

## Novel stability indicating high-performance liquid chromatography method for the separation and simultaneous quantification of acalabrutinib and its impurities in pharmaceutical formulation

Venu Gopal Kamani<sup>1</sup>, Sujatha M<sup>1</sup> ★, and Guna Bhushana Daddala<sup>2</sup>

<sup>1</sup>Department of Chemistry, Koneru Lakshmaiah Education Foundation, Vaddeswaram, Guntur - 522502, A.P., India

<sup>2</sup>Department of Analytical and Research, Piramal Pharma Ltd, Ennore, Chennai - 600057, Tamil Nadu, India

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**Abstract:** This study reports for the first time about a stability indicating RP-HPLC method for qualitative and quantitative determination of acalabrutinib in bulk and dosage form and in presence its impurities 1, 2 and 3. The chromatographic separation was carried on Zorbax XDB-C18 (250×4.6 mm; 5 μ id) as stationary phase, Phosphate buffer pH 6.4 and methanol 80:20 (v/v) as mobile phase at a flow rate of 1.0 mL/min, UV detection was carried at wavelength of 238 nm and the analysis was completed with a run time of 15 min. In these conditions the retention time of acalabrutinib and its impurities 1, 2 and 3 was observed to be 3.50, 4.83, 8.40 and 9.93 min respectively. The method was validated for system suitability, range of analysis, precision, specificity, stability and robustness. Spiked recovery at 50 %, 100 % and 150 % was carried for both standard and impurities and the acceptable % recovery of 98-102 was observed for acalabrutinib and both impurities studied and the % RSD in each spiked level was found to be less than 2. Stability tests were done through exposure of the analyte solution to five different stress conditions i.e expose to 1N hydrochloric acid, 1 N sodium hydroxide, 3 % peroxide, 80 °C temperature and UV radiation at 254 nm. In all the degradation condition, standard drug acalabrutinib was detected along with both the impurities studied and the degradation products were successfully separated. In the formulation analysis there is no other chromatographic detection of other impurities and formulation excipients. Hence the developed method was found to be suitable for the quantification of acalabrutinib and can separate and analyse impurities 1 and 2.

**Key words:** acalabrutinib, impurity analysis, HPLC, forced degradation study, formulation analysis

### 1. Introduction

Acalabrutinib (*Fig. 1(a)*) is a second generation novel bruton tyrosine kinase (BTK) inhibitor prescribed for the treatment of mantle cell lymphoma also

known as non-hodgkin lymphoma in adults who have received at least one prior therapy.<sup>1</sup> Its is more selective and more potent with very less side effects than ibrutinib which is the first class BTK inhibitor.<sup>2</sup> The high therapeutic activity and toxicity of acala-

★ Corresponding author

Phone : +91-9908925641

E-mail : [sujathaenviero@kluniversity.in](mailto:sujathaenviero@kluniversity.in)

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brutinib is due to its high plasma proteins bound (97.5 %) that greatly influence the distribution of drug.<sup>3</sup> The BTK enzymatic inhibition activity of acalabrutinib is due to the formation of covalent bond in the BTK active with cysteine residue (Cys481) and activation of downstream signaling proteins and inhibits malignant B-cell proliferation.<sup>4,5</sup> Headaches, low red, white blood cells, low platelets, feeling tired, diarrhea and weight gain are the possible side effects associated with the usage of acalabrutinib.<sup>6</sup>

In the field of pharmaceutical analysis, analytical methods are focused on the investigation of drug content in bulk drug materials, drug products and formulations. Analytical methods also used for the detection of impurities, degradation compounds in bulk drug and biological samples contain known drug and its metabolites. The literature survey for the available analytical methods confirms that very few analytical methods are reported for the estimation of acalabrutinib in various samples. Two HPLC (High

Performance Liquid Chromatography) assay methods<sup>7,8</sup> reported for the estimation of acalabrutinib in formulations. LCMS (Liquid chromatography–mass spectrometry) bio-analytical method reported for the pharmacokinetic study of acalabrutinib Sprague Dawley rats.<sup>9</sup> One analytical method reported for the evaluation of protein binding efficiency of acalabrutinib using spectroscopic and computational studies.<sup>10</sup> One UPLC-MS/MS (Ultra-performance liquid chromatography tandem mass spectrometer) method reported for the simultaneous estimation of acalabrutinib, ibrutinib, and their metabolites in beagle dog plasma.<sup>11</sup> Hence the present work intended for the development of a simple HPLC method for the separation and quantification of related impurities of acalabrutinib in bulk drug and pharmaceutical formulations. Based in the availability, the impurities 1, 2 & 3 of acalabrutinib were selected for the study and the molecular structure of the selected impurities was given in Figs. 1(b), 1(c) and 1(d) respectively.

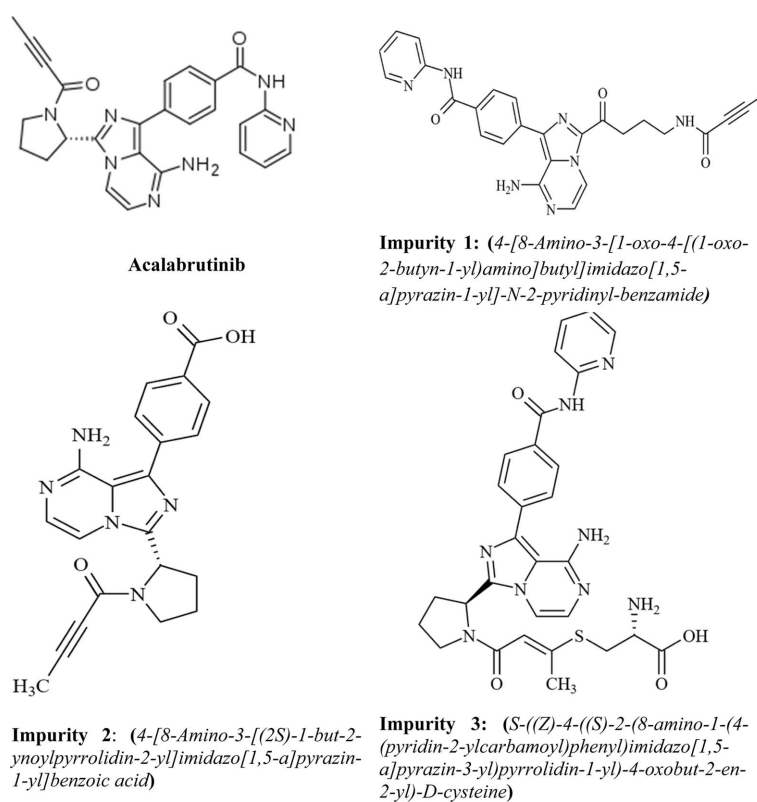


Fig. 1. Molecular Structure of acalabrutinib and its impurities.

## 2. Materials and Methods

### 2.1. Reagents

The working standard drug acalabrutinib (98.79 % purity) and its Impurities (1, 2 & 3) studied were obtained from AstraZeneca Pharma India Limited, Bangalore, Karnataka, India. Methanol (HPLC Grade) and Acetonitrile (HPLC grade) were purchased from Merck chemicals, Mumbai. Ultra-Pure (Milli-Q<sup>®</sup>) Water was used during the study. All the other chemicals used during the study are of analytical reagent grade and were purchased from Merck chemicals, Mumbai.

### 2.2. Instrumentation

HPLC studies were carried on Agilent (USA) 1100 series HPLC instrument coupled with Quaternary pump (G1311 A), Thermostatic auto sampler (G 1329A) with injection volume of 0.1-1500  $\mu$ L and programmable UV (ultraviolet) detector (G 1314 A). Agilent chem. station LC software was used for integrating the chromatograms.

### 2.3. Preparation of solutions

#### 2.3.1. Phosphate Buffer (pH 6.4) solution

Buffer solution was prepared by mixing of sodium phosphate dibasic heptahydrate (9.579 g) and sodium phosphate monobasic monohydrate (8.868 g) in 1000 mL HPLC grade water and the pH of was adjusted to 6.4 using 0.1 N hydrochloric acid.

#### 2.3.2. Acalabrutinib and impurity solutions

A stock solution of 1 mg/mL was prepared for standard and impurities separately by accurately weighing 50 mg of acalabrutinib/impurities and was dissolved in 50 mL of methanol. Required dilutions for were prepared from the stock solution and from the prepared dilutions, 10 mL of selected concentrations of acalabrutinib, impurity 1, 2 & 3 were mixed separately and the mixture solution was used for method development and validation studies.

#### 2.3.3. Formulation solution

Five tablets of acalabrutinib with brand Calquence<sup>®</sup> (100 mg) were powdered and from the tablet powder,

an amount of the powder equivalent to 10 mg of acalabrutinib was weighed and was dissolved in 10 mL of methanol. Then the content was filtered (0.45  $\mu$  membrane filter) and diluted to standard concentration and was used for the assay.

### 2.4. Method development

Prior to the initiation of the method development in HPLC, the suitable wavelength for simultaneous detection of acalabrutinib and its impurities was determined using UV-visible spectrophotometer. Acalabrutinib and its impurities at a concentration of 10  $\mu$ g/mL was used for the determination of wavelength maxima separately. The overlay UV absorption spectra of acalabrutinib and its impurities confirms the suitable wavelength for the detection of acalabrutinib and its impurities in HPLC.

The stationary phase that give best separation of acalabrutinib and its impurities was confirmed by change in different stationary phases like zodiac c18 column, prontoSIL ODS C18 column, zorbax XDB-C18 column and phenomenex luna C18 columns of 150 mm and 250 mm was studied. The mobile phase composition with different organic modifiers like methanol and acetonitrile was studied in combination with different pH modifiers like acetate buffer, phosphate buffer in different pH ranges was studied. Based on the separation of the compounds, the mobile phase was optimised. The flow rate of the mobile phase was also optimised in the range of 0.7 to 1.2 mL/min. The optimised conditions that produce best separation of acalabrutinib and its impurities were confirmed and proceed for further validation.

## 3. Method Validation

The method developed for the analysis of acalabrutinib and its impurities was validated as per the available literature and ICH guidelines.<sup>12,13</sup>

### 3.1. System suitability

Freshly prepared standard solution at recovery levels of acalabrutinib and the impurities was analysed

in the developed method. The system suitability was evaluated by summarizing the parameters such as relative retention time (RRT), asymmetric factor (tail factor), plate count (number of theoretical plates) and resolution factor.

### 3.2. Sensitivity

The method sensitivity for the detection of impurities of acalabrutinib was evaluated in terms of limit of detection (LOD) and limit of quantification (LOQ). The standard solution containing acalabrutinib and its impurities was evaluated at very low concentrations and the LOD and LOQ was confirmed based on signal to noise ratio (s/n). The s/n of 3 and 10 was considered as LOD and LOQ respectively.

### 3.3. Linearity and range

Standard solution at a concentration range of 50-1000 µg/mL of acalabrutinib and 0.1-10 µg/mL of three impurities was prepared and analysed in the method. The calibration curve was plotted by taking concentration prepared on X-axis and peak area obtained on Y-axis. Based on the correlation of points in the curve that produce best correlation coefficient for standard and impurities separately was considered as the range of analysis in the method.

### 3.4. Precision

Intraday and interday precision study has performed by analysing the standard solution containing acalabrutinib and its impurities six times in the same day for intraday precision and six times in three different days for interday precision. The % relative standard deviation (RSD) of the peak area was calculated and the % RSD of <2 was considered as acceptable.

### 3.5. Ruggedness

The standard solution was analysed six times in the same day with change in the analyst was performed for the evaluation of the ruggedness of the method. The % RSD of peak area obtained for standard and impurities was calculated separately and the % RSD of <2 was considered as the method is rugged.

### 3.6. Robustness

The effect of change in the optimised conditions for the analysis of acalabrutinib and its impurities have evaluated in robustness study. In this change in mobile phase composition ( $\pm 5$  mL), detector wavelength ( $\pm 5$  nm) and pH of the mobile phase ( $\pm 0.1$  factor) have studied. In each changed condition, the % change compared with optimised condition was calculated for both impurities and acalabrutinib and % change of <2 was considered as acceptable as per guidelines.

### 3.7. Recovery

In recovery/accuracy study, 50 %, 100 % and 150 % spiked levels of known standard concentration (100 %) in the calibration range was performed and the % recovery was calculated in each spiked level by comparing with calibration results. The % recovery of 98-102 was considered as acceptable.

### 3.8. Force degradation studies

50 mg of standard drug acalabrutinib was mixed with 50 mL of 0.1 N HCl (hydrochloric acid), 0.1 N NaOH (sodium hydroxide) and 3 % hydrogen peroxide solution separately for 24 h in acidic, basic and oxidative degradation study. Then the solution was neutralised and diluted to standard concentration. 50 mg of standard drug acalabrutinib was kept in an air oven at 60 °C for 24 h in thermal degradation study and kept under UV (ultraviolet) light at 254 nm for 24 h for photolytic degradation study. Then it was diluted to standard concentration and the degradation solutions were analysed in the developed method. The number of degradation compounds formed and the % degradation was calculated by comparing with unstressed results of acalabrutinib.

### 3.9. Formulation analysis

The formulation solution of acalabrutinib with brand Calquence® (100 mg) was analysed in the developed method. The % assay was calculated by comparing the formulation results with that of the calibration curve results.

#### 4. Results and Discussions

As the literature survey confirms that there is no analytical method available for the separation and estimation of acalabrutinib and its impurities in pharmaceutical formulations. Hence the present work aimed to develop a simple HPLC method for the separation and quantification of related impurities of acalabrutinib in tablet formulations as well as bulk drug. The systematic trails performed and method rejection resign during the optimization study was given in *Table 1* and the optimization chromatogram was given in *Fig. 2*.

Prior to validation of the developed method, repeatability and system suitability of the developed method was determined at recovery levels of acalabrutinib and its impurities. From the results obtained, the system suitability parameters like RRT, tail factor,

number of theoretical plates and resolution were evaluated and results confirms that all the parameters are in the acceptable limit (*Table 2*) hence the method was found to be acceptable.

The sensitivity of the method for the detection of impurities was evaluated prior to the construction of calibration curve. The LOD was observed to be 0.03  $\mu\text{g/mL}$  and LOQ was obtained as 0.10  $\mu\text{g/mL}$  for the impurities of acalabrutinib in the developed method. This proved that the method can detect the impurities up to very low concentration of 0.03  $\mu\text{g/mL}$  and can quantify up to 0.10  $\mu\text{g/mL}$ . The calibration curve was constructed from LOQ concentration of the impurities and standard concentration was considered such that the solution contains 0.2 % of the impurities in the study.

Ten points calibration curve was within the concentration range of 50-500  $\mu\text{g/mL}$  for acalabrutinib and

*Table 1.* Method development conditions tried during optimization process

S No	Mobile Phase composition	Result	Conclusion
1	MP: pH 6.2 acetate buffer, methanol 50:50 (V/V); SP: Zodiac c18 (100×4.6 mm; 3.5 $\mu$ id) column; WL: 238 nm; FR: 1.0 mL/min	No separation was observed. Three merged peaks were identified and 4 <sup>th</sup> compound not detected	Method Rejected
2	MP: pH 6.2 acetate buffer: methanol 70:30 (V/V); SP: Zodiac c18 (250×4.6 mm; 3.5 $\mu$ id) column; WL: 238 nm; FR: 1.0 mL/min	Impurity 1 and 2 are merged and one impurity not identified. acalabrutinib and impurity 3 were separated but the separation was found to be poor	Method Rejected
3	MP: pH 5.4 acetate buffer: methanol 75:25 (V/V); SP: ProntoSIL ODS C18 (250×4.6 mm; 5 $\mu$ id); WL: 238 nm; FR: 1.0 mL/min	Two merge peaks representing the acalabrutinib and its three impurities observed. Impurity 1 and two are merged and not separated. acalabrutinib and impurity 3 are merged and split peak was observed	Method Rejected
4	MP: pH 5.4 Phosphate buffer: methanol 20:80 (V/V); SP: Phenomenex Luna C18 (250×4.6 mm; 5 $\mu$ id); WL: 238 nm; FR: 1.0 mL/min	Impurity 2 and 3 are clearly separated and detected. Impurity 1 and acalabrutinib were merged	Method Rejected
5	MP: pH 5.4 Phosphate buffer: methanol 40:60 (V/V); SP: Zorbax XDB-C18 (250×4.6 mm; 5 $\mu$ id); WL: 238 nm; FR: 1.0 mL/min	acalabrutinib, impurity 1, 2 & 3 were separated and identified. The separation was found to be very poor and the peak area response was found to be less and not acceptable system suitability for all the compounds	Method Rejected
6	MP: pH 5.0 Phosphate buffer: methanol 20:80 (V/V); SP: Zorbax XDB-C18 (250×4.6 mm; 5 $\mu$ id); WL: 238 nm; FR: 1.0 mL/min	Clear separation of impurity 1 and 2 observed but the tail factor of the peaks was found to be very high. acalabrutinib and impurity 3 are merged.	Method Rejected
7	MP: pH 6.2 Phosphate buffer: methanol 60:40 (V/V); SP: Zorbax XDB-C18 (250×4.6 mm; 5 $\mu$ id); WL: 238 nm; FR: 1.0 mL/min	Acalabrutinib and its three impurities were well resolved and retained but the tail factor of compounds was found to be very high with less number of theoretical plates.	Method Rejected
8	MP: pH 6.4 Phosphate buffer: methanol 80:20 (V/V); SP: Zorbax XDB-C18 (250×4.6 mm; 5 $\mu$ id); WL: 238 nm; FR: 1.0 mL/min	Well resolved & retained peaks with acceptable system suitability observed	Method Accepted

MP = Mobile phase composition; SP = Stationary phase; WL = Detector wavelength; FR = Flow rate of mobile phase

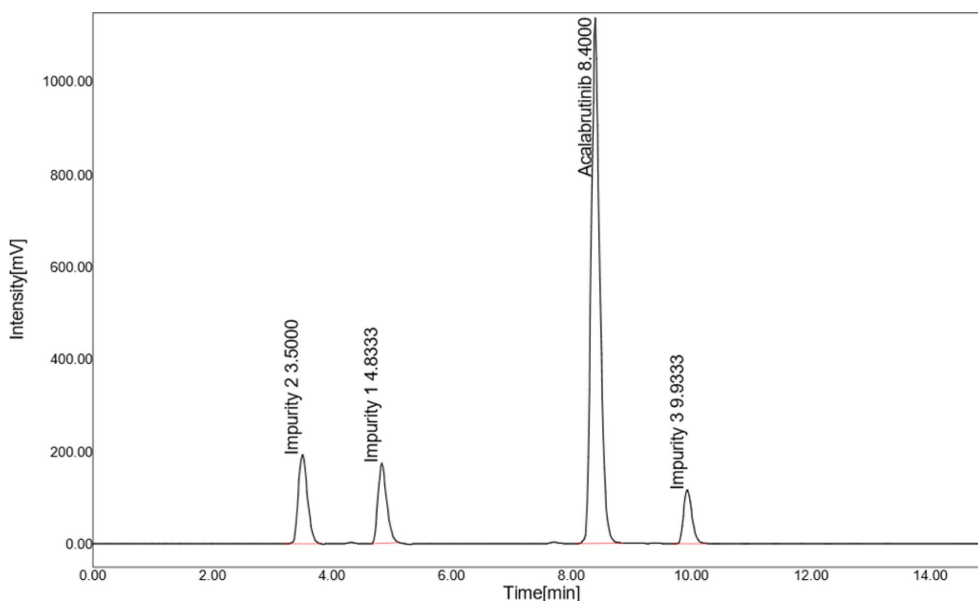


Fig. 2. Optimised chromatogram of acalabrutinib, impurity 1, 2 & 3.

Table 2. System suitability results

Compound	Concentration in $\mu\text{g/mL}$	Retention Time (min)	RRT	Theo plate	Tail Factor	Resolution
Acalabrutinib	150	8.4389 $\pm$ 0.042	---	3981	1.10	12.66
	200	8.4556 $\pm$ 0.082	---	4179	1.09	12.66
	250	8.4500 $\pm$ 0.060	---	4066	1.10	12.67
Impurity 1	0.3	4.8611 $\pm$ 0.035	0.5760 $\pm$ 0.001	5354	1.06	5.21
	0.4	4.8389 $\pm$ 0.010	0.5723 $\pm$ 0.004	5283	1.06	5.27
	0.5	4.8778 $\pm$ 0.079	0.5773 $\pm$ 0.012	5370	1.05	5.24
Impurity 2	0.3	3.5111 $\pm$ 0.019	0.4161 $\pm$ 0.001	7866	0.98	---
	0.4	3.5056 $\pm$ 0.010	0.4146 $\pm$ 0.005	7782	0.99	---
	0.5	3.5333 $\pm$ 0.017	0.4182 $\pm$ 0.004	7905	0.98	---
Impurity 3	0.3	10.0055 $\pm$ 0.048	1.1857 $\pm$ 0.004	9128	0.91	6.24
	0.4	9.9500 $\pm$ 0.029	1.1768 $\pm$ 0.013	9041	0.90	6.23
	0.5	9.9778 $\pm$ 0.063	1.1809 $\pm$ 0.013	9380	0.90	6.25

# Values given in table is the average  $\pm$  standard deviation of three replicate experiments

0.1-1  $\mu\text{g/mL}$  for impurity 1, 2 & 3. The regression equation was found to be  $y = 5563x - 2565.8$  ( $R^2 = 0.9996$ ),  $y = 103312x + 669.41$  ( $R^2 = 0.9997$ ),  $y = 122449x + 1401.4$  ( $R^2 = 0.9994$ ) and  $y = 84413x + 605.98$  ( $R^2 = 0.9997$ ) for acalabrutinib, impurity 1, 2 and 3 respectively. The calibration curve was found to be linear with very high correlation coefficient for both impurities and standard acalabrutinib. Linearity results were given in Table 3.

Precision was carried at a concentration of 250  $\mu\text{g/mL}$  of acalabrutinib and 0.5  $\mu\text{g/mL}$  of impurity 1, 2 and 3. The % RSD of peak area values in the replicate analysis of standard solution in intraday, interday precision and ruggedness was calculated and was found to be 0.22, 0.71, 0.19 and 0.32 in intraday precision, 0.25, 1.36, 0.32 and 0.71 in interday precision and 0.21, 0.23, 1.13 and 0.25 in ruggedness respectively for acalabrutinib, impurity 1, 2 and 3

Table 3. Linearity results

S. No	Acalabrutinib		Impurity 1		Impurity 2		Impurity 3	
	Con <sup>#</sup>	Peak Area (mV)	Con <sup>#</sup>	Peak Area (mV)	Con <sup>#</sup>	Peak Area (mV)	Con <sup>#</sup>	Peak Area (mV)
1	50	254592.4	0.1	11485.1	0.1	13476.9	0.1	9114.2
2	100	562913.1	0.2	20925.5	0.2	26301.1	0.2	17648.9
3	150	813686.7	0.3	31415.8	0.3	38298.1	0.3	26348.5
4	200	1142691.5	0.4	42901.7	0.4	50381.4	0.4	33949.8
5	250	1387446.9	0.5	53193.4	0.5	63677.7	0.5	42696.7
6	300	1676262.8	0.6	62526.7	0.6	75007.2	0.6	51995.4
7	350	1944173.5	0.7	72205.4	0.7	88477.8	0.7	59897.6
8	400	2226354.9	0.8	83593.3	0.8	100288.1	0.8	68489.5
9	450	2505141.9	0.9	93493.8	0.9	110974.5	0.9	76642.3
10	500	2759315.2	1	103837.4	1	121999.6	1	84152.1

#Con = Concentration in µg/mL

Table 4. Robustness results

S. No	Changed Condition	Acalabrutinib		Impurity 1		Impurity 2		Impurity 3	
		Peak Area (mV)	% Change	Peak Area (mV)	% Change	Peak Area (mV)	% Change	Peak Area (mV)	% Change
1	Standard	1387447	---	53193	---	63678	---	42697	---
2	MP 1	1376278	0.81	52911	0.53	63466	0.33	42541	0.36
3	MP 2	1382771	0.34	53059	0.25	63352	0.51	42636	0.14
4	pH 1	1383951	0.25	52854	0.64	63413	0.42	42508	0.44
5	pH 2	1376680	0.78	52955	0.45	63185	0.77	42319	0.88
6	WL 1	1385310	0.15	52884	0.58	63750	-0.11	42408	0.68
7	WL 2	1389737	-0.17	52998	0.37	63892	-0.34	42504	0.45
8	FR 1	1374153	0.96	52795	0.75	63605	0.11	42378	0.75
9	FR 2	1372312	1.09	53127	0.13	63341	0.53	42601	0.23

MP (Mobile Phase) 1: Phosphate buffer: methanol 85:15 (v/v), MP 2: Phosphate buffer: methanol 75:25 (v/v); WL (Wavelength) 1: 233 nm, WL 2: 243 nm; pH 1: 6.3, pH 2: 6.5

respectively. Hence the method developed for the analysis of acalabrutinib and its impurities was found to be rugged and precise.

The % change in all the changed conditions in robustness study was found to be with the acceptable limit of less than 2 (Table 4) for acalabrutinib and its impurities studied. This confirms that the method is found to be robust as there is no considerable change in the separation and detection of acalabrutinib and impurities when small change in the developed method conditions.

Accuracy of the method was determined spiked recovery studies by spiking 50 %, 100 % and 150 % concentrations of target 100 µg/mL for acalabrutinib, 0.2 µg/mL for impurity 1, 2 and 3. The % recovery

in was calculated for both standard and impurities in each analysis and the % RSD was calculated for each spiked level. The acceptable % recovery of 98-102 was observed for acalabrutinib and both impurities studied and the % RSD in each spiked level was found to be less than 2 (Table 5) which is the acceptable limit. Hence the method was found to be accurate.

The different stress exposed and unstressed standard solution of acalabrutinib was analysed in the optimised conditions. The % degradation was calculated by comparing with unstressed samples and was found that the % degradation was found to very high in acidic stress followed by oxidative degradation. In acid degradation condition the drug was found to be degraded up to 9.91 % in 24 H of stress expose. In

Table 5. Recovery results

S. No.	Compound	Recovery Level	Concentration in $\mu\text{g/mL}$			Amount found Mean $\pm$ SD	% recovered Mean $\pm$ SD	% RSD of Recovery
			Target	Spiked	Final			
1		50 %	100	50	150	149.363 $\pm$ 0.080	99.58 $\pm$ 0.053	0.05
2	Acalabrutinib	100 %	100	100	200	199.290 $\pm$ 0.324	99.65 $\pm$ 0.162	0.16
3		150 %	100	150	250	248.347 $\pm$ 0.392	99.34 $\pm$ 0.157	0.16
4		50 %	0.2	0.1	0.3	0.296 $\pm$ 0.002	98.78 $\pm$ 0.509	0.52
5	Impurity 1	100 %	0.2	0.2	0.4	0.394 $\pm$ 0.002	98.50 $\pm$ 0.500	0.51
6		150 %	0.2	0.3	0.5	0.496 $\pm$ 0.001	99.20 $\pm$ 0.200	0.20
7		50 %	0.2	0.1	0.3	0.296 $\pm$ 0.001	98.67 $\pm$ 0.333	0.34
8	Impurity 2	100 %	0.2	0.2	0.4	0.395 $\pm$ 0.002	98.83 $\pm$ 0.382	0.39
9		150 %	0.2	0.3	0.5	0.493 $\pm$ 0.003	98.60 $\pm$ 0.529	0.54
10		50 %	0.2	0.1	0.3	0.296 $\pm$ 0.001	98.78 $\pm$ 0.192	0.19
11	Impurity 3	100 %	0.2	0.2	0.4	0.395 $\pm$ 0.002	98.75 $\pm$ 0.500	0.51
12		150 %	0.2	0.3	0.5	0.495 $\pm$ 0.001	98.93 $\pm$ 0.231	0.23

\*Values given in table are the average  $\pm$  standard deviation for three replicate experiments

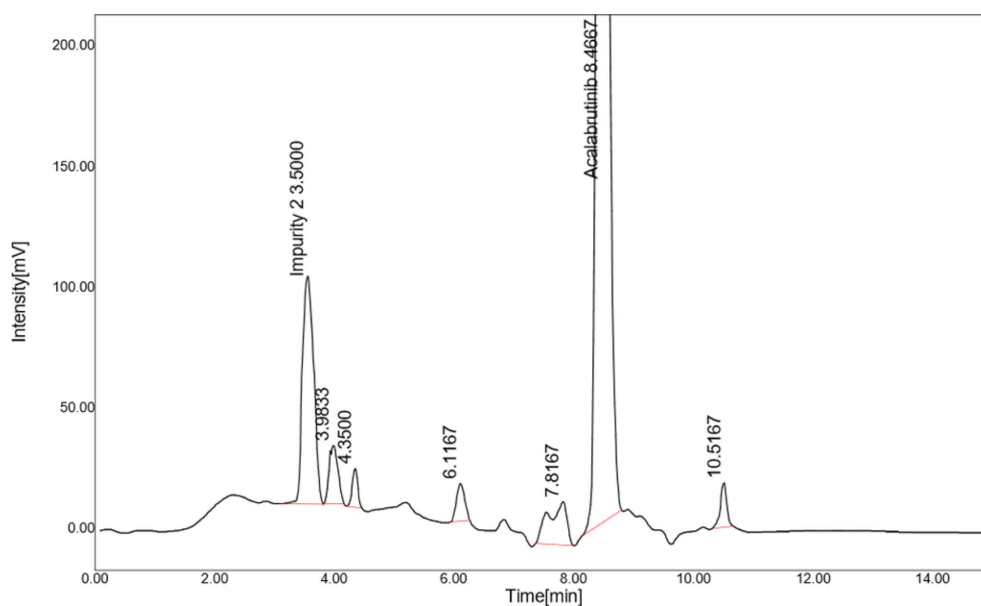


Fig. 3. Acid degradation chromatogram of acalabrutinib.

this stress study, five additional degradation products were observed at a retention time of 3.98, 4.35, 6.11, 7.81 and 10.51 min along with impurity 2 (Fig. 3). In base degradation study, the drug was found to be degraded up to 5.73 % in 24 H of stress expose and three additional peaks at a retention time of 3.08, 4.18 and 7.93 min along with acalabrutinib and impurity 2 (Fig. 4). In oxidative degradation study four additional peaks was detected at a retention time of 5.71, 6.68,

7.71 and 11.51 min and the % degradation was found to be 9.46 (Fig. 5). In this stress study, impurity 1 and 3 identified corresponding to the standard spiked chromatogram. In photolytic degradation, the % degradation of acalabrutinib was found to be 8.07 and four additional degradation products were detected at a retention time of 3.01, 6.20, 7.76 and 11.11 min along with two impurities (1 and 2) in the study (Fig. 6). In thermal degradation study, the % degradation



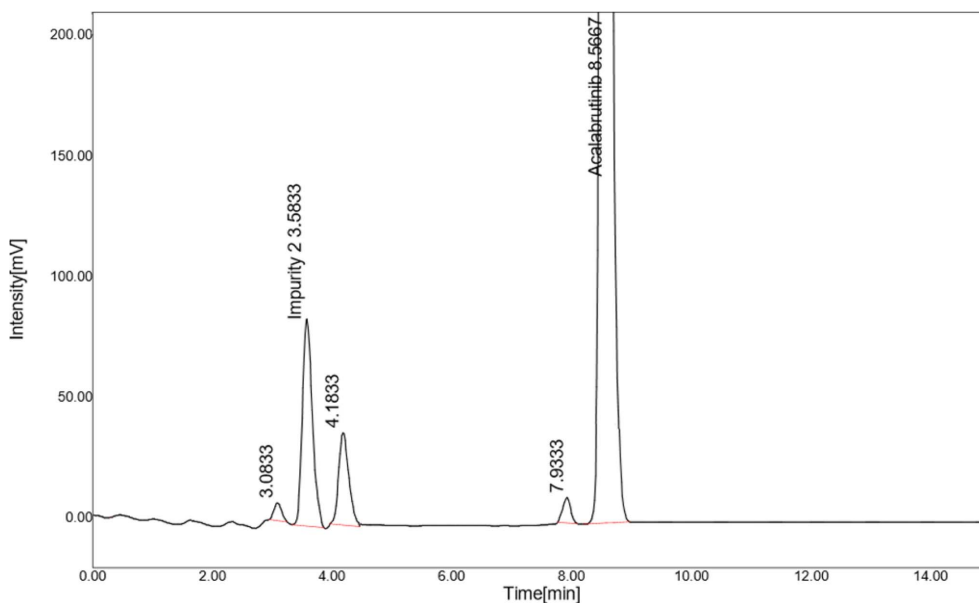


Fig. 4. Base degradation chromatogram of acalabrutinib.

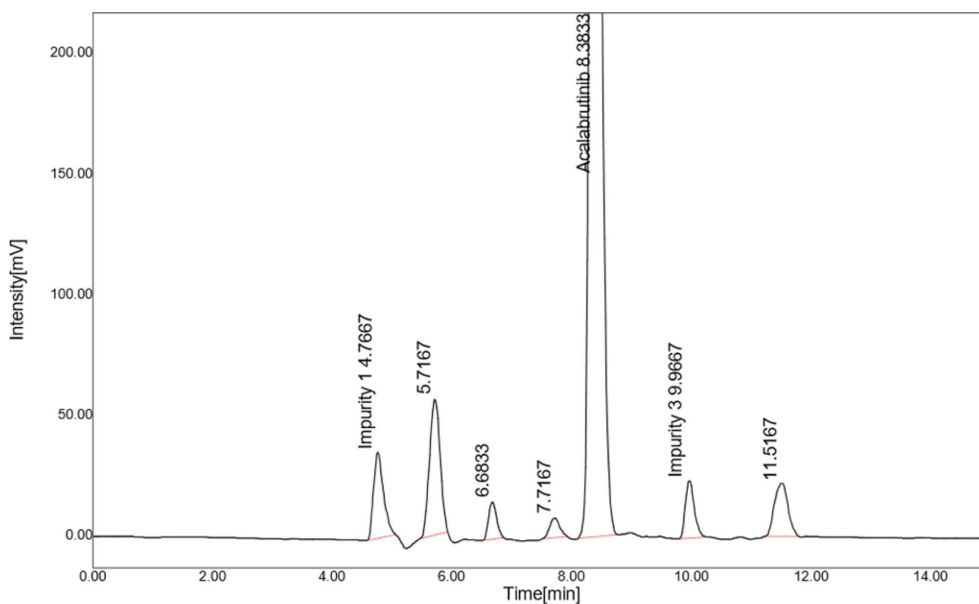


Fig. 5. Oxidative degradation chromatogram of acalabrutinib.

was found to be 5.49 and, in this condition, four additional degradation products were observed at a retention time of 1.58, 4.33, 7.73 and 11.78 min along with imp 3 studied (Fig. 7). In all the degradation condition, standard drug acalabrutinib was detected along and the degradation products were successfully

separated in the optimised conditions and there is no change in retention time of the acalabrutinib in all the stress studies and impurities in the detected stress study. Hence the method can separate and quantify the potential impurities in acalabrutinib.

The % drug and impurity content was calculated

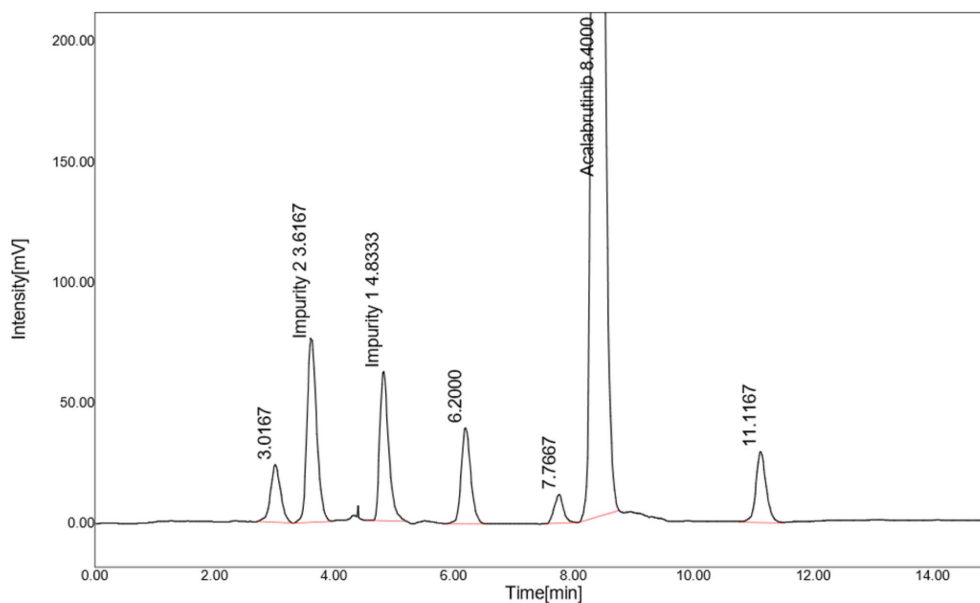


Fig. 6. Photolytic degradation chromatogram of acalabrutinib.

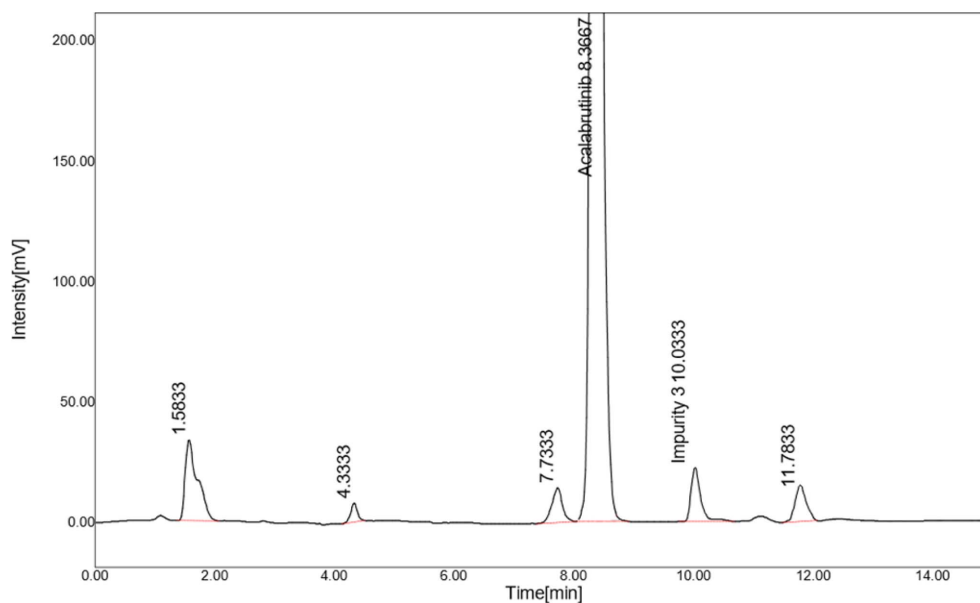


Fig. 7. Thermal degradation chromatogram of acalabrutinib.

using the peak area values observed for the formulation sample solution and compared with standard calibration results. The % assay was found to be 98.79, 0.13, 0.27 and 0.14 % of acalabrutinib, impurity 1, 2 and 3 respectively. The formulation chromatogram was present in Fig. 8. In the formulation chromatogram

there is no other chromatographic detection of other impurities and formulation excipients detected and hence these are not interfere the results. Hence the developed method was found to be suitable for the quantification of acalabrutinib and can separate and analyse impurities.

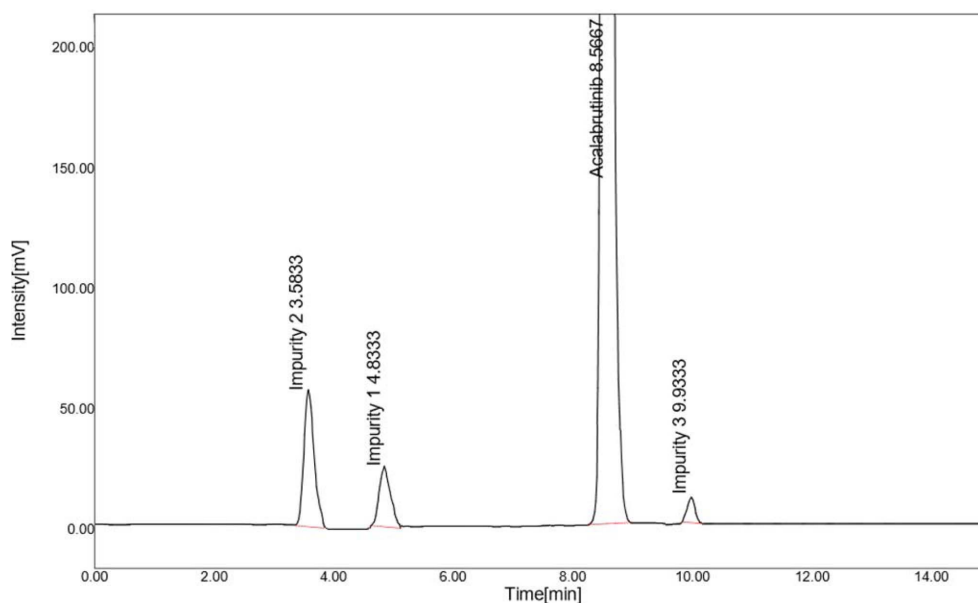


Fig. 8. Chromatogram obtained for the analysis of acalabrutinib and its impurities in Calquence®.

## 5. Conclusions

A novel stability indicating RP-HPLC method was developed for the separation, identification and quantification of acalabrutinib and its impurities 1, 2 and 3. The proposed method achieves satisfactory separation of acalabrutinib from impurities and the degradation products with extended linear range, high recoveries and rapid analysis time. The proposed method ensured the accurate determination of acalabrutinib and its impurities in pharmaceutical formulations. Hence the method is simple, convenient and suitable for the analysis of acalabrutinib and impurities 1, 2 and 3 in bulk and in pharmaceutical formulations.

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**Authors' Positions**

Venu Gopal Kamani : Associate Professor  
Sujatha M : Associate Professor  
Guna Bhushana Daddala : Scholar