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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR DETERMINATION OF NEW ANTICANCER AGENT ACALABRUTINIB IN BULK AND ITS PHARMACEUTICAL FORMULATION

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ABSTRACT

A novel isocratic reverse phase liquid chromatography method for determination of Acalabrutinib was developed and validated after optimization of various chromatographic conditions.Chromatographic conditions used are stationary phase kromosilC18 column (250×4.6 mm5m) with mobile phase 0.1% O-Phosphoric acid and methanol in ratio of 50:50 and flow rate was maintained at 1.0 ml/min, detection wavelength was 236nm,column temperature was set to 30^0C and diluents was mobile phase conditions were finalized as optimized method.System suitability parameters were studied by injecting the standard six times and results were under acceptance criteria. Linearity study was carried out between 25% to 150% levels, R value was found to be 0.4 for repeatability and 0.8for intermediate precision.LOD and LOQ are 0.73µg/ml respectively. The retention time of Acalabrutinib was 2.6min. The linearity for Acalabrutinib was in the range of 25-150 µg mL-1 with coefficient of correlation 0.999. The proposed method was validated with respect to linearity, accuracy, precision and robustness.

KEYWORDS: Acalabrutinib, RP-HPLC, Validation.

INTRODUCTION

The chemical name of the active substance is 4- [8-amino-3-[(2S)-1(but-2-ynoyl) pyrrolidine-2-yl]imidazo[1,5-a]pyrazin-2-yl)benzamide.. The molecular formula of active substance is $C_{26}H_{23}N_7O_2$, its relative molecular mass 465.517 g/mol.

Accalabrutinib is a solid form new molecular entity, soluble in water, soluble in 15mg/ml ethanol,soluble in DMSO and DMF at 25 mg/ml. The pH of a saturated acalabrutinib solution in water is 4.2 at 23.5 °C, its pKa is 12.34.

Acalbrutinib is claimed to be a novel cancer pharmacologic agent with high affinity and potency inhibitor of Bruton Tyrosine Kinase (BTK) proposed for treatment of patients with mantle cell lymphoma(MCL). Both Acalabrutinib and its active metabolit ,ACP-5862,act to form a covalent bond with cystiene 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.

Acalabrutinib is currently indicated for the treartment of adult patient with Mantle Cell Lymphoma (MCL) who have received at least one prior therapy.

Literature review reveals that only two analytical methods has been carried on acalabrutinib drug i.e UV[1] and Flourimetry.

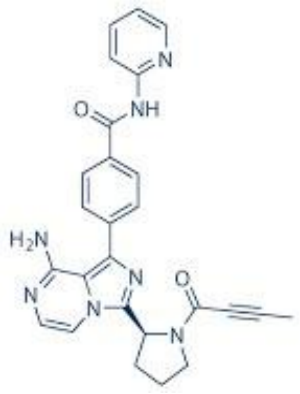

Fig 1: Structure of Acalabrutinib.

MATERIALS AND METHODS

2.1. Chemicals and Reagents

HPLC grade Acalabrutinib from Acerta Pharma Pvt Ltd, Mumbai.Chemicals and Whatman GFC filter were used in the study.Analyticaly pure ASP was procured as gratis

sample from Sun Pharmaceutical Pvt. Ltd., [Baroda, India]. Water HPLC grade was obtained from Rankem laboratories Capsule formulation [Acalabrutinib] (100mg), Sun pharmaceuticals Ltd., Sikkim, India] containing labeled amount 100 mg of acalabrutinib capsule was purchased from local market.

2.2. Equipments

The instrument was a Water Alliance 2695 separation module, having water 2996 photodiode array detector in isocratic mode. The system was connected with the help of Empower2 software in a computer system for data collection and processing. The analytical column used is BDS C18

2.3. Chromatographic condition

The mobile phase consists of a mixture of 0.1% O-Phosphoric acid and methanol in ratio of 50:50 was filtered through 0.45 µm nylon membrane filter before use. The injection volume was 20 µL with a flow rate 1 mL min-1 and detection wavelength 236 nm having ambient condition and run time 5 min.

2.4. Standard preparation

Stock solutions were prepared by accurately weighing 25 mg of ACT and transferring to 25 ml volumetric flasks containing 3 ml of methaol and water mix. The flasks were sonicated for 10 min to dissolve the solids. Volumes were made up to the mark with ethanol and water mix which gave 1000 µg mL-1. Aliquots from the stock solutions were appropriately diluted with mobile phase to obtain working standards of 100 µg mL-1 of drug. Typical standard chromatogram of acalabrutinib.

2.4. Sample preparation

Twenty tablets were weighed and crushed to fine powder. The powder equivalent to 25mg of Acalabrutinib was taken in a 25 mL volumetric flask and made up with methanol. The resultant mixture was filtered through 0.45 µm nylon filter. From this filtrate 10mL of solution was pipette out into 100 ml standard flask and made up with mobile phase is shown in Fig.[2]

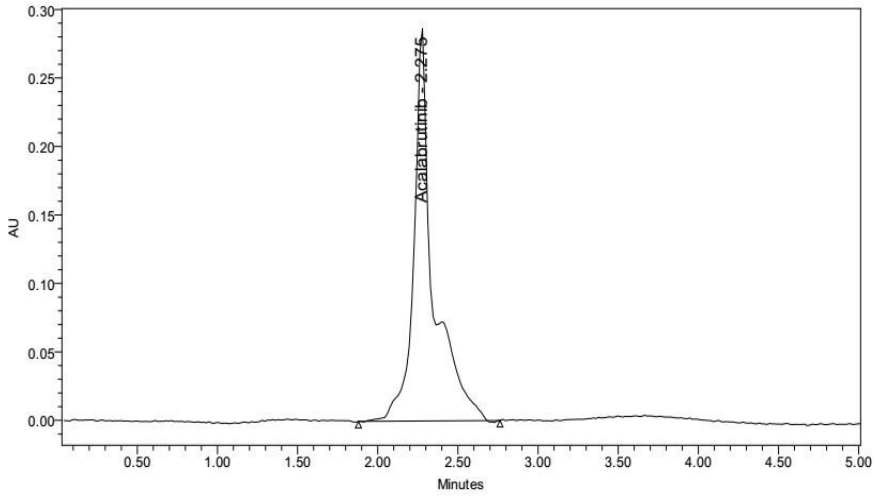

Fig 2: standard chromatogram of Acalabrutinib.

Degradation Studies: Degradation studies were performed with the formulation and the degraded samples were injected. Assay of the injected samples was calculated and all the samples passed the limits of degradation

Degradation procedure[2,3]

Oxidation

To 1 ml of stock solution of Acalabrutinib 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 60^0c. For HPLC study, the resultant solution was diluted to obtain (100ppm) solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies

To 1 ml of stock ssolution Acalabrutinib 1 ml of 2N Hydrochloric acid was added and refluxed for 30mins at 1c .The resultant solution was diluted to obtain (100ppm) solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali Degradation Studies

To 1 ml of stock solution Acalabrutinib 1 ml of 2 N sodium hydroxide was added and refluxed for 30mins at 60^0c. The resultant solution was diluted to obtain (100ppm) solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies

The standard drug solution was placed in oven at 105^0c for 6 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to (100ppm) solution and10µl were injected into the system and the chromatograms were recorded to assess the

stability of the sample**.**

Photo Stability studies

The photochemical stability of the drug was also studied by exposing the (1000ppm) solution to UV Light by keeping the beaker in UV Chamber for 7days or 200 Watt hours/m^2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain (100ppm) solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral Degradation Studies

Stress testing under neutral conditions was studied by refluxing the drug in water for 6hrs at a temperature of 60ºc. For HPLC study, the resultant solution was diluted to (100ppm) solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

RESULTS AND DISCUSSION

3.1. Estimation of Acalabrutinib in capsule dosage form

The HPLC procedure was optimized with a view to develop precise and stable assay method. Acalabrutinib was run in different mobile phase composition C18 columns Waters Xbridge [100 mm x 4.6 mm i.d.,5 µm] [Kromosil 150 mm x 4.6 mm i.d.,5 µm], column-Hiber [250 mm x 4.6 mm 5 µm] at ambient temperature [25º and 30º C].The flow rate was also varied from 0.5 mL to 1 mL min-1. The mobile phase consists of and a mixture of 0.01 N Ortho phosphoric acid [pH adjusted to 4.5] [50 volumes water] and methanol [50volumes] was filtered through 0.45 µm nylon membrane filter before use. The Column used is Hiber c18, 5 µm column having 250×4.6 mm i.d.

The sample solution was chromatographed similar to standard solution and concentrations of acalabrutinib in capsule for samples were calculated using regression equation. Typical sample chromatogram of acalabrutinib is shown in Fig [3].

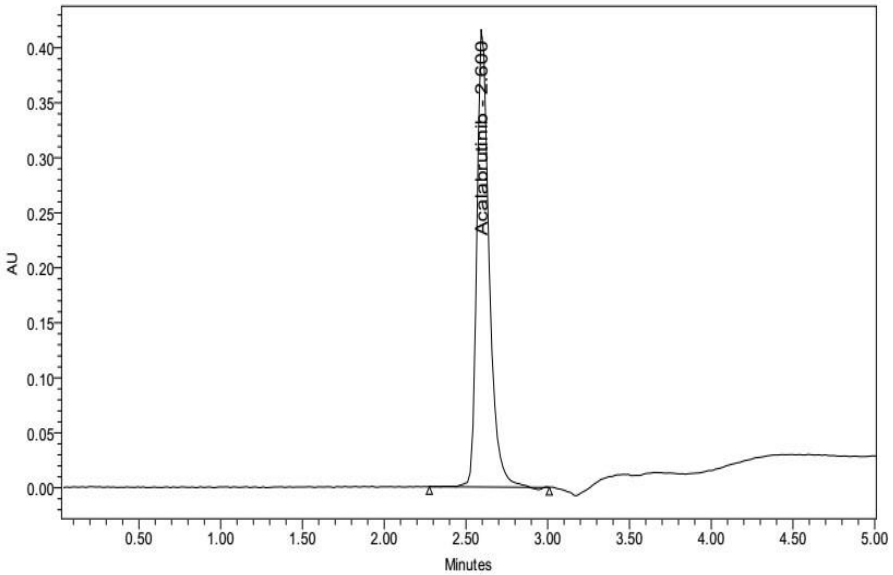

Fig 3: chromatogram of Acalabrutinib sample.

3.2. Method Validation[5,6,7]

The described method has been validated for the assay of Acalabrutinib using following parameters.

Accuracy

The accuracy of the method was determined by recovery experiments. Placebo was spiked with known quantities of Standard drugs at levels of 50 to150% of label claim. The recovery studies were carried out 3 times and the Percentage recovery and standard deviation of the percentage recovery were calculated and presented in Table.

Table1: Results of accuracy studies.

% Level	Amount Spiked(µg/mL)	Amount recovered(µg/mL)	% Recovery	Mean %Recovery
50%	50	50.144596	100.29	100.15%
	50	49.951503	99.90	
	50	50.116809	100.23	
100%	100	100.30382	100.30	
	100	100.74756	100.75	
	100	100.59414	100.59	
150%	150	150.27825	100.19	
	150	149.26287	99.51	
	150	149.34396	99.56	

The mean %recovery is well within the acceptance limit, hence the method is accurate

System suitability studies

The system suitability test was carried out on freshly prepared stock solution of Acalabrutinib to check various parameters such as column efficiency, tailing factor and number of theoretical and presented in Table 2. The values obtained were demonstrated the suitability of the system for the analysis of the drug. System suitability parameter may fall within 3% standard deviation range during routine performance of the method.

Table 2: System Suitability Studies.

	Peak Name	RT	Area	USP Plate Count	USP Tailing
1	Acalabrutinib	2.593	2073746	5825	1.33
2	Acalabrutinib	2.594	2061997	5939	1.35
3	Acalabrutinib	2.595	2066892	5979	1.30
4	Acalabrutinib	2.596	2088747	5888	1.30
5	Acalabrutinib	2.596	2072030	5596	1.30
6	Acalabrutinib	2.598	2060912	5977	1.30
Mean			2070721		
Std. Dev.			10225.4		
% RSD			0.5		

LOD and LOQ

The LOD and LOQ of the developed method were determined by injecting progressively low concentrations of the standard solutions using the developed RP-HPLCmethod. The LOD is the smallest concentration of the analyte that gives a measurable response [signal to noise ratio of 3]. The LOD for Acalabrutinib was found to be 0.24 µg mL-1.The

LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified [signal to noise ratio of 10]. The LOQ was 0.74 µg mL-1 for Acalabrutinib respectively.

Linearity and Range

Linearity was studied by preparing standard solution at five different concentration levels. The linearity range was found to be 0-150 µg mL-1. 20 µL of each solution was injected into chromatograph. Peak areas were recorded for all the chromatogram. Calibration curve was constructed by plotting peak areas [Y axis] against the amount of drugin µg mL-1 [X axis]. Peak area of linearity range and the parameters were calculated and presented in Table 3. The linearity curve of Acalabrutinib was shown in Fig [4].

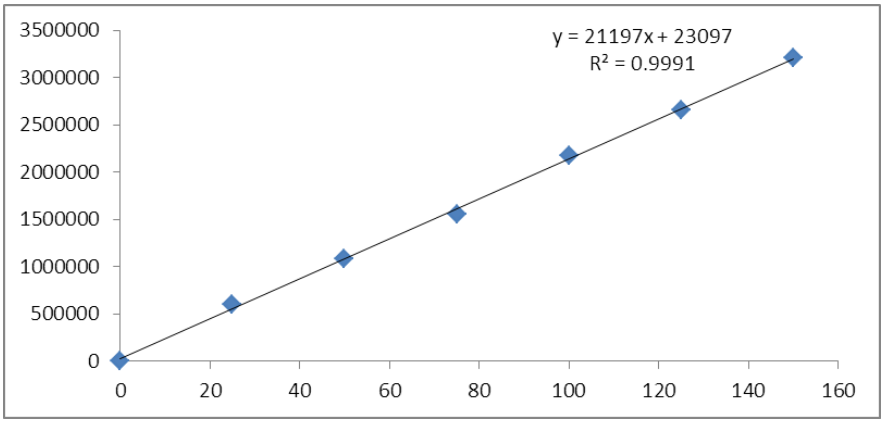

Fig 4: The linearity curve of Acalabrutinib concentartion vs peak area.

Table 3: Result of Linearity.

S.no	Pipetted from stock [mL]	Volume of flask [Ml]	Concentration µg/mL	% linearity level	Peak area
1	0	0	0	0	0
2	0.25	10	25	25	214387
3	0.5	10	50	50	406311
4	1	10	100	100	823006
5	1.25	10	125	125	1059709
6	1.50	10	150	150	1292803

Specificity

Specificity of the method was determined by injecting the diluted placebo. There was no interference of placebo with the principle peak, hence the developed analytical method was specific for Acalabrutinib in tablet dosage form.

Precision

System precision: The system precision of the method was established by six replicate injections of the standard solution containing Acalabrutinib. The percentage RSD were calculated and presented in Table 4. From the data obtained, the developed RP-HPLC method was found to be precise.

LOD: Detection limit of the Acalabrutinib in this method was found to be 0.241µg/ml.

LOQ: Quantification limit of the Acalabrutinib in this method was found to be 0.73µg/ml.

Table 4: System precision results.

S.No	Peak Area
1	2062328
2	2072525
3	2083059
4	2062408
5	2063168
6	2073211
AVG	2069450
STDEV	8349.6
%RSD	0.4

Robustness

Robustness of the method was determined by making slight change in the chromatographic condition. It was observed that there were no marked changes in the chromatograms, which. The results of robustness were presented.

Table 5. Method Robustness of Acalabrutinib in Dosage Forms

Parameter	%RSD
Flow Minus	0.7
Flow Plus	0.6
Mobile phase Minus	0.9
Mobile phase Plus	0.6
Temperature minus	0.5
Temperature plus	0.4

ASSAY OF MARKETED FORMULATION

Standard solution and sample solution were injected separately into the system and chromatograms were recorded and drug present in sample was calculated using before mentioned formula.

Table 6: Assay of Formulation.

Sample No	%Assay
1	99.20
2	99.69
3.	100.19
4.	99.20
5.	99.24
6.	99.72
AVG	99.54
STDEV	0.40
%RSD	0.4

Table 7: Degradation Data of Acalabrutinib.

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	8.65	0.220	0.285
2	Alkali	7.94	0.266	0.293
3	Oxidation	6.06	0.299	0.278
4	Thermal	3.88	0.268	0.288
5	UV	1.28	0.283	0.294
6	Water	1.28	0.242	0.292

CONCLUSION

Stability indicating RP-HPLC method was developed for the estimation of Acalabrutinib in pharmaceutical dosage form. The developed method was validated and found to be specific, accurate, precise, linear and robust. The drugs was Acalabrutinib stable under different forced degradation conditions. The developed method can be used for the rapid quantification of Acalabrutinib in its pharmaceutical dosage form.

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