DEVELOPMENT AND VALIDATION OF UV SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF ACALABRUTINIB

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Abstract

The goal of this work was to create a new, fast, and accurate UV spectrophotometric method for quantifying acalabrutinib in pure and capsule dose forms. Water & ethanol were used in the ratio of 70:30 as a diluent. The highest absorbance of acalabrutinib was measured at 294 nm, and the linearity ranged from 2.5 to 15 g/ml. The regression equation for acalabrutinib was y= 0.0583x + 0.0053, with a correlation value of 0.9995. The percentage of recovery ranged from 99.87 to 100.71 percent. The relative standard deviation for intraday precision and interday precision was determined to be less than two. Acalabrutinib's LOD and LOQ were determined to be 0.006 g/ml and 0.197 g/ml, respectively. The spectrometric technique was validated in accordance with ICH criteria and was found to be suitable for routine quantitative measurement of acalabrutinib in pure and capsule dose forms.

Keywords*: Acalabrutinib, Method development, Ethanol*

1. Introduction

Acalabrutinib is chemically 4-{8-amino-3-[(2S)-1-(but-2-ynoyl) pyrrolidin-2-yl] imidazo[1,5-a] pyrazin-1-yl}-N-(pyridin-2-yl) benzamide. The molecular formula is $C_{26}H_{23}N_7O_2$ with molecular weight 465.517 g/mol. It is an FDA-approved irreversible second-generation Bruton's tyrosine kinase (BTK) inhibitor for the treatment of adult patients with mantle cell lymphoma (MCL) who have had at least one prior therapy. 1 Acalabrutinib has also been used in clinical trials to treat myelofibrosis, ovarian cancer, multiple myeloma, and Hodgkin lymphoma. In 2017, the FDA approved calquence (acalabrutinib) developed by Astra Zeneca.[2] Because it was deliberately developed to be more effective and selective than ibrutinib, acalabrutinib is also called a second generation BTK inhibitor. It is theoretically intended to have fewer negative effects due to reduced impact on targets other than BTK.[3] Acalabrutinib also has a role as non-specific protein-tyrosine kinase inhibitor, an antineoplastic agent and an apoptosis inducer. The chemical structure of acalabrutinib was displayed in Fig 1.

A review of the literature on acalabrutinib found that LC-MS/MS methods,[4 5] and RP-HPLC methods,[6 7] were published. No UV spectroscopic method has been reported till date. The study's goal was to develop a simple, specific, and accurate UV spectrophotometric method for quantifying acalabrutinib in both its pure and capsule dose forms.

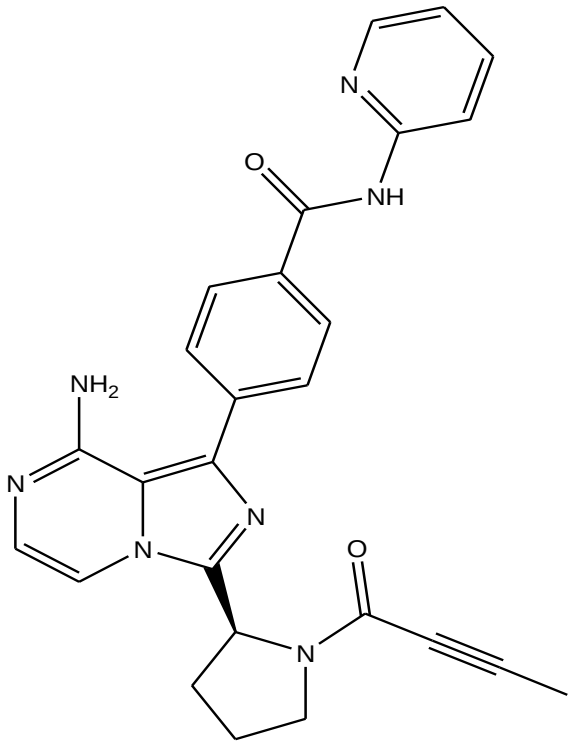

Figure 1. Structure of acalabrutinib

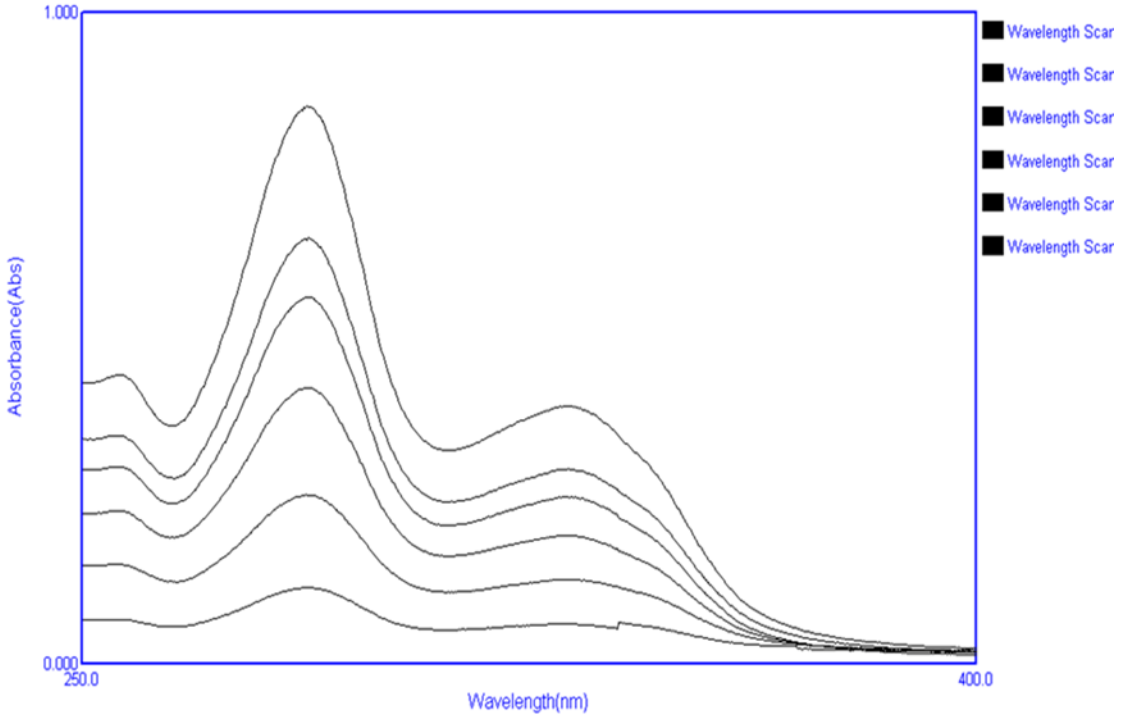

Figure 2. Overlain spectra of acalabrutinib

2. Materials and Methods

Instrumentation

The absorbance of acalabrutinib solution was measured using a PG Instrument T60 UV-VIS spectrophotometer with unique bandwidth of 2mm and 10mm. The above instrument consists of matched quartz cells combined with UV win 6 Software. For measuring purposes, an electronic balance was employed. Pipettes and volumetric flasks made of borosilicate glass were used in the experiment.

Chemicals and reagents

Spectrum pharma research solutions (Hyderabad, India) provided a free sample of acalabrutinib. The chemicals utilised were all of analytical grade.

Preparation of drug stock solution

Ten milligrams of acalabrutinib was accurately weighed and transferred to volumetric flask (10 ml). Then the volume was made up diluent and a concentration of 1000 µg/ml was achieved.

Preparation of working standard solutions

1ml of acalabrutinib was accurately weighed and transferred to volumetric flask (10 ml). The volume was then filled up with diluent and a concentration of 100 µg/ml was achieved.

Preparation of solvent

The solvent was prepared by using 70 ml of water and it was added to 30 ml of ethanol in a 100 ml volumetric flask.

Preparation of calibration curve

Six volumetric flasks of volume 10 ml were used in this experiment. From working standard solution 0.25 - 1.50 ml samples were taken into volumetric flasks and made up with water: methanol (70:30 v/v) to get 2.5-15 µg/ml solutions. The solutions were scanned with a UV-Visible spectrophotometer in the 200-400 nm UV range.

Method validation

Linearity

Appropriate volumes of samples from acalabrutinib, the usual working solution was transferred to a volumetric flask (10ml). The volume was adjusted to water: ethanol (70:30 v/v) and solutions with concentrations (2.5-15 µg/ml) were achieved. Each solution's absorbance was measured, and a calibration curve was created by graphing absorbance vs concentration. [8]

Accuracy

Accuracy was measured at 50%, 100%, and 150% by adding a known amount of acalabrutinib standard stock solution to the sample stock solution. The recovery was confirmed by estimating the medication in duplicate preparations at each prescribed concentration level and calculating the percent RSD. [9]

Precision

Precision was calculated by inter day and intraday variation. In intraday study, on the similar day three different solutions of same concentration were prepared and analysed for six times. In the inter-day precision investigation, the identical concentration solutions were made and analysed for three consecutive days, and absorbances were recorded. Percentage RSD was determined.[10]

Sensitivity

The lower limit of quantification and the limit of detection were derived by means of the subsequent equations based on the slope of the calibration curve and the SD of responses. [11]

LOD = 3.3 × Standard deviation (SD) / slope

LOQ = 10 × Standard deviation (SD) / slope [12]

Robustness

Two samples of same concentration (10µg/ml) were prepared and analysed by small adjustments in the UV wavelength settings (±2 nm).[13]

Assay

Acalabrutinib (Brand name: calquence 100mg) was used for assay. Ten capsules were weighed, and ten mg were transferred to a volumetric flask (100 ml) and dissolved in diluent. The flask was sonicated for 10 minutes. The solution was filtered and diluted with water. Aliquots of sample solutions were placed in a volumetric flask of 10 ml. Diluent was used to modify the volume. The absorbance was measured at 294nm.

3. Results and Discussion

Linearity

The ability to obtain test findings that were proportional to analyte concentration in samples within an appropriate range was termed as linearity. The developed approach showed linearity in the 2.5-15 g/ml range. The overlain spectra of acalabrutinib was plotted in fig 2. For acalabrutinib, the linearity equation was found to be y = 0.0583x + 0.0053, with a correlation coefficient of 0.9995. From the obtained linearity data, the coefficient of correlation was found to be less than 1. Hence the results were within the acceptable limits. The linearity result was illustrated in table 1 and the graph was plotted in fig 3.

Table 1. Linearity data of acalabrutinib

Conc(µg/ml)	Absorbance
2.5	0.147
5	0.295
7.5	0.444
10	0.597
12.5	0.739
15	0.871

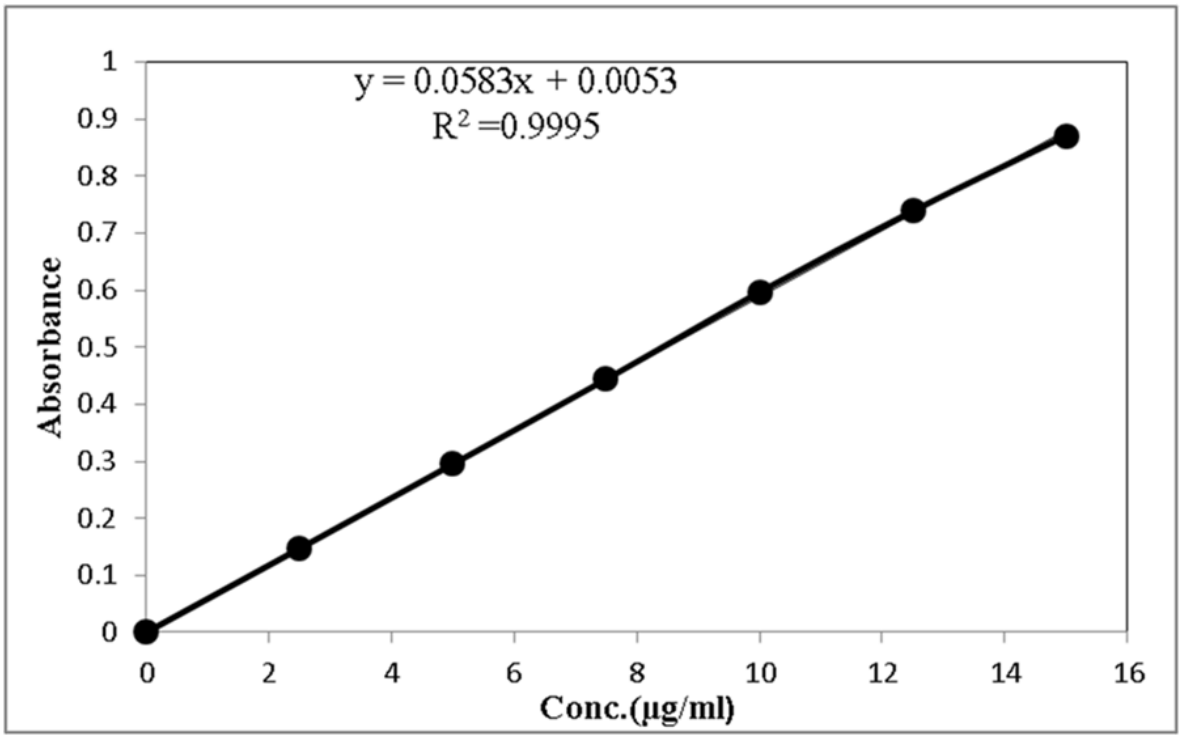

Figure 3. Calibration curve of acalabrutinib

Accuracy

Accuracy was the degree to which the measured value agrees with the accurate value. Mean percentage recovery of acalabrutinib was found between 99.87-100.71% and can be concluded that the results were within the limits. The observed data was within the range, indicating that the suggested analytical method had good recovery and accuracy. Accuracy results were shown in table 2.

Table 2. Accuracy data of acalabrutinib

% Of recovery level	Pure drug added(µg/ml)	Drug recovered(µg/ml)	*Recovery (%)	RSD (%)
50%	5.20	5.43	100.46	0.43
100%	10.07	10.07	99.87	
150%	15.00	15.10	100.71	

*Mean of three determinations at each level

Intraday precision

Precision was defined as the degree of agreement between individual test findings when the procedure was tested on several uniform samples. Percentage relative standard deviation for intraday precision was found between 0.21-1.41. The percentage relative standard deviation of precision studies was less than 2 and within the acceptable range. The intraday precision results were shown in table 3.

Table 3. Intraday precision

Concentration taken (µg/ml)	Absorbance	Concentration found (µg/ml)	% Assay	SD	%RSD
10	0.588	9.99	99.9	0.141	1.41
	0.587	9.97	99.7		
10	0.586	9.96	99.6	0.141	1.41
	0.588	9.99	99.9		
10	0.584	9.92	99.2	0.049	0.49
	0.588	9.99	99.9		
10	0.585	9.94	99.4	0.014	0.14
	0.586	9.96	99.6		
10	0.586	9.96	99.6	0.007	0.07
	0.587	9.97	99.7		
10	0.586	9.96	99.6	0.021	0.21
	0.588	9.99	99.9		

Inter day precision

Percentage relative standard deviation for interday precision was found to be 0.14-1.41. The percentage relative standard deviation of precision studies was less than 2 and within the acceptable range. The intraday precision was summarised in table 4.

Table 4. Inter day precision

Concentration taken (µg/ml)	Absorbance	Concentration found (µg/ml)	%assay	SD	%RSD
10	0.586	9.96	99.6	0.141	1.41
	0.585	9.94	99.4		
10	0.587	9.97	99.7	0.141	1.41
	0.588	9.99	99.9		
10	0.588	9.99	99.9	0.021	0.21
	0.586	9.96	99.6		
10	0.584	9.92	99.2	0.049	0.49

	0.588	9.99	99.9		
10	0.586	9.96	99.6	0.007	0.07
	0.587	9.97	99.7		
10	0.584	9.92	99.2	0.014	0.14
	0.585	9.94	99.4		

Robustness

Robustness was an estimation of its capacity to remain unchanged by little but planned changes in analytical process settings and provided a hint of its consistency throughout usage. %RSD were found to be 0.17 and can be reported that it was within the limit. The robustness data also represented that the values were within the limits The results of robustness were illustrated in table 5.

Table 5. Robustness data of acalabrutinib

Wavelength (nm)	Concentration (µg/ml)	Absorbance	Wavelength (nm)	Concentration ((µg/ml)	Absorbance
292	10	0.588	296	10	0.587
	10	0.589		10	0.589
	10	0.587		10	0.588
	Average	0.588		Average	0.588
	SD (±)	0.001		SD (±)	0.001
	(%) RSD	0.17		(%) RSD	0.17

Assay

Percentage purity was found to be 99.87%. According to the label claim, the drug content obtained from the values of sample solutions was found to be in the permissible range of 90–110 %. The assay data was illustrated in table 6. The developed technique was validated according to ICH guidelines. The summary of the results was tabulated in table 7.

Table 6. Assay data of acalabrutinib

Brand name	Available form	Label Claim	Concentration found	Assay
CALQUENCE	Capsule	100mg	99.87	99.87

Table 7. Summary of validation parameters

Parameters	Obtained values
Maximum absorbance (λ max)	294nm
Linearity(µg/ml)	2.5-15
Intercept(c)	0.0053
Slope(m)	0.0583x
Intra-day precision(%RSD)	0.25-0.71
Inter-day precision(%RSD)	0.29-0.85
Recovery (%)	99.87-100.71
LOD	0.006 µg/ml
LOQ	0.197 µg/ml

4. Conclusion

The new approach was validated by establishing its precision, accuracy, robustness, sensitivity, and specificity, demonstrating its applicability for routine acalabrutinib analysis.

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Conflict of interest

The authors declare that there is no conflict of competing financial interests.

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