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UPLC method development and validation of acalabrtunib in bulk and pharmaceutical dosage form

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Abstract

The objective of the study was to develop UPLC method for the determination of purity of Acalabrutinib in API and tablets its validation. UPLC is a better technique than HPLC in terms of performance and speed, so it was selected. The method was developed using Ammonium Acetate buffer and Methanol and XBridge Shield RP18 (3mmX 50mmX2.5µm) as a stationary phase at a flow rate of 0.7 ml/min. Validation was done by linearity, precision, and robustness studies. The precision was found to be within the limits. The linearity studies indicated the drug obeys Beer's law and revealed the specified range of linearity for drug was between 62.5 µg/ml and 187.5 µg/ml. The robustness was observed from the insignificant variation in the analysis by changes in flow rate, wavelength. It can be concluded that the proposed method was simple, precise, and robust and can be useful for determination of purity of Acalabrutinib in API and dosage form by using UPLC.

Keywords: UPLC, Acalabrutinib, Active pharmaceutical ingredient (API), method development, validation.

Full length article *Corresponding Author, e-mail: santoshillendula@gmail.com

1. Introduction

Acalabrutinib 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, ACP5862,act to form a covalent bond with cystiene residue (cys481) in the BTK active site, leading to inhibition of enzymatic activity [1]. 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 treatment of adult patient with Mantle Cell Lymphoma (MCL) who have received at least one prior therapy [2].

1.1. Mechanism of action

Mantle Cell Lymphoma (MCL) is a rare yet aggressive type of B-cell non-Hodgkin lymphoma (NHL) with poor prognosis. Subsequently, relapse is common in MCL patients and ultimately represents disease progression. Lymphoma occurs when immune system lymphocytes grow and multiply uncontrollably [3]. Such cancerous lymphocytes may travel to many parts of the body, including the lymph nodes, spleen, bone marrow, blood, and other organs where they can multiply and form a mass (es) called a tumor [4].

One of the main kinds of lymphocytes that can develop into cancerous lymphomas is the body's own B-lymphocytes (B-cells). Bruton Tyrosine Kinase (BTK) is a signalling molecule of the B-cell antigen receptor and cytokine receptor pathways [5]. Such BTK signaling causes the activation of pathways necessary for B-cell proliferation, trafficking, chemotaxis, and adhesion. Acalabrutinib is a small molecule inhibitor of BTK. 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 BTK enzymatic activity. As a result, acalabrutinib inhibits BTK-mediated activation of downstream signaling proteins CD86 and CD69, which ultimately inhibits malignant B-cell proliferation and survival (Figure 1).

1.2. Aim and Objective

The aim of the present research work was to develop an innovative analytical method for the estimation of Acalabrutinib in capsule dosage form by Ultra high performance liquid chromatography (UPLC). The developed method will be validated as per ICH Guidelines Validation parameter for the developed method as per ICH Guidelines [6].

1.2.1. Objective

The literature study reveals that there are numerous analytical methods reported for quantification of Acalabrutinib alone or combination with other drugs. The study includes UV spectrophotometry, HPTLC, LC/MS, HPLC and UPLC.UPLC a special version of HPLC with the advantage of technological strides lead to a very significant increase in resolution, sensitivity and efficiency with faster results. The intrinsic worth of the method in terms of very low solvent consumption, more robust method with greater confidence, substantial cost reduction make the technology environment friendly. Objective of the present research work is to develop a simple UPLC method with better resolution and to quantify the drug with a short retention time in the selected dosage form.

1.3. Plan of work

The plan of the proposed work includes the following steps:

- The extensive survey of literature for Acalabrutinib regarding their physico- chemical properties and analytical methods forms the basis for the development of new UPLC method.
- Selection of suitable solvent for extraction of analyte present in the formulations.
- To develop initial chromatographic conditions by selection of suitable column and appropriate wavelength and pH to optimize the method.
- Development of UPLC method for the quantification of Acalabrutinib.
- Validate the developed analytical method as per the ICH guidelines .

2. Materials and methods

2.1. Methodology

2.1.1. Method development

2.1.1.1. Selection of Detection Wavelength

20 mg of Acalabrutinib was dissolved in 10ml DMSO and makeup with mobile phase. The solution was scanned from 200-400 nm the spectrum was obtained. The overlay spectrum was used for selection of wavelength for Acalabrutinib. In the entire UV visible region the drug were strongly absorbed at 231 nm. So this wavelength was selected for further studies (Figure 2).

2.2. Method validation

2.2.1. Preparation of solution

2.2.1.1. Preparation of Buffer Solution

0.5mM Ammonium acetate buffer was prepared by dissolving 38.5 g of Ammonium acetate in 1000 mL distilled water. The solution was filtered through 0.45 µ nylon filter (Figure 3).

2.2.1.2. Preparation of Mobile Phase

Prepare a mixture of buffer and methanol in the ratio of 55:45 Filter through 0.45µ Membrane filter and degas (Table 3).

2.2.1.3. Preparation of standard solution

2.2.1.3.1. Standard stock preparation

Stock was prepared by 25 mg of Acalabrutinib transferred in 20 ml volumetric flask add 10ml of DMSO and makeup with mobile phase (1250 µg/mL) (Table 4).

2.2.1.3.2. Standard preparation

Pipette out 2 ml of standard stock preparation into 20ml volumetric flask and makeup with mobile phase (125µg/mL).

2.2.1.3.3. Preparation of sample solutions

20 capsules were accurately weighed and average weight was found. Weigh accurately about 146.22 mg (25 mg of Acalabrutinib) transferred into 20 mL volumetric flask. About 10 mL of DMSO was added and sonicate in an ultrasonic bath for 15 min and then volume make up with mobile phase. Then pippete out 2ml of above solution and volume makeup with mobile phase. The solution filtered through 0.45µm nylon syringe filter. The amount of Acalabrutinib present in capsule were calculated by using the following formula: Area of Sample X Weight of STD X Potency of STD X Dilution Factor (Table 1 & 2).

2.2.1.3.4. Validation of developed method

The developed method was validated according to ICH guidelines. The method was validated in terms of specificity, system suitability, linearity, precision, accuracy and robustness Table 5).

2.2.2. System suitability

2.2.2.1. Suitability-001

2.2.2.2. Report

The % RSD for the area of five injections results should not be more than 2% (Figure 5).

2.2.2.3. Specificity

2.2.2.4. Accuracy

The closeness of agreement between the true values which is accepted either conventional new value or an accepted reference value and the value found. Based on the comparison to reference standard method (Table 6).

2.2.2.5. Precision

Precision of an analytical method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple sampling of a homogenous sample. Precision of analytical method is usually expressed as the standard deviation and relative standard deviation (Table 7).

2.2.2.6. Ruggedness

To evaluate the intermediate precision (also known as ruggedness) of the method precision as performed on different day by using different make column and different analyst (Figure 5).

2.2.2.7. Linearity and range

2.2.2.8. Preparation of standard Solution

The linearity of the method was performed by preparing the concentration range of 62.5-187.5µg/mL for Acalabrutinib, from standard stock solution. Calibration curves were constructed by plotting concentration versus area of Acalabrutinib (Figure 6).

2.2.2.9. Acceptance criteria

Correlation coefficient should be not less than 0.999 (Table 8).

2.2.2.10. Result

The developed method for Acalabrutinib was found to linear between the range (Figure 7) (Table 9).

2.2.2.11. Robustness

The robustness of the method was analysed by changing experimental, chromatographic condition. Altering in flow rate (0.7±1 mL/min), changes in column oven temperature (40±5 °C), Changes mobile phase buffer pH (3.5±0.2), changes in mobile phase composition and changes in wavelength allowable limits from actual chromatographic condition. It was noted that there is no recognizable change in mean RT and RSD and parameters fall within the limit of ≤ 2. The theoretical plate, tailing factor, resolution are found to be good. This method is robust with variability condition. The analytical condition result are shown in table 11.

2.2.2.12. Solution stability

Evaluated the stability of analytical solution by injecting the standard and sample solution at 24 hours. The results are summarized in the table 12 for standard and table 13 for sample solutions .

2.2.2.13. Acceptance Criteria

1. The % difference for % assay of standard and sample preparation between initial and different time intervals should be within ± 2.0 (Table 10).

2.2.2.14. Conclusion

The % of assay difference between the initial and respective time points of standard and sample solutions meet the acceptance limit for 24 Hours (not above 25°C). From the above results, it is concluded that the standard solution and sample solution are stable for 24 hours at room temperature (not above 25°C).

2.2.2.15. Assay of proposed method procedure

2.2.2.15.1. Preparation of sample

20 capsules were accurately weighed and average weight was found. Weigh accurately about 146.22 mg (25 mg of Acalabrutinib) transferred into 20 mL volumetric flask. About 10 mL of DMSO was added and sonicated in an ultrasonic bath for 15 min and then volume make up with mobile phase. Then pippete out 2ml of above solution and volume makeup with mobile phase. The solution filtered through 0.45µm nylon syringe filter. Separately inject both the standard and sample preparations and record the peak area responses. The % RSD is not more than 2.0. The results are summarized in the table 14.

2.2.2.15.2. Acceptance criteria

Assay value should be in the range of 90% to 110%

2.2.2.15.3. Conclusion

Test result is showing that the test method is precise. The percentage assay of Acalabrutinib is found to be 99.86 %. Results are within the limits.

3. Results and discussion

The scope of the present work is the optimization of the chromatographic condition and to develop new RP-UPLC method. A series of mobile phase were tried and the mobile phase comprising of mixture of ammonium acetate buffer : Methanol (55:45 v/v) was chosen as an ideal mobile phase, since it gave a good resolution and peak shapes with perfect optimization. The detection was carried out at 231 nm. The flow rate was optimized at 0.7 ml/min. So, finally we optimize the last trail and validated as per ICH guidelines. The optimized results are given below.

3.1. Chromatogram of optimized condition

UPLC : UPLC Thermo Fisher Scientific
Column : XBridge Shield RP18 (3mmX 50mmX2.5µm)
Flow rate : 0.7 ml/ min
UV Detection : 231 nm
Injection Volume : 10µL
Temperature : 25ºC
Run Time : 5 min.
Mobile phase : Ammonium acetate buffer : Methanol (55:45v/v)
Diluents : DMSO + Mobile phase

The retention time of Acalabrutinib was found to be 2.391 min respectively with a run time of 5 mins, theoretical plate for Acalabrutinib were 7971.38 respectively. Thus to summarize, the developed UPLC method of analysis was found to be accurate and precise, as depicted by the statistical data of analysis (Figure 8). This method reveals an admirable performance in terms of speed and sensitivity. Furthermore, the present method offers a number of advantages viz., reduction in operation cost, less solvent consumption and decreased process cycle time by maintaining the resolution performance. The assay percentage obtained values were in the range of 90-110% which were within acceptance criteria. The amount present found to be Acalabrutinib 99.86 % Accuracy result were within limits and RSD was 0.959, 0.22 and 0.226 for Acalabrutinib respective concentration of 50%, 100%, 150%, the acceptance criteria are 98-102%. Precision may be considered at three levels: repeatability the low % RSD (≤ 2%) for Acalabrutinib indicated that the method is precise (Figure 9). Method precision was found to be within limits and RSD was found to be 0.802 for Acalabrutinib. The acceptance criteria was 90-110% and RSD NMT 2. Ruggedness result were found to be within limits and RSD was 0.33 for Acalabrutinib. The calibration was linear in concentration range of Acalabrutinib 69.5-187.5 µg/ml and the correlation coefficient was 0.999 for Acalabrutinib. Robustness studies also proved by result were within limits, acceptance criteria were RSD and tailing factor should be less than 2. There was no significance change observed in RT after individually changing the condition of flow rate of mobile phase, and wavelength. Calculation for all other system suitability parameters met the acceptance criteria and the data generated are comparable with the normal conditions (Table 15). The low values of RSD indicates the method was precise and accurate. The solution stability was found to be stable for 24 hours at 25^0C. The newly developed method was found to be simple, accurate, economical and rapid. This novel method can be applied for routine laboratory analysis for quality control of bulk and pharmaceutical formulations [7].

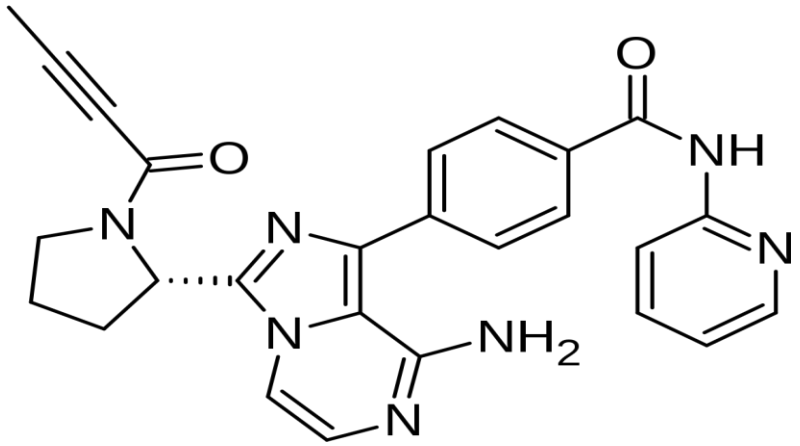

Figure 1: Structure of Acalabrutinib

Table 1: List of the chemicals and reagents

Materials	Make
Acabrunat capsule	NATCO Pharma Ltd. MFG DATE:FEB 2023 EXP DATE:JAN 2025
Working standard for Acalabrutinib	Synpharma research lab, Hyderabad.
Ammonium acetate	Sd fine- Chem ltd, Mumbai, India
Methanol (UPLC grade water)	Loba Chem, Mumbai, India

Table 2: Equipments

EQUIPMENTS USED	MAKE
UV-Spectrophotometer	Shimadzhu
UPLC	Thermo Fisher Scientific
Ultrasonicater	Labindia
Analytical balance	Sartorius
PH meter	Labindia

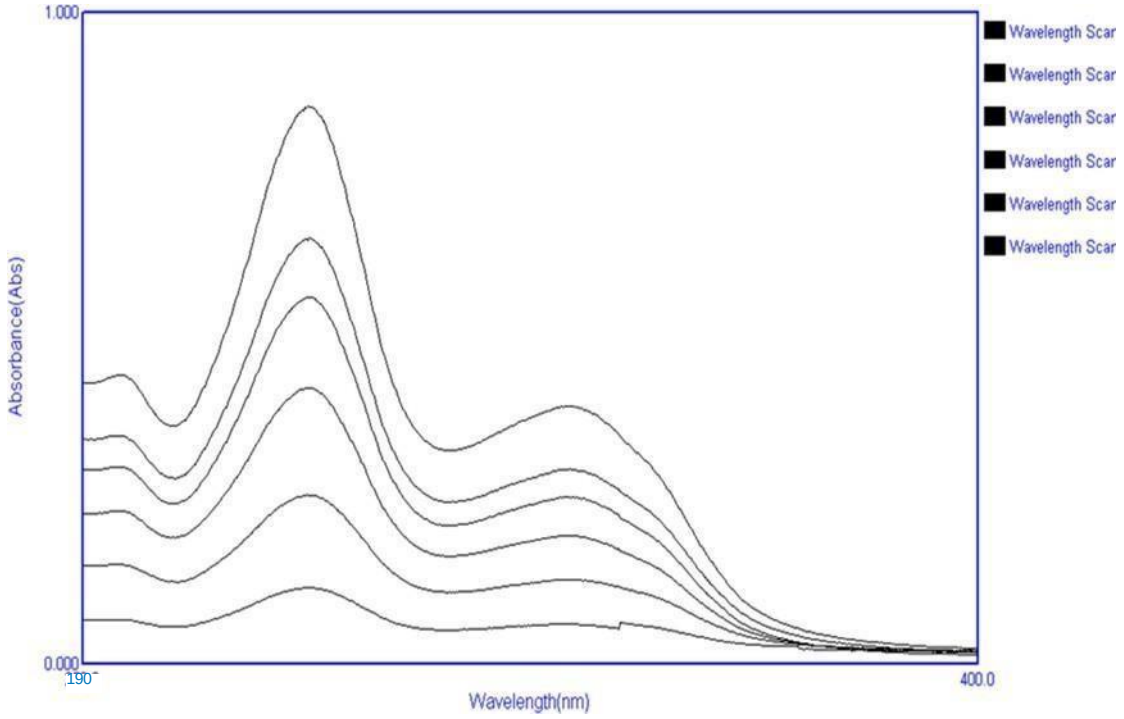

Figure 2: UV spectrum of Acalabrutinib

Table 3: Selection of Mobile Phase and Column

Trail No	Column	Mobile phase composition	λMax (nm)	Flow rate (ml/min)	Observation
1	Luna 3UC18100A (50mm x 3mm, 3µm)	Ammonium acetate buffer: Methanol(55:45v/v)	231	0.3	Poor peak shape
2	Thermo Scientific (50 mm x4.6 mm, 5 µm)	Ammonium acetate buffer: Methanol(55:45v/v)	231	0.5	Fronting is observed
3	Xterra MS C-18 (50mm x 4.6mm, 3.5µm)	Ammonium acetate buffer: Methanol(55:45v/v)	231	0.7	Less theoretical plate count
4	ACQUITY CSH C-18 (50mm x 2.1mm, 1.7µm)	Ammonium acetate buffer: Methanol(55:45v/v)	231	0.7	The peak was eluted at 3.683 min an attempt was made reduce the retention time
5	XBridge Shield RP18 (3mmX 50mmX2.5µm)	Ammonium acetate buffer: Methanol(55:45v/v)	231	0.7	good separation peaks symmetry are found to be satisfactory

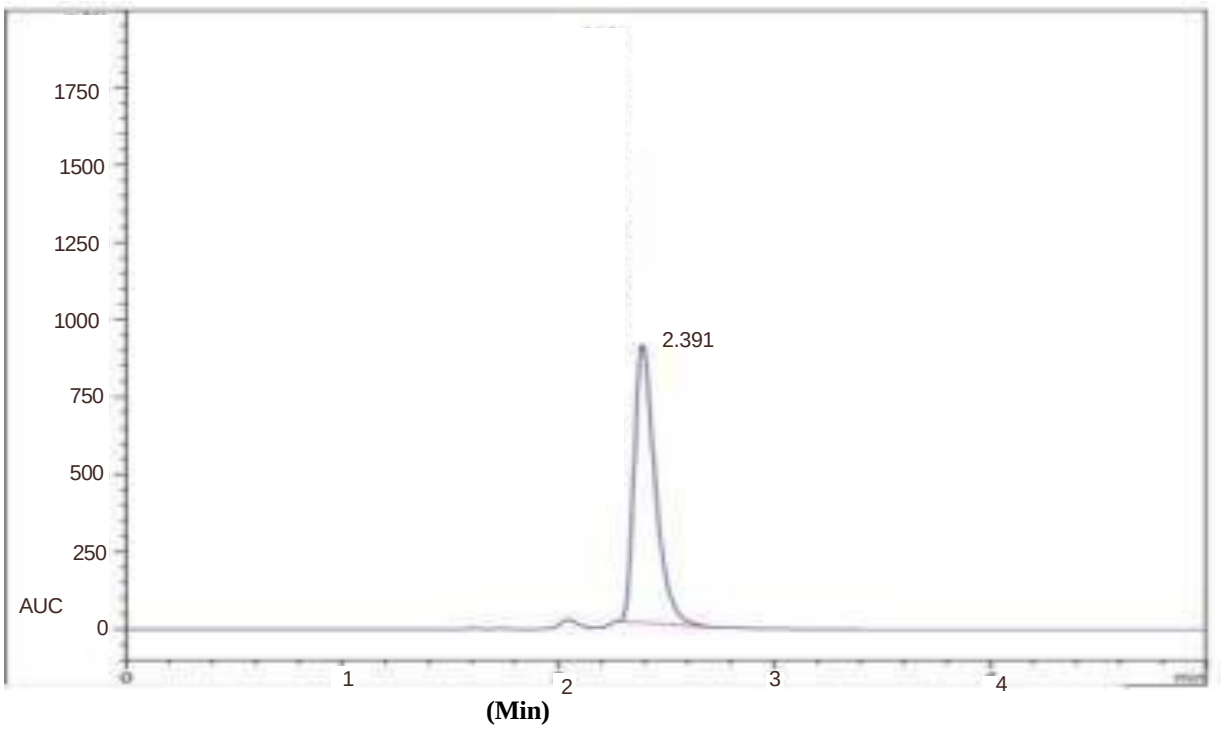

Figure 3: Chromatogram of optimized method

Name	RT	Area
ACALABRUTINIB	2.391	6023.448

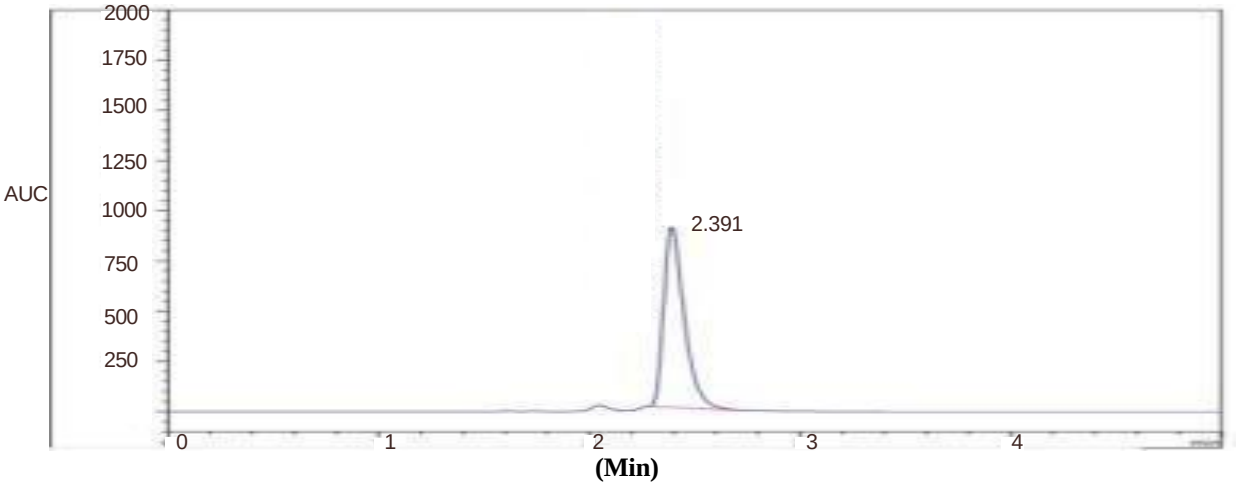

Figure 4: Chromatogram of system suitability-001

Table 4: System suitability results

Injection ID	ACALABRUTINIB		
	Theoreticalplates	**Rt**	**Area**
1	7971.38	2.391	6094.831
2	7964.02	2.391	6130.308
3	7973.15	2.391	6047.247
4	7960.40	2.392	6144.261
5	7968.71	2.392	6161.495
Average		2.391	6115.628
SD		0.0005	45.4081
% RSD		0.0229	0.7425

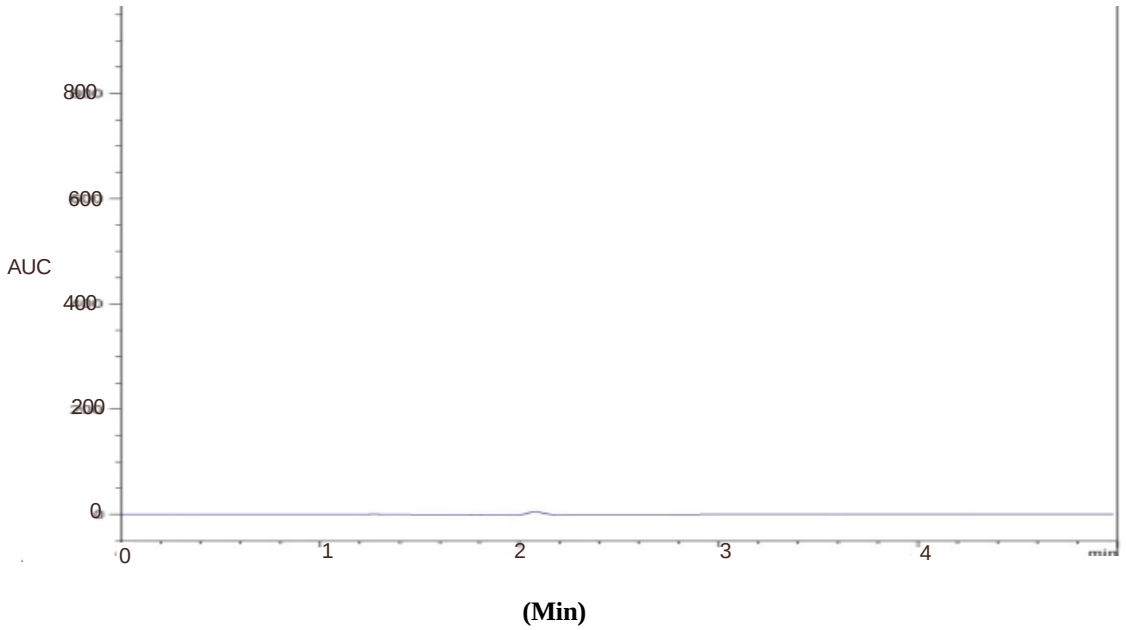

Figure 5: Specificity Chromatogram of Blank

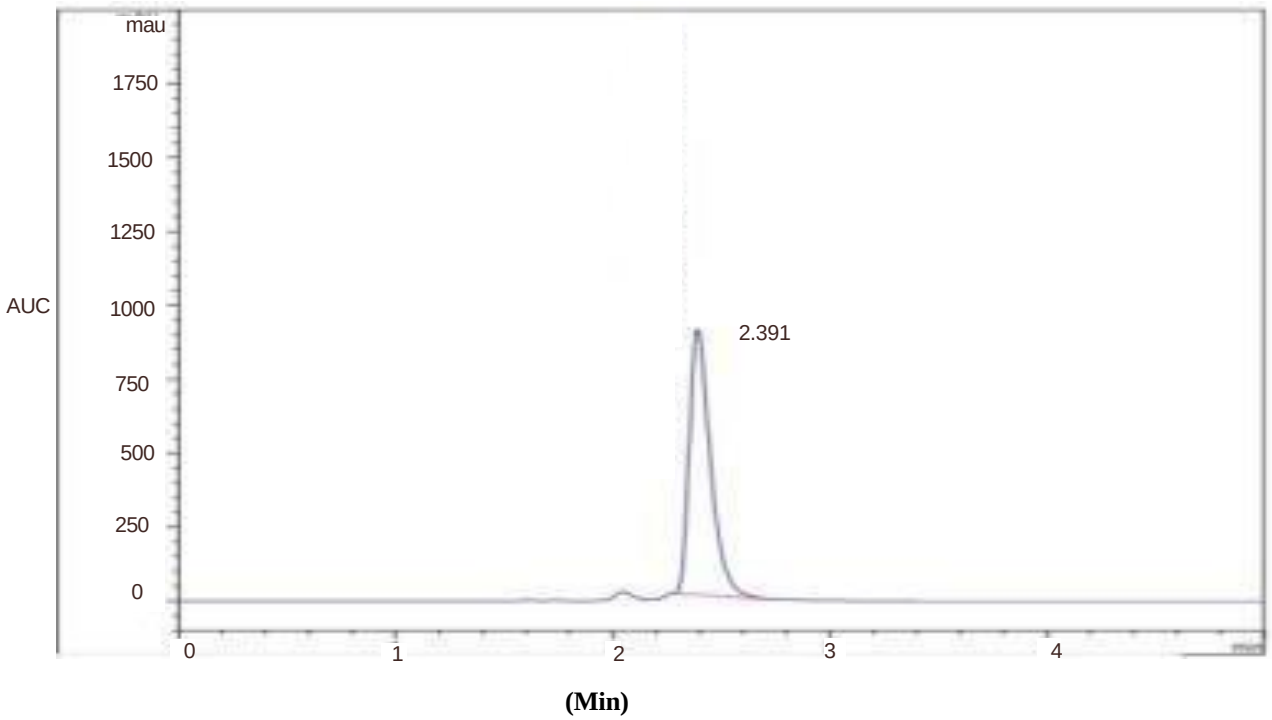

Figure 6: Specificity Chromatogram of Acalabrutinib

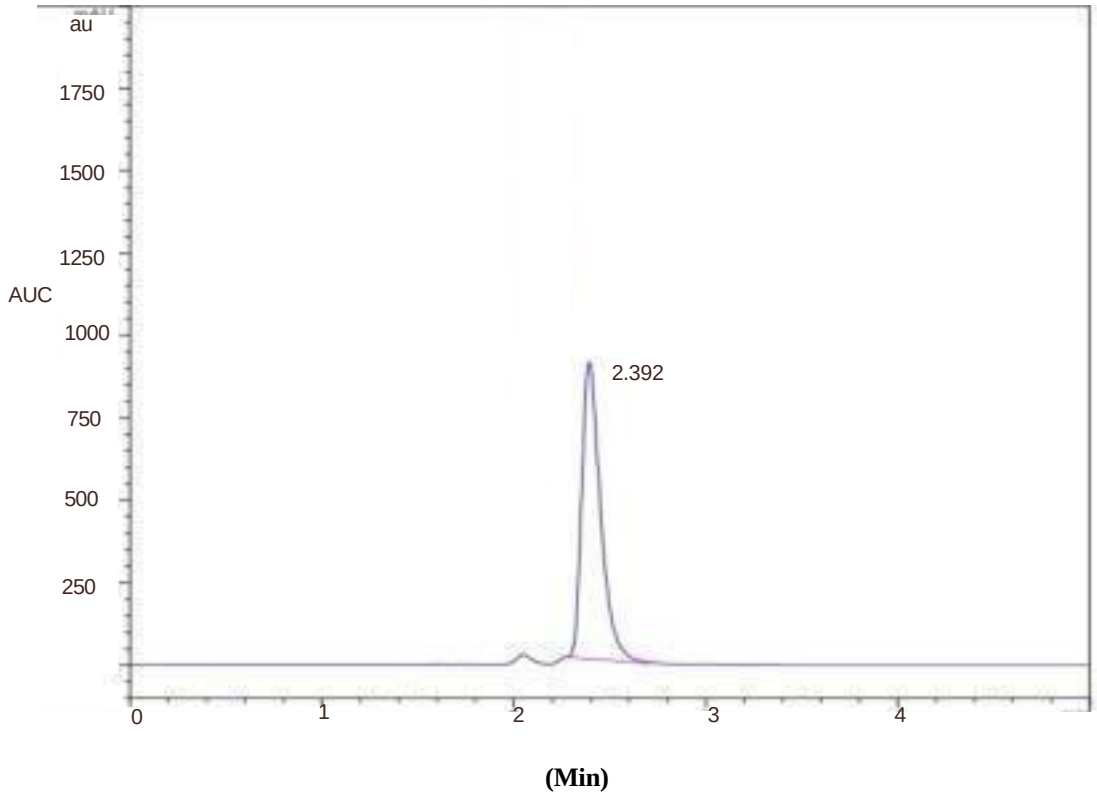

Figure 7: Specificity Chromatogram of Acalabrutinib

Table 5: Results of Accuracy-Acalabrutinib (50%)

S.NO	SAMPLEID	Conc. Spiked (µg/ml)	Drug		Calculated conc'n (µg)	% Recovery
			PEAK AREA	RETENTION TIME		
1	C 1	62.5	3028.192	2.391	62.41	99.85
2	C 2	62.5	3050.525	2.391	62.25	99.65
3	C 3	62.5	3078.419	2.392	61.32	98.11
MEAN			3052.379	2.391	61.99	99.20
STD DEV			25.16476	0.0005	0.588586	0.95212
%RSD			0.8244	0.0241	0.949434	0.959767

Table 6: Results of Accuracy-Acalabrutinib (100%)

S.NO	SAMPLEID	Conc. Spiked (µg/ml)	Drug		Calculated conc'n (µg/ml)	% Recovery
			PEAK	RETENTION		
1	C 1	125	6206.661	2.391	124.52	99.65
2	C 2	125	6185.477	2.392	124.23	99.23
3	C 3	125	6213.884	2.391	124.63	99.54
MEAN			6202.007	2.391	124.46	99.47
STD DEV			14.7642	0.0005	0.2066	0.21
%RSD			0.2381	0.0241	0.1660	0.22

Table 7: Results of Accuracy-Acalabrutinib (150%)

S.NO	SAMPLE ID	Conc. Spiked (µg/ml)	Drug		Calculated conc'n (µg/ml)	% Recovery
			PEAK AREA	RETENTION TIME		
1	C 1	187.5	9358.491	2.391	186.46	99.44
2	C 2	187.5	9364.791	2.392	186.91	99.68
3	C 3	187.5	9419.931	2.392	187.18	99.23
STD DEV			33.8008	0.0005	0.363731	0.225167
%RSD			0.3603	0.0241	0.194665	0.226412

Table 8: Precision results of Acalabrutinib

S.NO	Peak Retention Time	Peak area
1.	2.391	6169.336
2.	2.391	6076.421
3.	2.391	6112.390
4.	2.392	6183.911
5.	2.392	6210.565
6.	2.391	6137.234
MEAN	2.391	6148.31
STD DEV	0.0005	49.3278
%RSD	0.02	0.802299

Table 9: Results of Analyst Variation for Acalabrutinib

S.NO	Peak Retention Time	Peak area
Injection 1	2.391	6016.362
Injection 2	2.391	6022.849
Injection 3	2.392	6039.673
Injection 4	2.392	6068.921
Injection 5	2.391	6034.285
MEAN	2.3914	6036.418
STD DEV	0.0005	20.3589

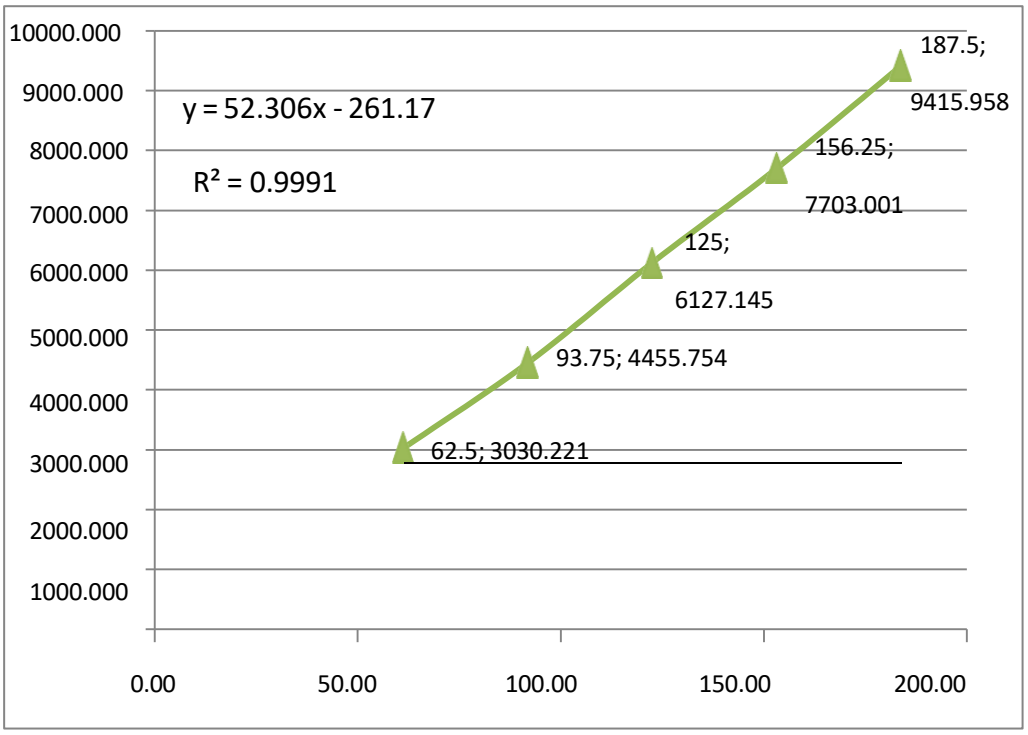

Figure 8: Linearity graph of Acalabrutinib

Linearity result for acalabrutinib

y=mX+c	
Intercept (c)	261.17
Slope(m)	52.306

Table 10: Linearity results of Acalabrutinib

Concentration (µg/ml)	Peak Retention Time	Peak area
62.5	2.391	3030.221
93.75	2.392	4455.754
125	2.391	6127.145
156.25	2.391	7703.001
187.5	2.392	9415.958

Table 11: Robustness results for Acalabrutinib

Drug name	Parameter	Chromatographic condition	
ACALABRUTINIB	**Flow rate Change**	**RT**	**AREA**
	0.6ml/min	2.485	6156.326
	0.7ml/min	2.391	6085.354
	0.8ml/min	2.289	6056.239
	Wavelength change ±2%	**RT**	**AREA**
	229 nm	2.392	6181.546
	231 nm	2.391	6074.523
	233 nm	2.391	6065.236

Table 12: Solution stability for Standard

Time in hours	% Assay	% Difference
Initial	99.88	-
24 Hours	99.63	0.25

Table 13: Solution stability for Sample

Time in hours	% Assay	% Difference
Initial	99.32	-
24 Hours	98.99	0.33

Table 14: Assay results

Label claim	Average weight	Area	Percentage assay
25mg	146.22 mg	6051.247	99.86

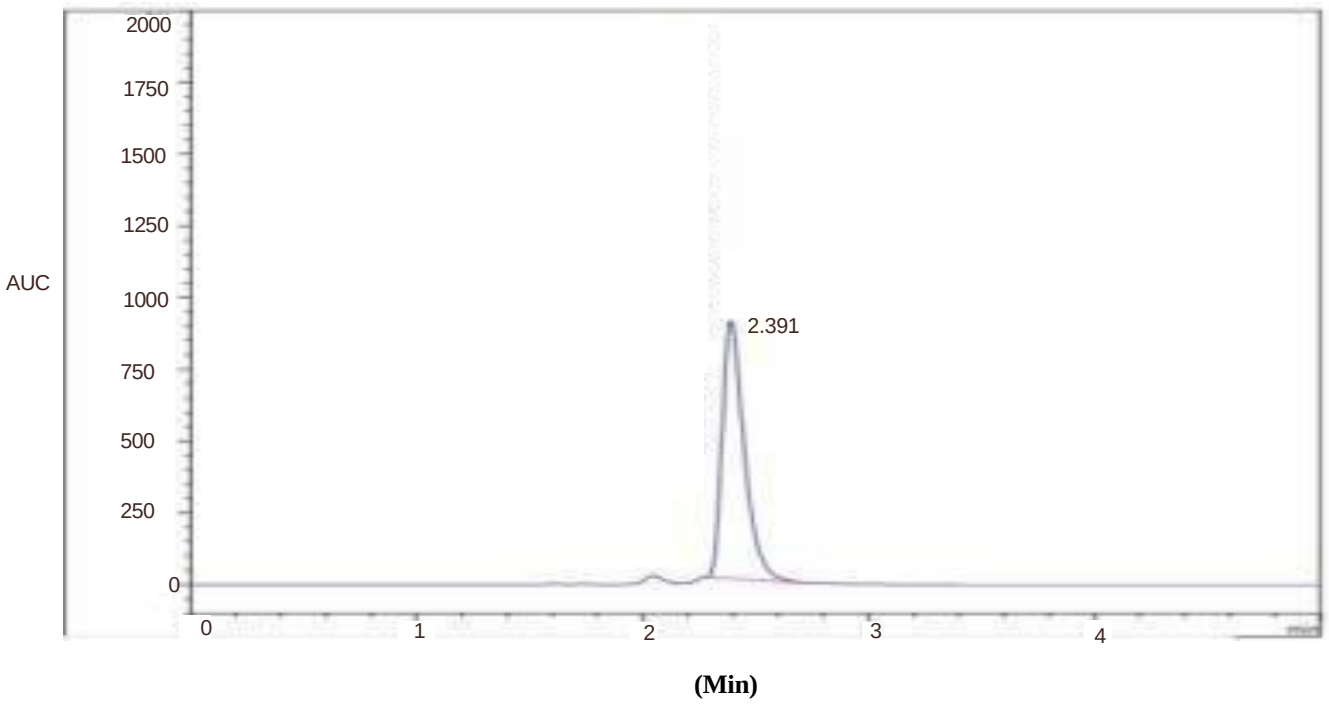

Figure 9: chromatogram of optimized condition

Name	RT	Area
ACALABRUTINIB	2.391	6023.448

Table 15: validation summary of the proposed method

S.NO	PARAMETERS	ACCEPTANCE CRITERIA	RESULTS
1	System suitability	Theoretical plate	7971.38
		Tailing Factor	1.2
2	Specificity	No interference was observedbetween placebo and blank withprincipal peak	Blank-Nil Placebo-Nil Standard-2.391 Sample-2.392
3	Accuracy	98-102%	99.37 %
4	Precision	%RSD NMT 2 %	0.802%
5	Linearity	Correlation coefficient NLT 0.999	0.999
6	Solution stability	%RSD NMT 2 %	Stable for 24 hours
7	Assay	90-110%	99.86%

Based on the result of the above studies, it is concluded that the method for determination of assay of Acalabrutinib 25 mg is precise, linear over the concentration range, accurate and more rugged. The method is robust with respect to variation in flow rate, and wavelength. Additionally, the method is specific for the quantization of assay of Acalabrutinib in capsule. So the current developed method can be easily used for the validation of pharmaceutical dosage form.

4. Summary and conclusion

The optimized method was suitable, linear, precise accurate and robust for the estimation of Acalabrutinib in capsule dosage form.The developed method is rapid, extremely small flow rate with relatively short run time. All these factors enable rapid quantification and estimation of the Acalabrutinib in capsule pharmaceutical formulation without any excipient interference. It can therefore be concluded that the developed method is more economical and practical. It can applied for the simultaneous analysis of the Acalabrutinib in research and quality control laboratories.

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Conflicts of interest

None to declare for all authors.

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