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Method Development and Validation for Quantitative Estimation of Acalabutinib in Capsule Dosage Form by RP-HPLC Method

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ABSTRACT

A simple and new isocratic high-performance liquid chromatography (HPLC) method was developed for the quantitative determination of Acalabrutinib in its tablet dosage form. The chromatographic separation was achieved on the Gemini LCC18 column (250mm x 4.6mm, 3µm). The mobile phase selected 25 MM Ammonium acetate (pH-4): Acetonitrile in the ratio of 50: 50 v/v at flow rate 1.0ml/min with column temperature maintained at 40°C and 10µl injection volume. The detection was carried out at 230nm. The retention time of Acalabrutinib was found to be at 3.51 min. The developed HPLC method was validated as per the ICH guideline. The HPLC method was linear over the range of 5-20 µg/ml with a regression coefficient of 0.9997. The results of validation parameters indicate that the developed HPLC method was specific, accurate, precise, rapid, reliable, and reproducible, therefore, it can be applied for routine quality control analysis of Acalabrutinib in its tablet dosage form.

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.[2]

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 is soluble in organic solvents such as ethanol, dimethyl sulfoxide (DMSO), dimethylformamide (DMF). The solubility of acalabrutinib in ethanol is 15mg/ml and solubility in DMSO and DMF is 25mg/ml. Its pKa value is 12.34. [3]

Acalabrutinib is a tyrosine kinase inhibitor being developed by Acerta Pharma. On November 21, 2019, Acalabrutinib was approved by Food and drug administration (FDA). [4]

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. [5] 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. [6]

From the literature survey, it was found that few chromatographic methods were developed for the estimation of Acalabrutinib in bulk and pharmaceutical preparations.[7,8] Hence there was a need to develop new, simple, rapid, precise, and accurate reverse phase chromatographic methods to estimate Acalabrutinib in the capsule dosage form. The proposed method was optimized and validated according to International Conference on Harmonization (ICH) guidelines. [9]

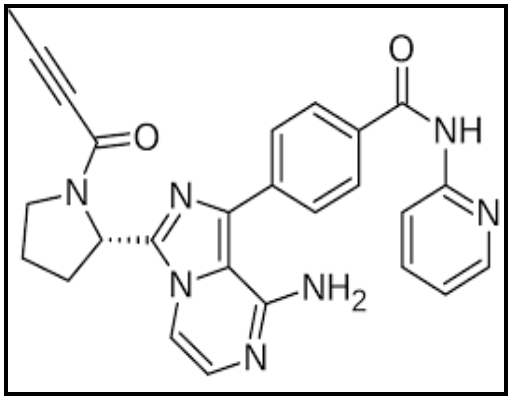

Fig No. 1: Structure of Acalabrutinib

MATERIALS AND METHODOLOGY:

Chemicals and Reagents –

An analytically pure Acalabrutinib working standard was procured from Central Drug Testing Laboratory, Mumbai with defined potency of 98.6 % (as is basis). CALQUENCE (100 mg) Film-coated tablets were received as a gift sample from Assistant Drugs Controller Office, Air Cargo, Mumbai.

Acetonitrile, HPLC Grade from Rankem, Ammonium acetate AR Grade, Glacial Acetic Acid AR Grade, and Millipore water were used during the analysis.

Instrument-

Thermo Scientific Dionex Ultimate 3000 was connected to a computer. Thermo Scientific Dionex Chromelon HPLC using software data system version 7.2.6 with LC instrument control was used for chromatography.

Determination of wavelength -

About 10.0 mg of working Acalabrutinib was transferred to the 20ml volumetric flask (500ug/ml) and the volume was made up to the mark with diluent {Mixture of Acetonitrile and water (50:50)}. The aliquot portions of standard stock solutions of Acalabrutinib have diluted appropriately with diluent to obtain concentrations of 10ug/ml of the drug. The solution was scanned in the range of 200-400nm. The average absorbance maximum of Acalabrutinib was found at 230nm. As a result, 230nm was chosen as the final wavelength for the method development and validation.

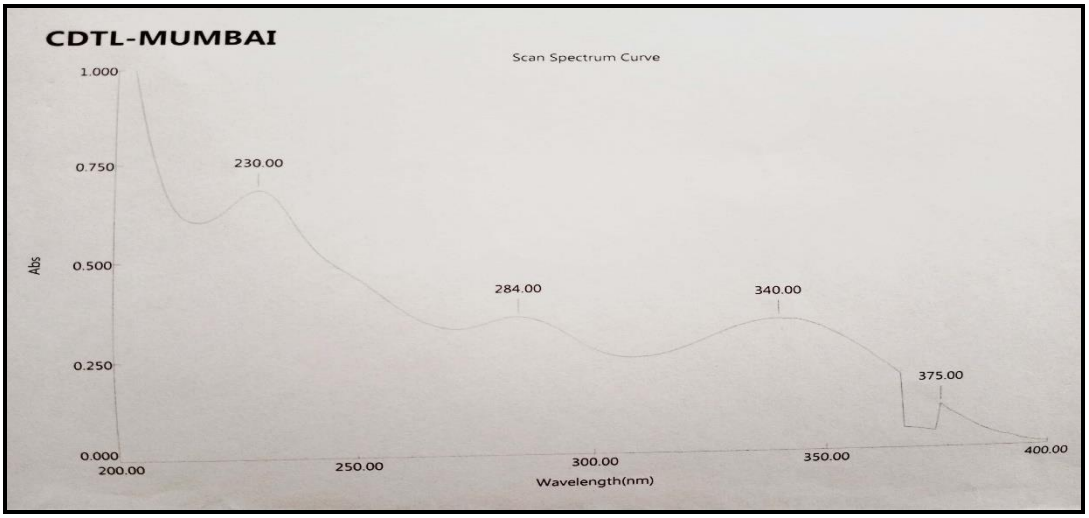

Fig No. 2: Acalabrutinib UV Spectrum

Optimized chromatographic conditions -

The chromatographic separation was carried out on the Gemini LC C18 column (250mm x 4.6mm, 3µm) at 40°C with a mixture of 25 MM Ammonium acetate (pH-4) and acetonitrile in the ratio of 50:50% v/v contain as mobile phase. The detection was carried out at 230 nm, 10µl injection volume was selected with the flow rate 1ml/min. The retention time was found to be 3.51 minutes.

Preparation of Mobile phase -

The mixture of 25 MM Ammonium Acetate buffer (pH 4 with Glacial Acetic Acid) and acetonitrile in the ratio of (50:50) v/v. It was then sonicated using an ultrasonic bath for 10 minutes and was filtered using a 0.2 u nylon filter.

Diluent Preparation -

Based on the Molecular structure and chemical nature of Acalabrutinib, a Mixture of Acetonitrile and water (50:50) was selected as a diluent for the preparation of standard and sample preparation.

Preparation of standard solution -

Accurately weighed about 10mg of Acalabrutinib standard was transferred in a 20ml volumetric flask and dissolved by sonication in sufficient diluent then volume made with diluent (500ug/ml). Then 1ml from the above stock solution was diluted up to 50ml with the same diluent (10ug/ml) which is treated as 100% target concentration.

Preparation of sample solution-

Ten capsules of CALQUENCE (100mg) were weighed and the average weight was calculated. Weight equivalent to 1 tablet was poured in 200ml of the volumetric flask; 50 ml of diluent was added and sonicated for 15 minutes. Final dilution was made up to 200 ml with diluent (500ug/ml). Then 1ml from the above stock solution was diluted up to 50ml with the same diluent (10ug/ml).

VALIDATION OF DEVELOPED METHOD:-

The developed RP-HPLC method of Acalabrutinib was validated as per ICH guidelines for parameters such as specificity, linearity, accuracy, the limit of detection, the limit of quantification, robustness.

System suitability:-

It is an integral part of method development. A blank preparation (single injection) and standard preparation (six replicate) at the working concentration (10ug/ml) were injected into the HPLC and the chromatograms were recorded to evaluate the parameters like area, retention time, number of theoretical plates, and tailing factors.

Table No. 1: System suitability

SR. NO.	Area	Retention time	Tailing factor	Theoretical Plates
1	6935.97	3.56	1.06	2323
2	6950.62	3.57	1.05	2338
3	6933.74	3.57	1.06	2362
4	6962.72	3.57	1.06	2319
5	6997.39	3.57	1.06	2328
6	6915.28	3.57	1.05	2328
MEAN	6949	3.570	1.05	2333
SD	28.53	0.0015	0.0051	15.569
% RSD	0.4106	0.0421	0.4887	0.6673
Limit	NMT 2.0%	NMT 1.0%	NMT 2.0%	NLT 2000

Specificity -

It is the ability of the analytical method to measure the response of the analyte and have no interference from other extraneous components and well-resolved peaks are obtained. For specificity blank, standard drug solution (10ug/ml) and sample solution (10ug/ml) were injected into the HPLC and their chromatogram was recorded. It reveals that the peaks obtained in the standard and sample solution at working concentration are only because of drugs, as blank has no peak at the retention time of Acalabrutinib. Accordingly, it can be concluded that the method is said to be specific.

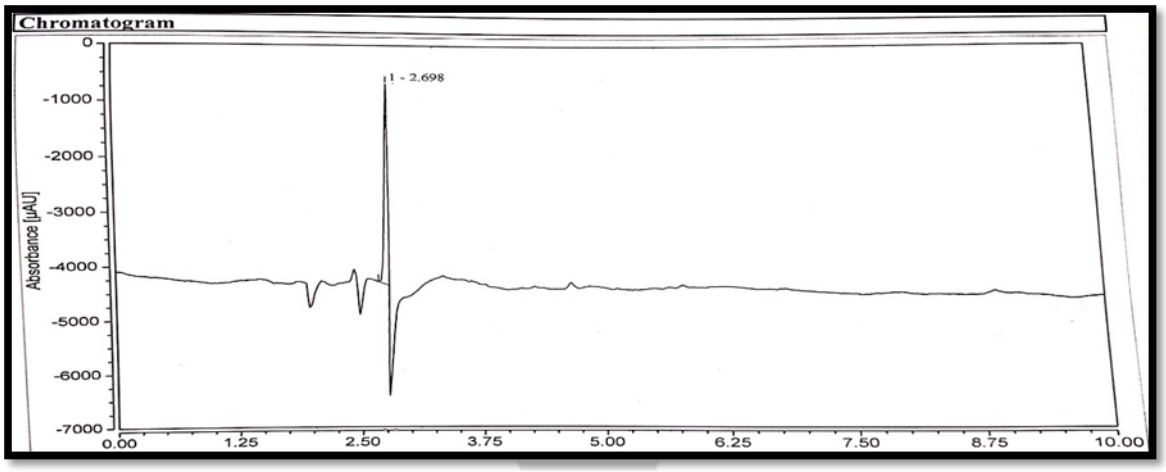

Fig. No. 3: Chromatogram of Blank Solution

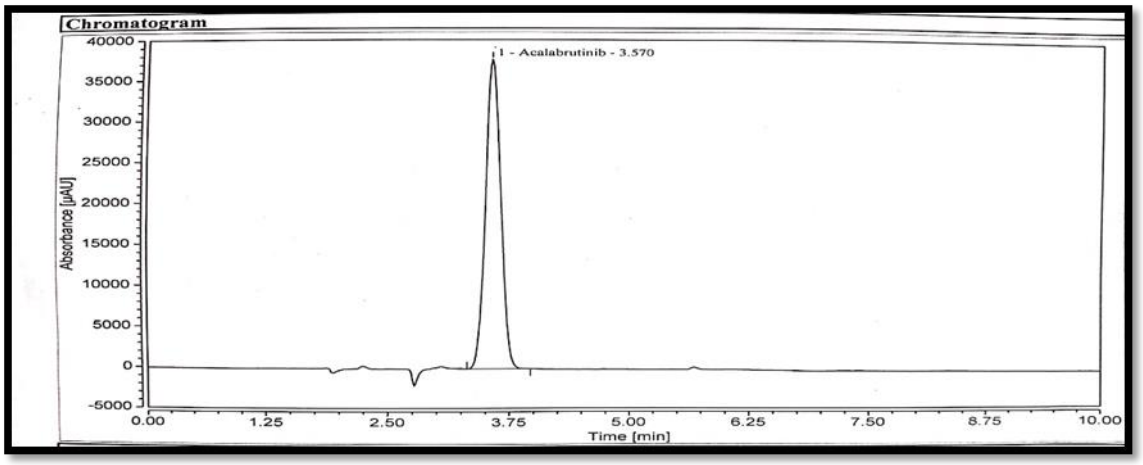

Fig. No. 4: Chromatogram of Acalabrutinib Standard Solution

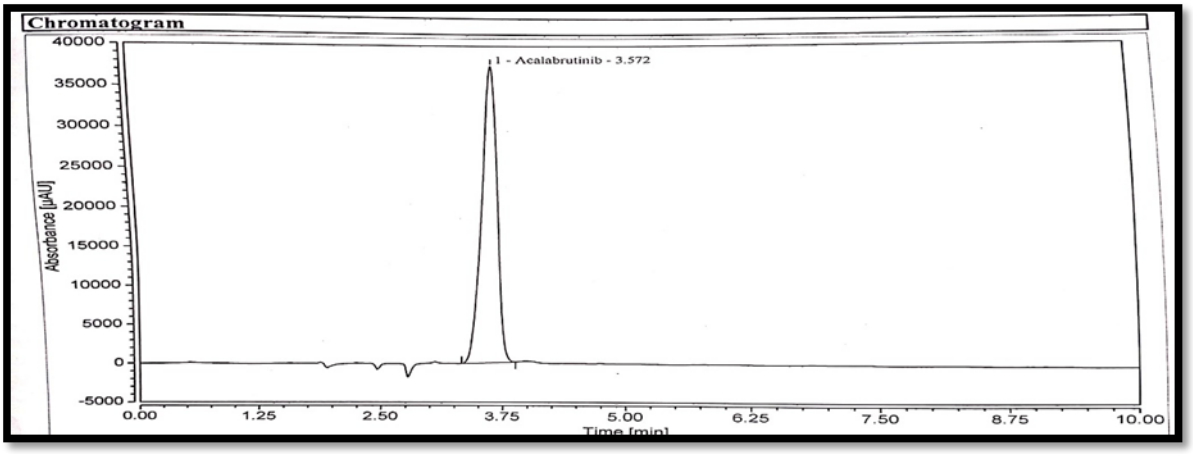

Fig. No. 5: Chromatogram of Acalabrutinib Sample solution

Linearity -

Linearity was studied by preparing a standard solution at six different concentration levels. The linearity range was found to be 5-20 ug/ml prepared from the standard stock solution of 500 ug/ml. A calibration curve was plotted by taking the concentration level of the drug on X-axis and the corresponding peak area on Y-axis and linearity was found to be 0.9997. The developed method is showing good linearity over the range of 5-20 ug/ml.

Table No. 2: Linearity

Linearity level	Concentration (ug/ml)	Peak Area
1	5	3810
2	7.5	5568
3	10	7283
4	12.5	9220
5	15	11111
6	20	14811

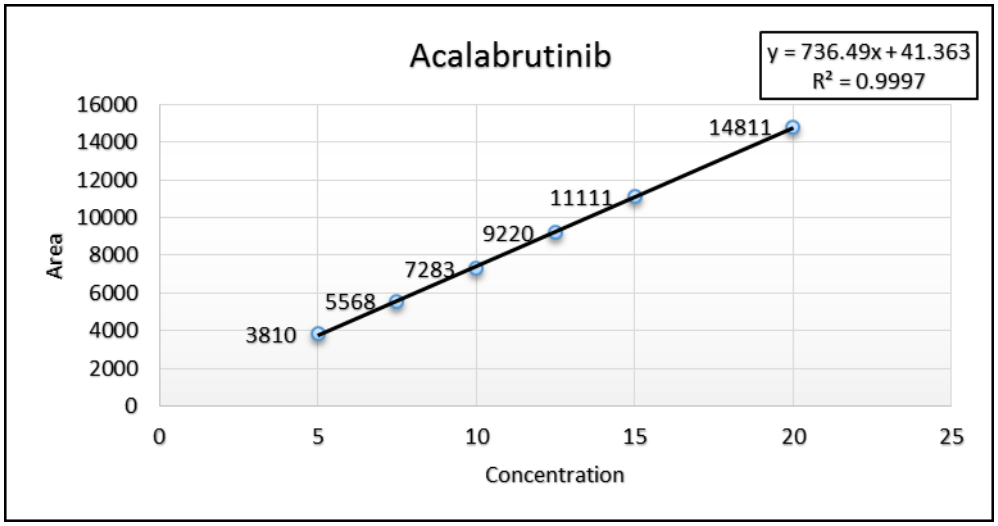

Fig. No. 6: Linearity Curve of Acalabrutinib

Precision:-

System Precision- six injections of standard solution (10ug/ml) were injected into the HPLC. The standard deviation and relative standard deviation of 6 replicate injections were calculated and reported.

Method Precision-6 injections of the sample solution (10ug/ml) were injected into the HPLC. The standard deviation and relative standard deviation of 6 replicate injections were calculated and reported.

Table No. 3 (a): System precision of Standard sample

System Precision (Standard)	
Injection No.	**The area at 230 nm**
1	7019
2	7028
3	6936
4	6933
5	6887
6	6988
MEAN	6965
SD	55.56
% RSD	0.7976
Limit	NMT 2.0%

Table No. 3 (b): Method precision of sample

Method Precision (sample)	
Injection No.	**The area at 230 nm**
1	6954
2	6959
3	6927
4	6955
5	6966
6	6886
MEAN	6941
SD	29.8
% RSD	0.4293
Limit	NMT 2.0%

Accuracy -

Accuracy is defined as the closeness of agreement between a measured quantity value and a true quantity value. Accuracy was determined by the method of recovery experiments, by the determination of % mean recovery of the sample at 3 different levels (110, 120, and 130%). At each level, three determinations were performed. The Percentage recovery and standard deviation of the percentage recovery were calculated.

Table No. 4: Accuracy of standard solution

% Level added	STD Spiked (ug/ml)	Amount Recovered (mg)	% Recovery	Mean % Recovery
110	5	112.10	101.9	101.7
110	5	111.68	101.5	
110	5	111.90	101.7	
120	10	122.44	102.0	102.0
120	10	122.41	102.0	
120	10	122.46	102.0	
130	15	132.50	101.9	101.9
130	15	132.56	102.0	
130	15	132.39	101.8	
			MEAN	101.73
			SD	0.1543
	Limit	NMT 2.0%	% RSD	0.1516

Robustness -

The robustness of the method was established by a deliberate change in detection wavelength by ±2nm, change in the Temperature by ±2°C, change in mobile phase composition by ±5ml and flow rate by ±0.2ml in the estimation of the capsule. The reproducible results were obtained which proves that method is robust.

Table No. 5: Robustness of Acalabrutinib

Parameter	Change in parameter	% Estimation	Mean	SD	% RSD
Wavelength (±2nm)	228	101.17	101.127	0.4371	0.4322
	230	100.67			
	232	101.54			
Temperature (±2°C)	38	98.06	99.8948	1.5923	1.5940
	40	100.94			
	42	100.67			
Flow rate (±0.5 ml/min)	0.8	100.96	101.01	0.7121	0.7050
	1	100.32			
	1.2	101.74			
Mobile phase	45,55	101.99	100.50	1.6139	1.6059
	50,50	100.7			
	55,45	98.8			
		limit			NMT 2.0%

Assay-

Six sample preparation of Acalabrutinib were prepared and injected into the HPLC. The mean, standard deviation, and % RSD of assay percentage of Acalabrutinib sample solution was calculated. The limit for the assay is not less than 98% and not more than 102% of the labeled content.

Table No. 6: Assay of Acalabrutinib

Sr. No.	Weight of standard (mg)	Sample Weight (equivalent to100 mg)	Area of standard at 230 nm	Area of sample at 230nm	% Assay
1	10.3	1 capsule	6980	6915	100.3
2		1 capsule		6959	100.9
3		1 capsule		6939	100.7
4		1 capsule		6942	100.7
5		1 capsule		6979	101.2
6		1 capsule		6955	100.9
				Mean	100.7
				SD	0.3089
	Limit- 2.0%			% RSD	0.3065

.RESULTS AND DISCUSSION:

Simple and precise RP-HPLC methods have been developed for the determination of Acalabrutinib in tablet dosage forms. The optimized chromatographic condition was found satisfactory to yield a well retained, sharp, and symmetrical peak at 10 min. The results of linearity studied over the concentration range 5-20 µg/mL showed the linear detector response with a correlation coefficient of 0.9997. Good recovery of the spiked drug was obtained by the standard drug addition method at each added concentration, indicating that the method was accurate.

In the case of specificity, no interference was observed from blank (diluents) and at the retention time of the Acalabrutinib peak. Therefore, the developed HPLC method for the determination of Acalabrutinib is specific.

In the case of robustness, it was performed by changing various parameters such as wavelength, flow, temperature, and the ratio of mobile phase, and it was observed that the method was robust through the validation report, it can also be concluded that the system is suitable for analysis.

The number of Average theoretical plates was 2333 and the tailing factor was 1.09 for Acalabrutinib, which indicates the efficient performance of the column.

CONCLUSION:

The proposed RP-HPLC method developed for the qualitative and quantitative determination of Acalabrutinib in tablet dosage form was simple, selective, sensitive, accurate, precise, and rapid. The developed method is better than earlier published articles with respect to superior System Suitability Parameter such as Theoretical plates, tailing factor. The method was validated as per ICH guidelines and validation acceptance criteria were met in all cases. Thus, the described method is suitable for routine analysis and quality control of pharmaceutical formulation i.e., tablets dosage form.

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