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Stability indicating RP-HPLC method development and validation for the determination of Acalabrutinib in bulk drug and capsule dosage form

Anusha A.*, Pushpa Latha E. Uttam Prasad Panigrahy, Rama Mohan Reddy T., Abbulu K.
Department of Pharmacy Analysis, CMR College of pharmacy, Kandlakoya, Medchal dist., Telangana, India.

Abstract: Simple, rapid, accurate, precise and reproducible stability indicating RP-HPLC method for the estimation of Acalabrutinib in bulk and Capsule dosage form was developed and validated as per ICH guidelines. The separation was done using Zodiasil C_{18} 150 x 4.6 mm, 5 µ column. The mobile phase (Water and methanol 60:40%v/v) was pumped at 0.8ml/min and effluent was detected at 230nm using a PDA detector. The retention time was 2.76 ± 0.1 min and the method produced a linear response in the concentration range of 25-150 µg/ml (r^2- 0.9997). In recovery studies, %RSD from reproducibility was found to be below 2%. LOD and LOQ were 0.03 µg/ml and 0.08µg/ml respectively. The drug was subjected to different stress conditions such as acidic, alkaline, oxidative, photothermal and hydrolysis. The drug showed more degradation in acidic condition and no degradation was observed in hydrolysis and photo condition. The developed RP-HPLC method was found to be effective, sensitive and specific for the estimation of Acalabrutinib in bulk and Capsule dosage form.

Key words: Acalabrutinib; RP-HPLC; Validation

Introduction

Acalabrutinib (fig.1), is a highly selective Bruton's tyrosine kinase inhibitor, is associated with high overall response rates and used for treated chronic lymphocytic leukemia [1]. Acalabrutinib binds covalently to Cys481 in the ATPbinding pocket of BTK [2]. Acalabrutinib is chemically, (4-[8- amino-3-[(2S)-1-but-2-ynoylpyrrolidin-2-yl]imidazo[1,5-a]pyrazin1-yl]-N-(2-pyridyl)benzamide)] [3]. It is freely soluble in water at pH values below 3 but is practically insoluble in water at pH values above 6 [4]. Literature survey revealed that only one RP-HPLC based method has been reported for the estimation of Acalabrutinib [5]. The aim of the present work was to develop a novel, simple, rapid, sensitive, specific, accurate, precise, economic and reliable stability indicating RP-HPLC method for the estimation of Acalabrutinib in bulk and Capsule dosage form suitable for quality control analysis.

Materials and Methods

Chemicals

Acalabrutinib working standard was received as gift sample and sample Capsules (Label claim: 100mg; Calquence Capsule) were procured from Spectrum Labs pvt ltd, Hyderabad. HPLC grade Methanol and water were purchased from Merck Specialities Private Ltd., Mumbai and Rankem Laboratories, Haryana.

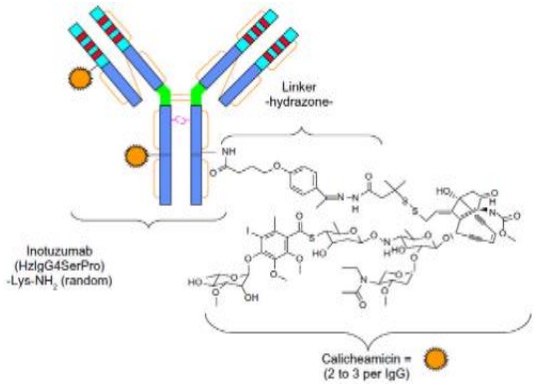

Figure 1: Structure of Acalabrutinib

Instrument

Waters HPLC 2965 system with auto injector and PDA detector integrated with Empower 2 Software was used for LC peak integration and Data processing.

Chromatographic Conditions

Chromatographic separation was performed on Zodiasil C_{18} 150 mm x 4.6 mm, 5 µm column at 30°C. The Capsule volume for standard and sample was 10 µL. Mobile phase used was water: Methanol

Corresponding Author:
E. Pushpa Latha,
Department of Pharmaceutical Analysis,
CMR College of Pharmacy, Hyderabad, India.
E-mail: latha.pushpa999@gmail.com

in the ratio 60:40 (v/v), with a flow rate 0.8 ml/min. samples were analysed by using PDA detector at wavelength 230 nm and the run time is 6 min.

Diluent: HPLC water: Methanol (60:40%v/v)

Preparation of standard stock solution
About 25 mg of Acalabrutinib working standard was accurately weighed and transferred into 25 ml volumetric flask, dissolved in diluent, filtered through 0.45 µm Ultipor N66 nylon filter and the volume was made up to the mark with the diluent to get 1000 µg/ml of Acalabrutinib.

Preparation of standard solution
Acalabrutinib (100 µg/ml) was prepared from the standard stock solution by pipetting out 1ml and made upto 10ml with the diluent. Accurately 10 µl was injected into the HPLC system and chromatogram was recorded.

Preparation of Sample solution
5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was poured into a 100 ml volumetric carafe, 50ml of diluents was added and sonicated for 25 min, further the volume was finished up with diluent and filtered by HPLC filters. 5ml of filtered sample stock solution was poured to 10ml volumetric flask and completed up with diluent. (100µg/ml of Acalabrutinib)

Validation of The Developed Method
The method developed was validated as per ICH guidelines [6] for linearity, accuracy, precision, LOD, LOQ, ruggedness and specificity.

Accuracy
The accuracy of the method was determined by calculating % recovery. A known amount of Acalabrutinib was added to placebo and the amounts were estimated by measuring the peak area. The studies were carried in triplicate manner (50%, 100%, and 150%).

Specificity
It is the ability of analytical method to measure the response of the analyte and have no interference from other extraneous components and well resolved peaks are obtained.

Linearity
Linearity solutions were prepared by pipetting out 0.25 – 1.5 ml from the Stock solution of Acalabrutinib into 6 different volumetric flasks and diluted to 10ml with diluent to get 2.5-15 µg/ml.

Limit of detection (LOD) and Limit of quantification (LOQ)
The LOD and LOQ for Acalabrutinib were found to be 0.03µg/ml and 0.08µg/ml respectively based on signal to noise ratio where s/n for LOD is 2.95:1 (limit-3:1) and LOQ is 9.8:1 (limit-10:1)

Robustness
Robustness of the method was determined by making slight changes in the composition of mobile phase ± 10%, flow rate by ± 0.1 ml/min and temperature by ±5° C. Retention time and chromatograms were determined for the drug.

System suitability
The system suitability parameters like Retention time, USP theoretical plates, USP tailing, and peak area and peak height were calculated.

Stability
Stability of both the standard and sample solutions was tested during analysis up to 24hours at room temperature.

Degradation studies
Acid degradation studies: 1 ml of Acalabrutinib stock solution was exposed to 2N HCL and refluxed at 60°C for 30 min.

Alkali Degradation studies: 1 ml of Acalabrutinib stock solution was exposed to 2N sodium hydroxide and refluxed at 60° C for 30 min.

Peroxide degradation: To 1ml of Acalabrutinib stock solution 1ml of 20% Hydrogen peroxide (H_2O_2) was added and kept for 30min.

Thermal degradation studies: The standard drug solution was placed in oven at 105°C for 6 hours to study dry heat degradation.

Photo stability studies: The photochemical stability of the drug was also studied by exposing the solution to UV light by keeping the beaker in UV chamber for 7 days or 200-Watt hours/m^2 in photo stability chamber.

Water degradation studies: stress testing under neutral conditions was studied by refluxing the drug in water for 6 hours at a temperature of 60°C.

Results and Discussions

Optimized Chromatographic Conditions:
To develop and establish a suitable RP-HPLC method for estimation of Acalabrutinib in bulk and Capsule dosage form, different preliminary tests were performed and different chromatographic

conditions were tested and optimized chromatographic conditions were developed which were given in Table-1. The final analysis was performed by using water: Methanol (60:40 v/v) at a flow rate of 0.8ml/min. samples were analyzed at 230 nm detector wave length and at an injection volume of 10µL using Zodiasil C18 150 mm x 4.6 mm, 5µ, with run time of 6 min. The proposed method was optimized to give sharp peak, retention time of 2.761 min with minimum tailing for Acalabrutinib. The optimized chromatogram was obtained as shown in (Figure 2).

Assay was performed for Capsule formulation and the mean % purity obtained was 100.18%. The result was shown in (Table 3) and the chromatogram of sample solution was shown in (Figure 3) respectively.

Validation

The developed method was found to be specific as no interference was observed in blank and placebo at the retention time of the drug.

The linearity was established (2.5-15 µg/ml) at six different concentrations each were injected in a duplicates and average areas were determined and linearity equations were obtained as y = 226879x + 8517.3, correlation coefficient (R^2) was determined as 0.9997. The Linearity calibration curves were plotted as shown in (Table 2 and Figure 4).

Three levels of Accuracy samples 50%, 100%, 150% were prepared and triplicates of Capsules were given for each level of accuracy and mean %Recovery was obtained as 100.36% was shown in (Table 5).

% RSD for precision studies was calculated from the corresponding peak areas obtained by injecting six times a known concentration of Acalabrutinib was obtained as 0.750% and 0.976% for repeatability and intermediate precision respectively which is within the limits (<2%). Hence the method was precise shown in (Table 5). The values were evaluated based on Relative standard deviation of response and slope of the calibration curve Acalabrutinib. The detection limit values were obtained as 0.03 µg/ml and Quantification limit were fund to be 0.08 µg/ml as given in (Table 1).

Robustness of the method was studied by changing the chromatographic conditions slightly and results were presented in (table 6). From the method developed it was observed that there were no significant changes in the retention time and area of the chromatograms by making slight alterations in temperature, mobile phase composition and flow rate. The % RSD was less than 1%, which demonstrated that the RP-HPLC method developed was robust. Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and the % degradation is calculated shown in (Table 7 and Figure 5-10).

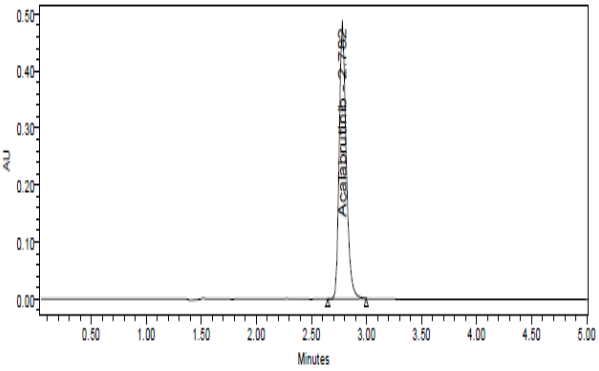

Figure 2: Standard Chromatogram of Acalabrutinib

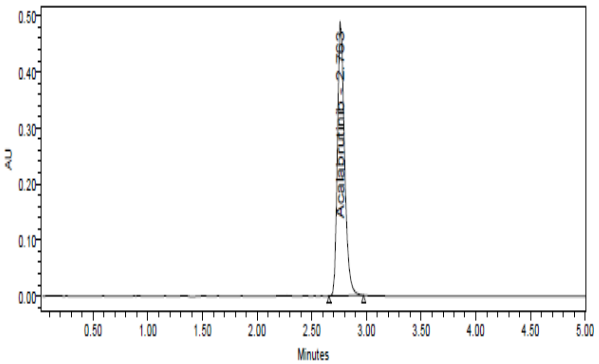

Figure 3: Sample Chromatogram of Acalabrutinib

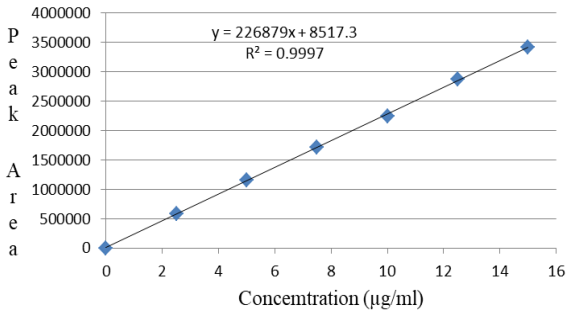

Figure 4: Linearity Curve of Acalabrutinib

Table 1: Optimized Chromatographic Conditions

Parameter	Condition
HPLC	WATERS HPLC with PDA detector
Column	Zodiasil C18 150mm x 4.6 mm, 5µm
Mobile phase	Water: Methanol (60: 40v/v)
Flow rate	0.8ml/min
Detector wave length	230nm
Column temperature	30°C
Capsule volume	10µL
Run time	6 min
Diluent	Water: Methanol (60: 40v/v)

Table 2: System suitability and validation parameters of the developed method

Parameter	Acalabrutinib
Theoretical plates	9238
Tailing factor	1.24
Retention time (min)	2.76
Linearity range (µg/ml)	2.5-15µg/ml
Regression equation Y=mx+c	y = 226879x + 8517.3
Slope (m)	226879
Intercept (c)	8517.3
Correlation coefficient	0.9997
Percent RSD	< 2
Precision Repeatability (n=6)	0.750
Intermediate Precision (n=6)	0.976
LOD (µg/ml)	0.033
LOQ (µg/ml)	0.08

Table 3: Assay Results of Acalabrutinib

Formulation	Label claim (mg)	Amount found (mg) (n=3) Mean ± SD	Assay	% RSD
Calquence	100 mg	100.18 ± 0.0027	100.18	0.749

Table 4: Accuracy results of Acalabrutinib

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean % Recovery
	50	50.05	100.09	
50%	50	50.76	101.51	
	50	49.96	99.91	
	100	98.31	98.31	
100%	100	100.99	100.99	100.36%
	100	101.49	101.49	
	150	152.42	101.62	
150%	150	146.38	97.59	
	150	152.58	101.72	

Table 5: Precision Result of Acalabrutinib

S.No.	Repeatability	Intermediate precision
1.	2080101	2060116
2.	2070509	2039318
3.	2097546	2048409
4.	2052511	2065415
5.	2086529	2015736
6.	2069767	2022743
Mean	2076161	2041956
S.D.	15572.3	19926.6
% RSD	0.750	0.976

Table 6: Robustness Data of Acalabrutinib

Parameter	Proposed	Modification	% RSD	Retention time (min)
Flow Rate (± 0.1ml/min)	0.8	0.9	0.3	1.910
		0.7	1.6	2.096
Mobile Phase (±10%) (W:M)	60:40	45:55	1.0	2.060
		65:35	1.0	2.838
Temperature (± 5°C)	30°C	35° C	0.4	1.926
		25° C	1.9	2.285

Table 7: Degradation Data of Acalabrutinib

S.No.	Degradation Condition	% Drug Degraded
1	Acid	3.36
2	Alkali	5.12
3	Oxidation	3.95
4	Thermal	1.05
5	UV	1.04
6	Water	1.04

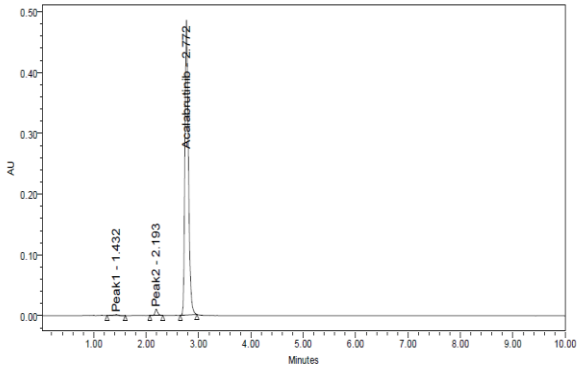

Figure 5: Acid degradation chromatogram of Acalabrutinib

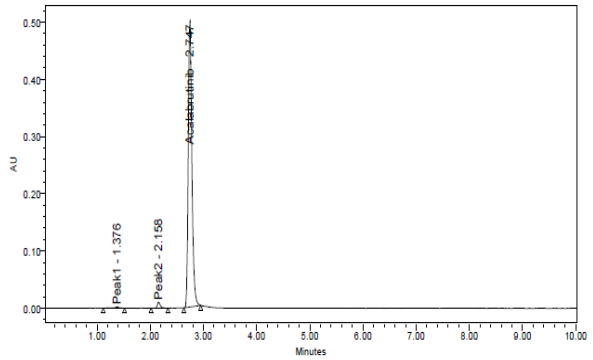

Figure 6: Base degradation chromatogram of Acalabrutinib

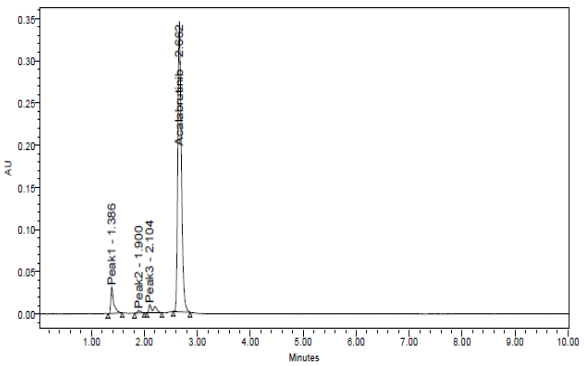

Figure 7: Peroxide degradation chromatogram of Acalabrutinib

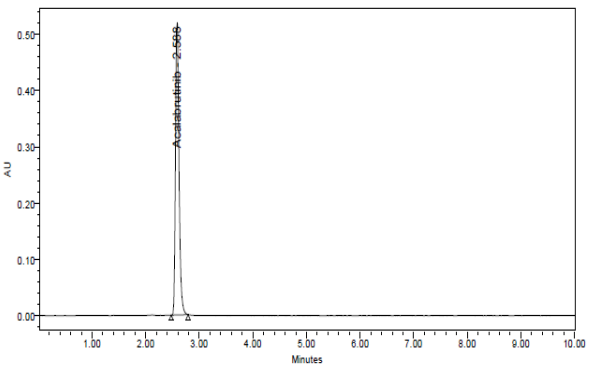

Figure 8: Thermal degradation chromatogram of Acalabrutinib

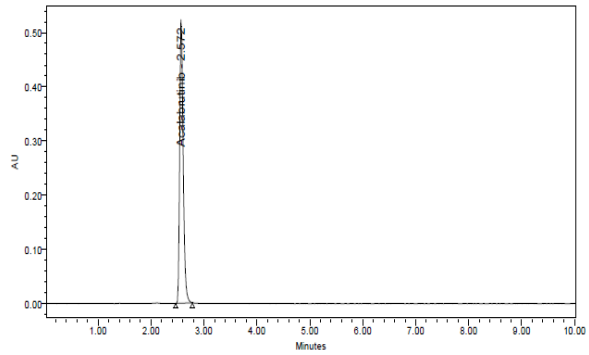

Figure 9: UV degradation chromatogram of Acalabrutinib

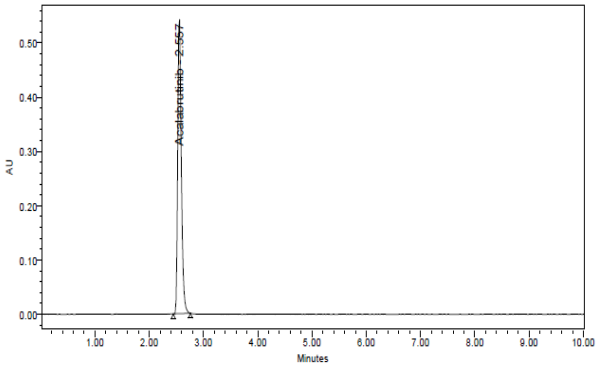

Figure 10: Hydrolytic degradation chromatogram of Acalabrutinib

Conclusion

A simple, Accurate, precise method was developed for the estimation of Acalabrutinib in bulk and pharmaceutical dosage form. Retention time of was found to be 2.761 min. % RSD was found to be 0.750 and 0.976 for repeatability and intermediate precision respectively. % Recovery was obtained as 100.36%. LOD, LOQ values were obtained from regression equations of Acalabrutinib was 0.03 µg/ml and 0.08 µg/ml respectively. Regression equation of Acalabrutinib is y = 226879x + 8517.3. Hence the method developed was simple and economical that can be adopted in regular Quality control analysis in Industries.

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