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DEVELOPMENT AND VALIDATION OF HPTLC METHOD FOR THE ESTIMATION OF ACALABRUTINIB IN TABLET DOSAGE FORM

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ABSTRACT:

The estimate of acalabrutinib in pharmaceutical tablet dosage form was accomplished through the development and validation of a unique, accurate, exact, sensitive, and appropriate High Performance Thin Layer Chromatography (HPTLC) method. The chromatographic separation of acalabrutinib was accomplished on a Silica gel 60 F254 HPTLC plate as the stationary phase. The mobile phase consisted of a 75: 15: 5: 1% v/v/v/v mixture of toluene, ethanol, ethyl acetate, and glacial acetic acid (GAA). At 230 nanometres, acalabrutinib was detected and quantified. It was discovered that the system produced a sharp peak and a well-resolved discrete band at an appropriate Rf value of 0.26. The suggested method's performance was verified in accordance with ICH requirements for robustness, linearity, accuracy, precision, LOD, and LOQ. A correlation coefficient of 0.9998 was discovered to indicate the linearity of the molecule within the 10-80ng/band range. It was discovered that the limits of detection and quantification were, respectively, 0.004 µg/ml and 0.14 ng/band. Since there was no prior HPTLC method for estimating acalabrutinib in pharmaceutical tablet dose form, the current method was innovative. It was also effectively employed for regular analysis and quality checks of the drug.

KEYWORDS: Acalabrutinib, High Performance Thin Layer Chromatography, Method development and validation, Quantification.

INTRODUCTION:

The chemical name of Acalabrutinib is 4-[8-amino-3-[(2S)-1-but-2-ynoylpyrrolidin-2- yl]imidazo[1,5-a]pyrazin-1-yl]-N-pyridin-2-ylbenzamide[1]. 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 freely soluble in water at pH below 3 and practically insoluble at pH above 6. Acalabrutinib (Calquence®) is a potent, selective, orally-administered, covalent inhibitor of Bruton tyrosine kinase (BTK) that was approved by USFDA in 2017 for treatment of mantle cell lymphoma. Acalabrutinib (ACA) is also used for chronic lymphocytic leukemia and Waldenstrom's macroglobulinemia. Acalabrutinib forms a covalent bond with a cysteine residue in the BTK active site, leading to inhibition of BTK enzymatic activity. Bruton's tyrosine kinase (BTK) has recently become a promising drug target for many diseases, especially hematopoietic malignancies and autoimmune diseases associated with B lymphocytes. Many BTK inhibitors are currently in different stages of clinical trials. Acalabrutinib is a BTK inhibitor established by Acerta Pharma and has been approved by the FDA for adult patients with mantle cell lymphoma who have received at least one prior

therapy [2]. Both Acalabrutinib and its active metabolite, ACP-5862, act to form a covalent bond with a cysteine residue (cys481) in the BTK active site, leading to inhibition of enzymatic activity. As a result, it inhibits BTK- mediated activation of downstream signaling proteins CD86 and CD69, which ultimately inhibits malignant B-cell proliferation and survival [3]. From the literature survey, it was found that few chromatographic methods were developed for the estimation of Acalabrutinib in bulk and pharmaceutical preparations [4,5,6]. Literature survey reveals that few instrumental methods were available for the estimation of acalabrutinib like HPLC. No HPTLC method was found during literature survey. Hence, present study aims to developed and validate rapid, precise, accurate, selective and economical HPTLC method for determination and estimation of acalabrutinib in tablet dosage form. The proposed method was optimized and validated according to International Conference on Harmonization (ICH) guidelines. [7]

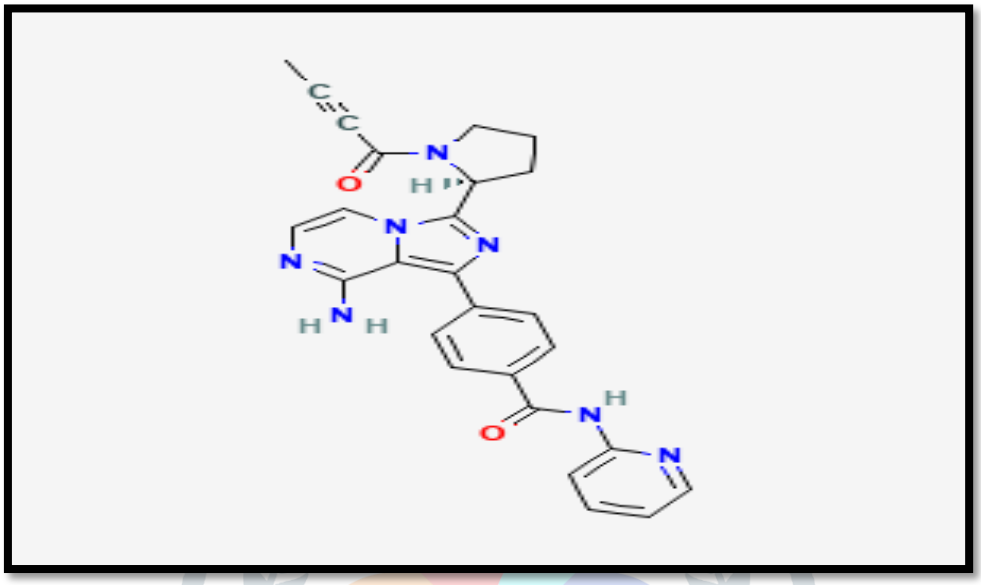

Figure no. 1: Structure of Acalabrutinib

INSTRUMENTATION:

Application was done by CAMAG Linomat syringe (100µl), a CAMAG Linomat 5 which sprayed samples on HPTLC plate in the form of bands with nitrogen gas generator. CAMAG TLC Plate Heater was used for heating plate. For the chromatographic separation of drug pre-coated HPTLC plates (silica gel 60 F_{254}) were used and linear ascending development was carried out in a CAMAG twin through glass developing chamber. Chromatographic scanning was carried out on CAMAG TLC scanner 4. For the visualization, imaging and documentation of chromatogram CAMAG TLC Visualizer 2 was used and for whole studies Vision CATS CAMAG HPTLC software version 4.0 was used.

CHEMICAL AND REAGENTS:

Acalabrutinib working standard with defined potency 99.03% and Calquence tablet 100mg (acalabrutinib tablet) were procured from Central Drug Testing Laboratory, Mumbai. Toluene of SRL grade, glacial acetic acid of E. Merk India Limited, ethyl acetate of lab grade molychem and ethanol of Gemini associates were obtained from Central Drug Testing Laboratory, Mumbai. The chromatographic separation of drug was performed on precoated HPTLC silica gel 60 F_{254} aluminium sheets (20x20cm with thickness 250µm) from Sigma Aldrich, Germany.

DETERMINATION OF WAVELENGTH:

By scanning an acalabrutinib standard solution between the 200.0 nm and 400.0 nm ranges of the near UV visible region, a UV spectrum was produced. Figure 2 displays the maximum absorbance (λ max) of acalabrutinib at 230 nm. Therefore, the same wavelength was chosen to analyse acalabrutinib in tablet form.

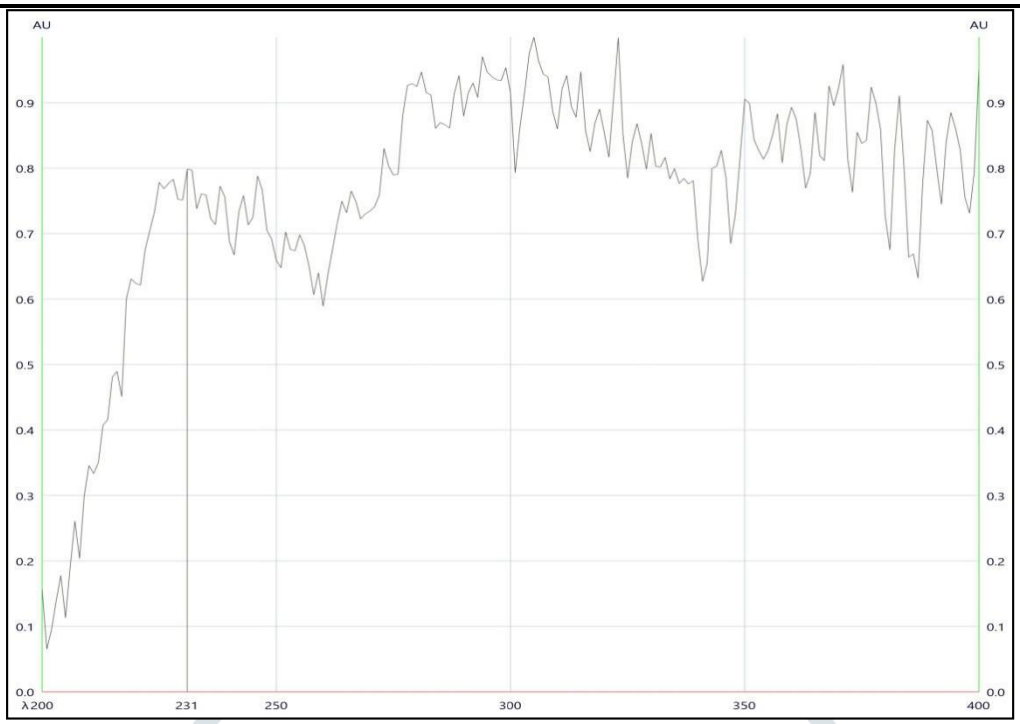

Figure no.2: UV spectra of Acalabrutinib

CHROMATOGRAPHIC CONDITION:

The HPTLC silica gel 60 F254 20x20cm aluminium plate, precoated, has a thickness of 250µm. A steady rate of 15 nl/s was used to spot standard and sample material onto the plate in the form of 8 mm long, narrow bands using a CAMAG Linomat syringe (100µl) and CAMAG Linomat 5 sample applicator with nitrogen gas aspirator. To prevent edge effect, the application positions X and Y were kept at 8 mm and 20 mm, respectively. The two bands were separated by 20 mm. For thirty minutes, a mobile phase comprising toluene, ethyl acetate, ethanol, and glacial acetic acid (75:15:5:0.5%v/v/v/v) was used to saturate a CAMAG twin through glass developing chamber. Following that, insert the plate into the saturated chamber to begin the chromatogram's linear ascending growth up to a run distance of 70 mm (solvent front). The plate was dried using a drier after development. Later, the plate was imaged and band visualisation was performed using CAMAG visualiser 2. Sharp peaks were seen by scanning the plate at 230 nm using a CAMAG TLC scanner 4 fitted with a deuterium light for densitometric scanning. The slit's dimensions were 6.0 x 3.0 mm, and its scanning speed was 100 nm/s in auto mode to maintain sensitivity. The data processing and peak area evaluation processes were carried out using Vision CATS CAMAG HPTLC software version 4.0.

PREPARATION OF SOLUTION:

Selection of Diluent:

Based on the solubility and chemical nature of acalabrutinib, ethanol was selected as diluent for preparation of standard and sample solutions.

Preparation of Standard Solution:

The standard solution was prepared by dissolving 5mg acalabrutinib standard into 100ml of volumetric flask. Sonicated and then made volume with diluent.

Preparation of Sample Solution:

Ten tablets of Acalabrutinib were weighed and average mean weight was calculated. The tablets were then triturated to get fine powder and weight equivalent to 1 tablet i.e. 417.2mg was transferred in 100ml volumetric flask and the sufficient amount of ethanol was added. 1 ml from above solution and transfer in 50ml volumetric flask. The content was sonicated for 10minutes and the final volume was made up to the mark with ethanol (50µg/ml).

OPTIMIZATION OF HPTLC METHOD:

The polarity, pKa, and chemical makeup of the molecule were taken into consideration when selecting mobile phases. The same parameters were used for the HPTLC after the procedure was refined for TLC. For the chromatographic separation of acalabrutinib, a precoated HPTLC silica gel 60 F254 aluminium plate with a thickness of 250µm was utilised. The mobile phase used in the first HPTLC trials consisted of Toluene, ethanol, ethyl acetate, and ammonia at a ratio of 70:15:15:0.5%v/v/v/v. On the other hand, low peak shape and a short Rf value were noted. Using a mobile phase consisting of Toluene, ethyl acetate, ethanol, glacial acetic acid (GAA) in the ratio of 75:15:5:0.5% v/v/v/v allowed for the observation of a good Rf value with a symmetrical and crisp peak shape after several trials. This method was used throughout the analysis.

METHOD VALIDATION:

Validation of present and optimized HPTLC method was done by checking the parameters such as Specificity, linearity, accuracy, precision, robustness, limit of detection and limit of quantification according to the ICH Q2 (R2) guidelines.

Specificity:

To ascertain the specificity of the current procedure, analyses were conducted on the Standard and Sample solutions. By comparing the Rf value and band spectra of the Acalabrutinib sample with the standard, the band was verified. To ascertain the peak purity of acalabrutinib, the spectra was evaluated at three distinct band regions: peak start (S), peak apex (M), and peak end (E).

Linearity:

The capacity of an analytical method to produce test findings that, within a certain range, are exactly proportionate to the analyte concentration in the sample is known as linearity. For the acalabrutinib linearity studies, three replicates of each concentration were made within the concentration range of 10-80 ng/band. Every band seen measured 5µl. A linear response with a 0.9994 correlation coefficient was attained. Figure No. 6 displayed the plot of peak areas vs corresponding concentrations, and Table No. 1 displayed the linearity results for acalabrutinib.

Precision:

The degree of agreement between several measurements made from repeated samplings of the same homogenous material under specified conditions is expressed as the precision of an analytical method. The suggested method's precision was confirmed by repeatability and intermediate precision experiments. Six applications of a 50ng/band standard solution of acalabrutinib were used to calculate the % RSD for repeatability. Every band observed on the HPTLC plate measured 2µl. Inter-day and intra-day precision served as validation for the intermediate precision approach. The analysis of three distinct levels of acalabrutinib on the same day—low, medium, and higher concentrations—was used to determine the intra-day precision. Additionally, three separate days were used to conduct inter-day precision. The findings of the research on repeatability and intermediate precision are displayed in Tables Nos. 2 and 3.

Accuracy:

The degree of agreement between the value found and the value regarded as true or conventionally true is expressed as the accuracy of an analytical technique. The standard addition approach of adding a known quantity of acalabrutinib standard solution at three distinct levels (e.g., 110%, 120%, and 130%) to a pre-analysed sample solution was used to conduct recovery tests. Three determinations were made at each concentration level, and the mean percentage recovery was computed and reported. The mean percentage recovery result, as indicated in Table No. 4, fell within acceptable bounds.

Assay:

An assay is an investigative approach used in the development and validation of the HPTLC method to quantitatively assess the amount of a certain medication in a given sample. Low S.D. values and high

percentage recoveries are shown in the assay findings. The results are displayed in Table No. 5 and the assay concentration is in relation to 50 ppm of sample with a spotting volume of 5µl.

Limit of Detection and Limit of Quantification:

The lowest concentration of analyte in the sample that can be identified but still need to be precisely measured is known as the detection limit of a particular analytical method. The lowest quantity of analyte in the sample that can be quantitatively measured with appropriate precision and accuracy is the quantification limit of any unique analytical method. Using the following calculations, the LOD and LOQ were determined based on the slope of the calibration curve and the regression line:

LOD = 3.3 x σ/s

LOQ = 10 x σ/s

Robustness:

Robustness is performed by small or deliberate change in the parameters that should not affect any method. For HPTLC method, robustness was established by making changes include change in mobile phase composition (±0.2%), change in chamber saturation time (±5min.), change in distance travelled by solvent front (±5mm) and the results was calculated and reported in table no.7.

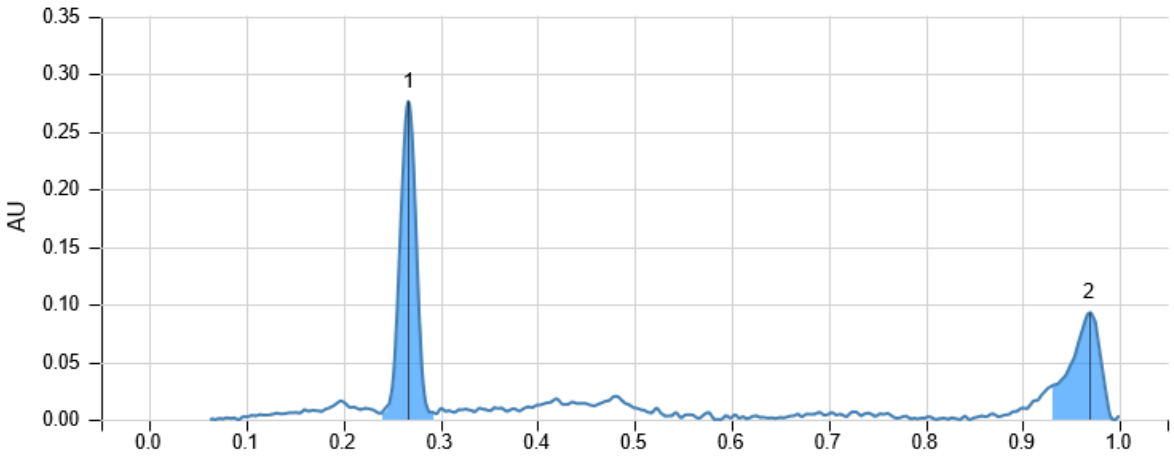

Figure no.3: HPTLC densitogram under optimized conditions showing Rf value of 0.23 for Acalabrutinib.

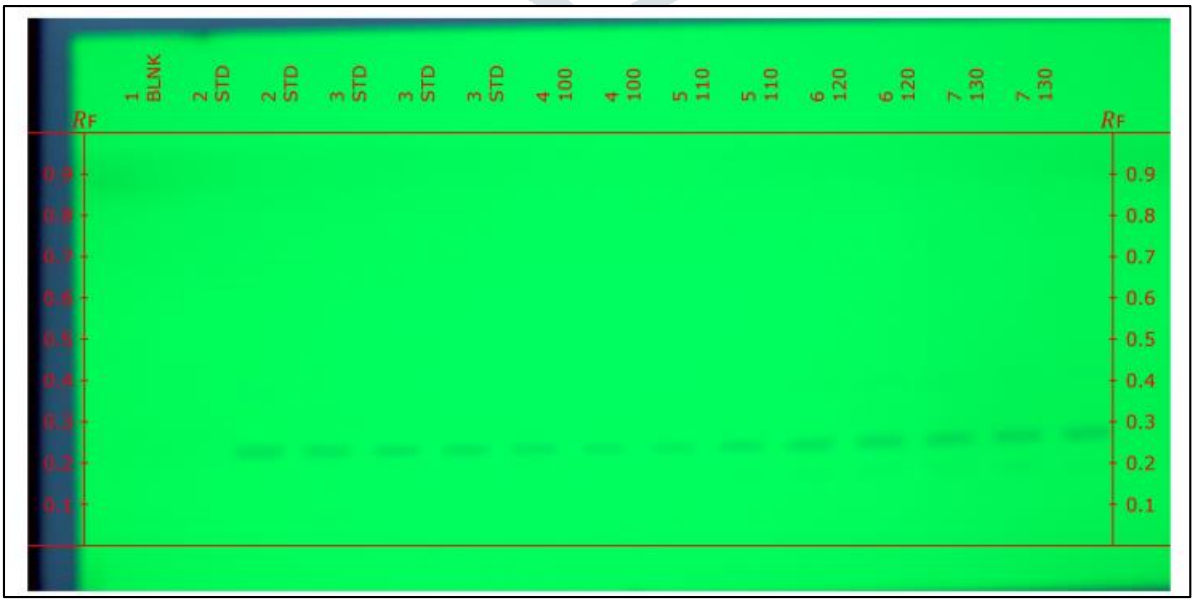

Figure no.4: Image of HPTLC plate taken at 230nm.

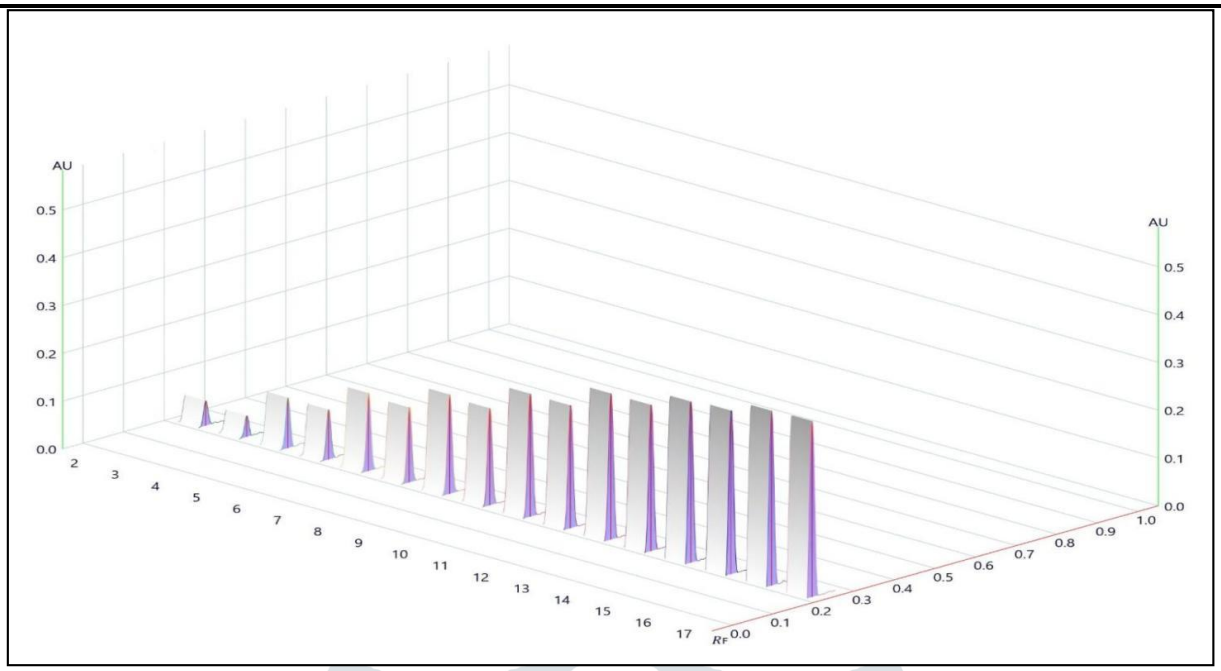

Figure no.5: 3-D densitogram for the linearity of Acalabrutinib

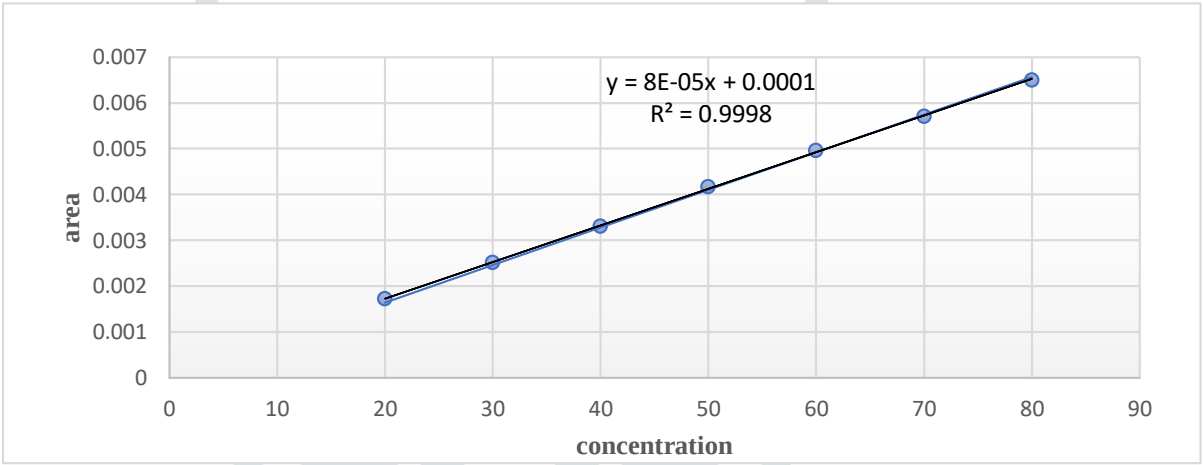

Figure no.6: Calibration curve of Acalabrutinib

Table no.1: Linearity data of Acalabrutinib

Concertation (ng/band)	Peak area
10ppm	0.00090
20ppm	0.00172
30ppm	0.00249
40ppm	0.00332
50ppm	0.00413
60ppm	0.00490
70ppm	0.00580
80ppm	0.00656

Table no.2: Precision (Repeatability)

Sr No.	Concentration ng/band	Area
1	500	0.0107
2	500	0.01067
3	500	0.01064
4	500	0.01068
5	500	0.01071
6	500	0.01063
	Mean	0.010671667
	SD	0.000032
	%RSD	0.29878

Table no.3: Intra-Day Precision and Inter-Day Precision

Concentration ng/band	Intraday Precision		Inter day Precision	
	Area± SD	% RSD	Area± SD	% RSD
25	0.00306 ± 5.7735E-06	0.188	0.00306 ± 5.7735E-06	0.188
50	0.00526 ± 1.1547E-05	0.219	0.00513 ± 4.16333E-05	0.811
75	0.00740 ± 3.21455E-05	0.437	0.00721 ± 0.000118462	1.643

Table no.4: Accuracy studies of Acalabrutinib

%Level added	Std spike	Amount recovered(mg)	% Recovery	Mean% Recovery
100%	0	99.71	99.71	
100%	0	99.79	99.79	99.81
100%	0	99.93	99.93	
110%	10	110.31	100.31	
110%	10	110.09	100.03	100.35
110%	10	110.71	100.71	
120%	20	120.87	100.87	
120%	20	120.75	100.75	100.57
120%	20	120.11	100.11	
130%	30	131.08	101.08	
130%	30	130.09	101.09	101.15
130%	30	131.29	101.29	
			Average	100.47
			Std Dev	0.554
			%RSD	0.551

Table no.5: Assay calculations

Sr. no.	Area of Standard at 230nm	Peak Area	% Assay
1	0.00339	0.00511	99.21
2		0.00509	99.03
3		0.00511	99.76
4		0.00512	100.12
5		0.00537	99.89
6		0.00539	100.73
Average			99.79
SD			0.619
% RSD			0.620

Table no.6: LOD & LOQ

Parameters	HPTLC method
Linearity range (ng/band)	10- 80ng/band
Regression equation	Y= 8E-05x + 0.0001
Detection wavelength (nm)	240
Correlation coefficient (R^2)	0.999
Limit of detection (ng/band)	0.004
Limit of quantification (ng/band)	0.14ng/band

Table no.7: Robustness Data of Acalabrutinib

Sr.NO	Parameters	Ratio	Average area	SD	%RSD
1	Change in Chamber Saturation Time (30 min.5+-min.)	25 min	0.00525	0.00003606	0.686
		35 min	0.0058	0.00000577	0.995
2	Changes in mobile Phase Volume (+- 2 percent of toluene)	73:16:6:0.5	0.00564	1.154	0.203
		77:14:4:0.5	0.00567	0.0054	0.735
3	Change In distance Travelled by solvent (80min.+-5min.)	75 D.T	0.0052	0.00003606	0.693
		85 D.T	0.0058	0.00001732	0.298

RESULTS AND DISCUSSION:

Acalabrutinib dose form estimate using high performance thin layer chromatography (HPTLC) was developed and verified as a unique, quick, easy, appropriate, and accurate method. Acalabrutinib's developed approach yielded results with a mobile phase. GAA (v/v/v/v) in toluene, ethyl acetate, and ethanol using HPTLC silica gel 60 F254 plates. To ascertain the peak purity of acalabrutinib, the spectra were compared at the peak start (S), peak apex (M), and peak end (E) positions. Acalabrutinib's linearity exhibits a continuous linear response within the 10-80ng/band range. In such case, the linear correlation coefficient (r2) was 0.9998. Repeatability and the intra-day precision approach were used to achieve the precision. Relative standard deviation (RSD) for the repeatability studies was determined to be 1.16%. It was discovered that the percentage RSD values for intra- and inter-day precision fell within allowable bounds. According to ICH requirements, the developed method was deemed to be precise, as evidenced by the % RSD values discovered within range. The average percent mean recovery for the recovery studies was found to be 100.47% at the levels of 110%, 120%, and 130%, and the mean percent RSD was found to be 0.551, all of which are within the acceptable range, indicating that the approach was accurate. Table No. 4 displays the recovery study outcomes. Table No. 5 displays the findings of the observed assay for Acalabrutinib in the Calquence tablet dosage form, which was found to be 100.46%, respectively. The new method's sensitivity was demonstrated by the findings that the Limit of Detection (LOD) was 0.004 ng/band and the Limit of Quantification was 0.14 ng/band. Because repeatable findings in the form of accurate Rf values and low% RSD values were produced, the procedure was proven to be robust. Small intentional adjustments to the parameters had no effect on the outcome.

Table no.8: Combined results of all parameters

Parameters	**Acalabrutinib**
Linearity Linearity range Correlation coefficient	 10-80ng/band 0.9998
Precision Repeatability Intra-Day Inter-Day	%RSD 0.298% 0.18-0.43% 0.18-1.64%
Accuracy (%level) 110% 120% 130%	 100.35% 100.57% 101.15%
Assay Mean recovery %RSD	 100.46% 0.519
LOD	0.004ng/band
LOQ	0.14ng/band
Robustness Change in Mobile phase Change in Saturation time Change in Distance travelled	%RSD 0.203-0.735% 0.686-0.995% 0.693-0.298%

CONCLUSION:

The current HPTLC technique is innovative, quick, accurate, exact, robust, and simple for both quantitative and qualitative measurement of acalabrutinib. In accordance with ICH Q2(R2) recommendations, the suggested approach was successfully designed and validated for the parameters of linearity, precision,

accuracy studies, assay, LOD & LOQ, and robustness. Every validation parameter's findings fell within the permissible range. Because of the developed method's ease of sample preparation, low solvent consumption, and high HPTLC bandwidth up to 15 samples per plate—which reduces costs and saves time, it should be given careful consideration. Therefore, regular analysis and quality control of acalabrutinib in pharmaceutical tablet dosage form can be performed using the established HPTLC approach.

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