simple analytical and robust HPLC method was developed for the determination of Acalabrutinib by using the QbD approach using Design Expert® software. The retention time of Acalabrutinib was found to be 2.343 min. %RSD of the Acalabrutinib was found to be 1.0%. %RSD of Method precision of Acalabrutinib was found to be 0.3%. %Recovery was obtained as 100.47% for Acalabrutinib.

LOD and LOQ values obtained from the regression equation of Acalabrutinib were 0.28 and 0.86. The regression equation of Acalabrutinib is y = 17109x + 5534.8. The method that was created was easy to use and cost-effective, making it suitable for routine quality control testing in industries. Both the retention times and the run time were reduced. Results which were obtained from the validation of the developed analytical method were within the limit as per ICH guidelines**.**

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CONFLICTS OF INTEREST: Nil

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