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RP- HPLC Method Development And Validation of Acalabrutinib In Bulk And Formulation & Greenness Assessment of Developed Method.

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Abstract
The present study was develop to asses a greenness approach for method development and validation which were verified for linearity, sensitivity, accuracy, precision, specificity, and robustness as per ICH guidelines. A simple UV method was develop & employed using acetonitrile as a diluent at a wavelength of 228nm. The linearity range was observed from 0-20 µg mL^{-1} with a correlation coefficient of 0.9993. The developed method was robust & precise with a %RSD value less than 2 for both intra-day (1.46%) and inter-day (1.52%) precision and results were statistically analyzed according to the ICH Q2 (R1) guidelines. Acalabrutinib was effectively separated using a HiQsil RP C18, 5 µm, 250 mmx4.6 mm i.d. column in an isocratic method of separation, utilizing Acetonitrile: water in the ratio of 75:25% v/v, with a flow rate of 1.0 mL/min and detection at 228 nm. The response was found to be linear in the drug concentration range of 0-50 µg mL^{-1} for Acalabrutinib. The correlation coefficient for (RP-HPLC) was found to be 0.9998 for Acalabrutinib. The value of 1.0438 µg mL^{-1} and 3.1630 µg mL^{-1} for LOD and LOQ for Acalabrutinib were found. The given approach demonstrated good % recovery for Acalabrutinib, indicating that it is very accurate. The method's specificity demonstrates a strong correlation between the retention times of the standard and sample solutions. As a result, the demonstrated approach precisely detects the analyte in the bulk sample with no interference from pharmaceutical dosage form excipients.The greeness of method was evaluated by tools like HPLC_EAT,AGREE, GAPI & COMPLEX GAPI.

Keywords: Acalabrutinib, RP-HPLC, ICH Q2 (R1) guidelines, %RSD, Greenness tools

I. Introduction

Acalabrutinib's molecular name is 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 chemical formula is $C_{26}H_{23}N_7O_2$, with a molecular weight of 465.517 g mol^{-1}. Acalabrutinib is described as a new cancer pharmacologic drug with a high affinity and potency inhibitor of Bruton Tyrosine Kinase (BTK) that has been proposed for the treatment of individuals with mantle cell lymphoma. It is given every 12 hours and can result in atrial fibrillation, various cancers, cytopenia, bleeding, and infection [1,2,3].

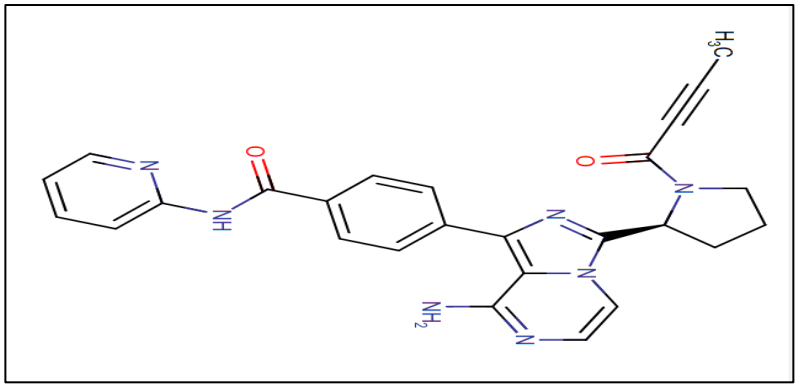

Figure 1. Structure of Acalabrutinib

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1.1 Materials and Methods

Active pharmaceutical ingredients :- Acalabrutinib and Its 100 mg formulation **was gifted by reputed pharma industry.**

Equipment

A Shimadzu UV 1800 was employed to record the UV spectra of the study which is a double beam spectrophotometer & JASCO Extrema lC system-4000 for HPLC analysis.

Analytical method development for Acalabrutinib

Determination of solubility for UV & HPLC analysis

A quantity of standard drug was dissolved in different solvents like water, methanol, ethanol and acetonitrile. UV method was employed for quantitative estimation of solubility & for selection of suitable solvent.

Acetonitrile was employed as the solvent. The solvent was selected based on solubility experiments performed on several solvents and a research of solubility choice solvents by UV spectrophotometer [4].

Preparation of standard stock solution

A 100 mL volumetric flask containing 100 mg of Acalabrutinib was precisely measured before being diluted with acetonitrile and sonicated to the appropriate strength, yielding a concentration of 1000 µg mL^{-1}, to create the standard stock solution. Using acetonitrile, an aliquot of 10 mL of the above standard stock solution was diluted up to the required concentration with acetonitrile in a volumetric flask of 100 mL, giving a concentration of 100 µg mL^{-1} (sub-stock solution).

Preparation of sample stock solution

Twenty-five individual formulation were precisely weighed and crushed into a fine powder for the sample stock solution. A volume equivalent to 10 mg was calculated, transferred, diluted with acetonitrile, and sonicated for 30 minutes in a volumetric flask with a total volume of 100 mL. To prepare the solution with a 100 µg mL^{-1} concentration, 1 mL of the sample stock solution mentioned above was transferred into a 10 mL volumetric flask and diluted with acetonitrile.

Selection of Detection Wavelength

The standardised solution of 10 µg mL^{-1} of Acalabrutinib was prepared and scanned over 400-200 nm range. After the scan was completed, it showed maximum absorbance at 228 nm, so the detection wavelength was selected as 228 nm.

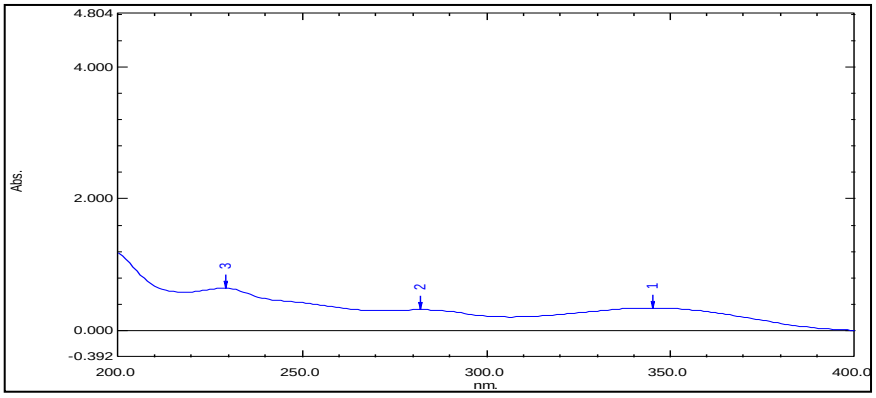

Figure 2 . UV spectra Of Acalabrutinib

1.2 Validation parameters of UV Spectrophotometer

The method was validated in terms of linearity, accuracy, precision, LOD, LOQ, and robustness.

Linearity

The different aliquots of Acalabrutinib in the range of 0-20 mL were transferred into a series of 10 mL volumetric flasks, and the volume was adjusted with acetonitrile to get concentrations of 0-20 µg mL^{-1}, respectively. The calibration curve is plotted as concentration vs absorbance [5].

Accuracy

At three distinct levels 80%, 100%, and 120% a known quantity of standard stock solution was added to the examined sample solutions. Using the suggested strategy, the answer was reanalyzed [6].

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Precision

Both intraday and interday fluctuations in the method's precision were examined. Intraday precision was determined by analyzing the 20 µg mL^{-1} of Acalabrutinib solutions three times on the same day. Interday precision was determined by analyzing the 20 µg mL^{-1} of Acalabrutinib solutions daily for 3 days over a week.

LOD & LOQ

The limit of detection and limit of quantification was used to determine the sensitivity of Acalabrutinib readings made using the suggested approach. Equation LOD = 3.3× σ/s and LOQ = 10× σ/s, LOD, where "σ" stands for standard deviation and "S" for slope, was used to get the LOD and LOQ.

Robustness

The degree to which test findings derived from the examination of the same sample may be repeated is a measure of statistical robustness.

Chromatographic Conditions for HPLC

Table I: Chromatographic Conditions

Sr no.	Specification	Description
1	**Equipment**	**JASCO Extrema lC system-4000**
2	**Software**	**CHROMNAV**
3	**Column**	**HiQsil C_{18} (250 x 4.6 mm, ID 5 µm)**
4	**Wavelength**	**228 nm**
5	**Column temperature**	**25°C**
6	**Flowrate**	**1.0 mL/min**
7	**Injection volume**	**10 µL**
8	**Run time**	**10 min**
9	**Mobile phase**	**Acetonitrile: Water (75:25)**
10	**Diluent**	**Acetonitrile**
11	**Elusion mode**	**Isocratic**

1.3 Selection of Mobile Phase

A range of solvents was screened to get the drug's sharp, well-resolved, and symmetrical peak. Preparations of standard solution and chromatographic conditions for the analysis were the same as discussed above in and respectively with respective changes in mobile phase composition. Various trials were conducted to select a suitable mobile phase for HPLC method development.

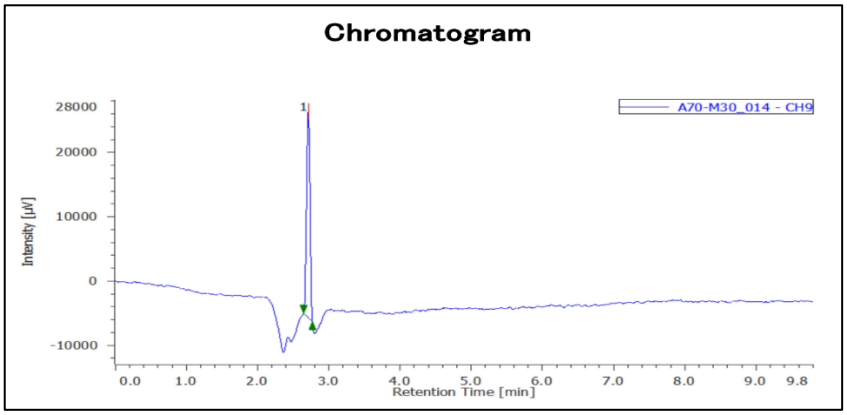

Figure 3.Trial 1- ACN:METHANOl in the ratio 70:30 v/v

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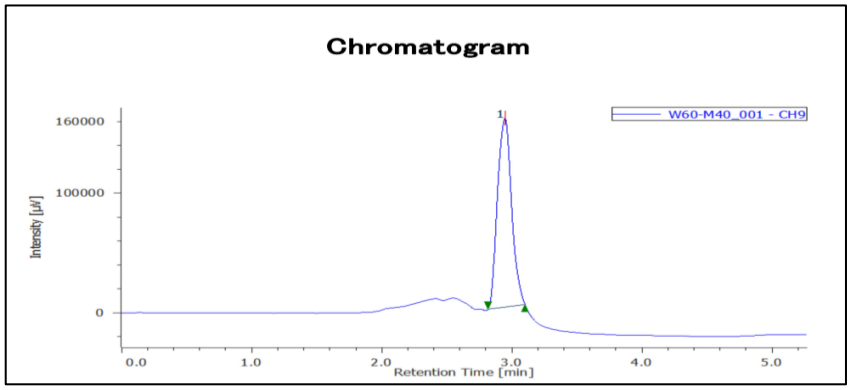

Figure 4.Trial 2- WATER:METHANOL in the ratio 40:60 v/v

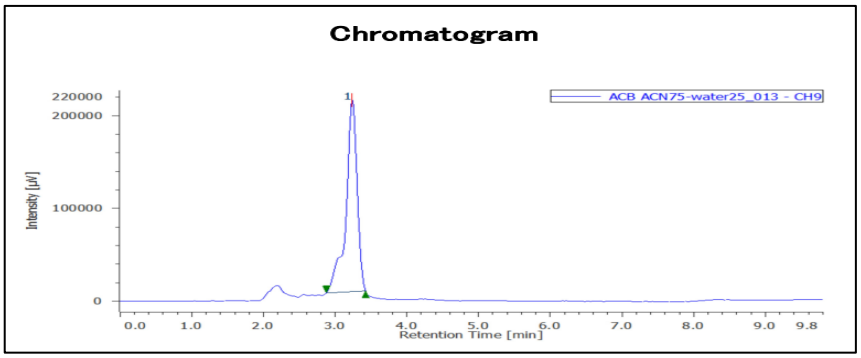

Figure No 5:Trial 3- ACN: WATER IN THE RATIO 75:25 v/v

For mobile phase selection, peak symmetry, tailing factor, peak height, No. of theoretical plates etc. were considered. So number of trials were performed for the selection of the mobile phase.

1.4 Analytical Method Validation for Acalabrutinib by HPLC Method

Specificity study

The following solutions were prepared and injected to prove that the method developed is specific.

Blank solution- Diluent used as a blank

Standard solution- Preparations of standard solution for the analysis were the same as directed in the method of analysis procedure.

Sample solution- Preparations of standard solution for the analysis were the same as directed in the method of analysis procedure. Interprete the identification of drug peak and interference study.

Linearity

To achieve a concentration of 1000 µg mL-1, correctly weigh 10 mg of the drug, put it to a 10 mL volumetric flask, and dilute it with solvent until the mark is satisfied. The drug's linear response was measured throughout a range of 0-50 µg mL- [7,8].

The peak area vs. Concentration calibration curve was plotted & the correlation coefficient and Y-intercept of the linearity curve were calculated.

Accuracy

The method's accuracy was determined by spiking with a known amount of drug X, resulting in sample solutions with drug concentrations of 80%, 100%, and 120% relative to the working concentration, which were then tested in triplicate using the method of analysis. The % recovery was calculated [9].

Precision

Repeatability

A 50µg mL-1 standard solution was meticulously produced and subsequently injected into the system six times. A chromatogram was produced and the %RSD of the Peak Area was calculated.

Intermediate Precision

The intermediate precision was calculated by measuring the responses of a standard peak on the same day and another day of the same solution concentration [10].

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LOD & LOQ
Six sets of linearity concentrations were analysed and LOD & LOQ were calculated using the following equations as per ICH guidelines, based on the response and slope of a regression equation [11].

Robustness
Each of the following parameters has been individually modified and their impact on the system suitability test has been examined [12,13]
- Change in flowrate ±0.2 mL/min
- Change in temperature ±5°C
- Change in wavelength ±2 nm

GREENNESS ESTIMATION
Tools like HPLC_EAT ,AGREE ,GAPI AND COMPLEX GAPI are primarily used in the analysis and validation of the methods greenness. HPLC_EAT shows the effects on health, safety & the environment that have been shown to be the most environmental friendly development[14]. The values in the range of 0 to 1 are notably displayed by AGREE. Red, yellow & Green are the colors used to elaborate the 12 AGREE principles. According to reports the GAPI & COMPLEX GAPI are greenness assessor tools that use a diaphragmatic approach to display the degree of environmental degradation for a range of analytical techniques[15,16].

II. RESULTS AND DISCUSSION
2.1 UV SPECTROPHOTOMETER
Linearity
The correlation coefficient for the linear curve obtained for standard preparations of Acalabrutinib was found to be 0.9993. A linear regression equation was found to be y=0.044x+0.007(R^2=0.9993). The relationship between the concentrations and area of the drug should be linear in the specified range and the correlation coefficient should not be less than 0.999.

Table II. Calibration Plot of Acalabrutinib

Concentration	Absorbance
0	**0**
4	**0.186**
8	**0.362**
12	**0.547**
16	**0.698**
20	**0.887**

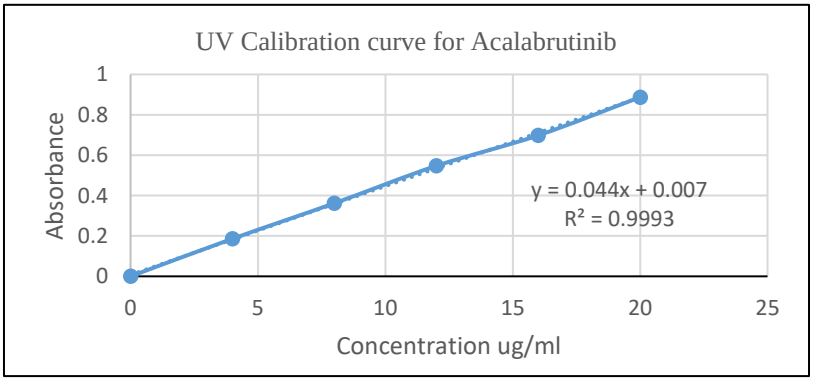

Figure 6. UV Calibration curve of Acalabrutinib

Accuracy
The %recovery was found to be 98.2%, 101.30%, and 101.37% for the levels of 80,100, and 120% respectively. % recovery should be in the range of 98-102%. The outcomes are depicted in Table 3.

Table III. Results of Acalabrutinib Accuracy (n=3 ±SD)

Level	Absorbance	calculated concentration	% recovery	Mean conc	standard Deviation	%RSD

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80%	**0.442**	**9.880**	**98.2%**	**9.82**	**0.052**	**0.53**
	0.439	**9.790**				
	0.438	**9.790**				
100%	**0.544**	**12.20**	**101.3%**	**12.15**	**0.207**	**1.71**
	0.532	**11.93**				
	0.550	**12.34**				
120%	**0.634**	**14.25**	**101.37%**	**14.20**	**0.133**	**0.93**
	0.625	**14.05**				
	0.636	**14.30**				

Precision

Relative standard deviation (%RSD) was used to express the developed method's precision. These findings demonstrate repeatability. The % RSD should not be more than 2. The outcomes of Precision are presented in Tables IV and V .

Table IV. Intraday Precision Results (n=6±S.D)

Concentration (µg Ml^{-1})	Absorbance	X^2	$(X-X)^2$	SD	%RSD
20	**0.889**	**20.04**	**0.2400**	**0.30**	**1.46**
20	**0.900**	**20.29**	**0.0576**		
20	**0.914**	**20.61**	**0.0064**		
20	**0.921**	**20.84**	**0.0961**		
20	**0.915**	**20.63**	**0.0100**		
20	**0.921**	**20.77**	**0.0576**		

Table V. Interday Precision Results (n=6±S.D)

Concentration (µg Ml^{-1})	Absorbance	X^2	$(X-X)^2$	SD	%RSD
25	**0.903**	**20.36**	**0.0049**	**0.31**	**1.52**
25	**0.914**	**20.61**	**0.0324**		
25	**0.905**	**20.40**	**0.0009**		
25	**0.921**	**20.77**	**0.1156**		
25	**0.914**	**20.61**	**0.0324**		
25	**0.882**	**19.88**	**0.3020**		

Detection Limit (LOD) and Quantification Limit (LOQ)

The results indicated that the LOD and LOQ for Acalabrutinib were 1.294 & 3.922 µg mL^{-1}.

Robustness

A standard solution containing 10 µg mL^{-1} was made by changing the wavelength and the effect was checked for the system suitability. The data obtained from these studies are illustrated in Table VI.

Table VI. Results of Acalabrutinib Robustness

CONCENTRATION (µg/mL)	WAVELENGTH	ABSORBANCE
10	**231**	**0.649**
10	**234**	**0.780**
10	**237**	**0.706**
10	**240**	**0.649**
10	**243**	**0.614**

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2.2 HPLC

System suitability parameters

System suitability tests were carried out as part of the validation procedure to make sure the HPLC system was operating dependably and consistently. Six duplicate injections of the standard solution at a concentration of 10 µg mL^{-1} were produced for each validation parameter.

Table VII . Results of System Suitability

SR no.	PARAMETER	RESULTS
1.	Retention time	2.950
2.	Theoretical plates	404534
3.	Tailing Factor	1.12
4.	NTP	2468

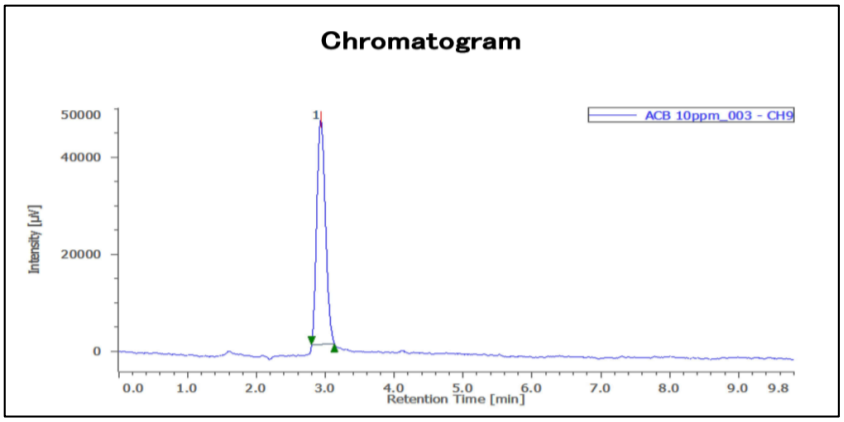

Figure 7. Standard chromatogram data of Acalabrutinib

Linearity

A series of dilutions were made using the standard stock solution to evaluate the linearity of the medication Acalabrutinib. The dilutions covered a concentration range of 0–50 µg mL^{-1}.

Table VIII. Linearity data for Acalabrutinib

Concentration	Absorbance
0	**0**
10	**404534**
20	**791061**
30	**1217986**
40	**1612778**
50	**2039938**

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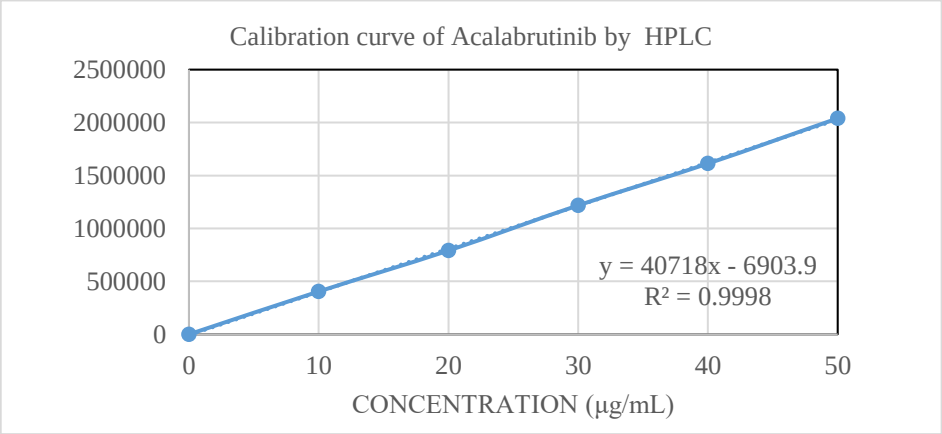

Figure 8. Calibration curve of Acalabrutinib by HPLC

Accuracy

Percentage drug accuracy of three different concentrations 80%, 100%, and 120%.The mean recovery percentage was then calculated based on the results obtained from the recovery studies.

Table IX. Results of accuracy by HPLC method

Level	Peak area	calculated concentration	% recovery	mean concentration	standard deviation	%RSD
0.8	1068816	26.07	100.50%	26.13	0.053	0.20
	1071721	26.15				
	1073110	26.18				
1	1239480	30.27	99.98%	29.99	0.239	0.79
	1224375	29.90				
	1221283	29.82				
1.2	1581215	38.66	101.87%	38.71	0.129	0.33
	1589186	38.86				
	1579245	38.62				

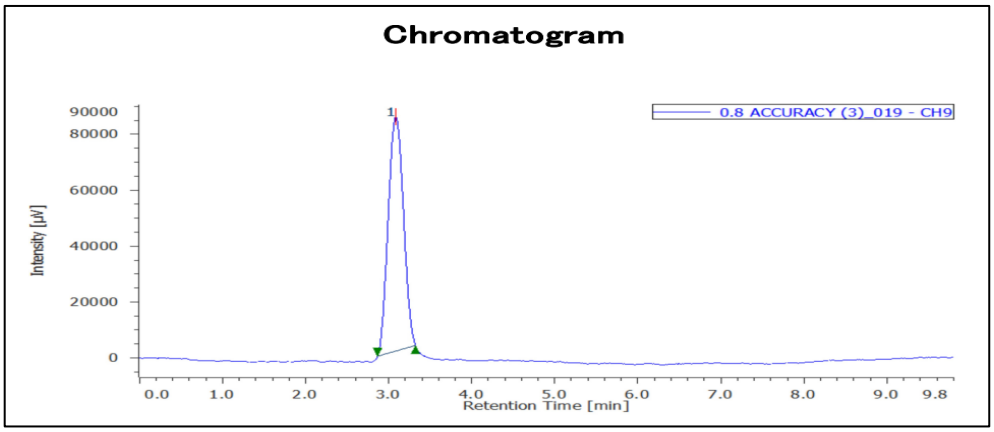

Figure 9. Chromatogram of Acalabrutinib for accuracy [80%]

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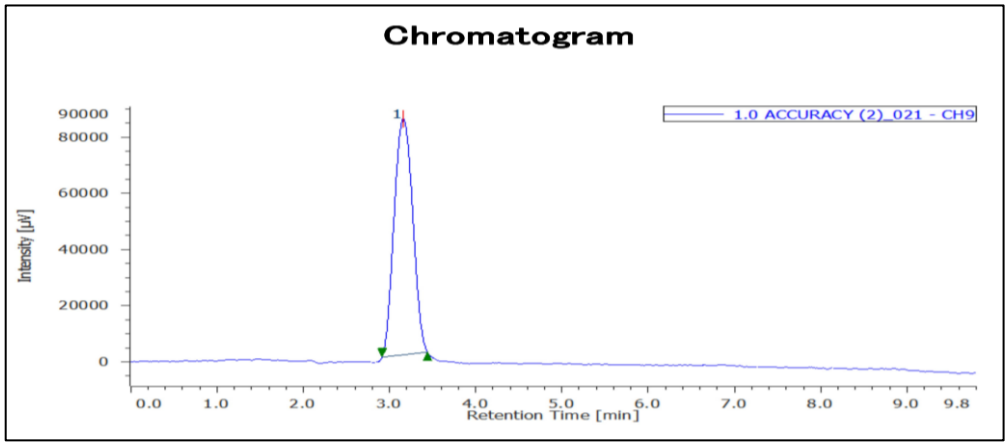

Figure 10. Chromatogram of Acalabrutinib for accuracy [100%]

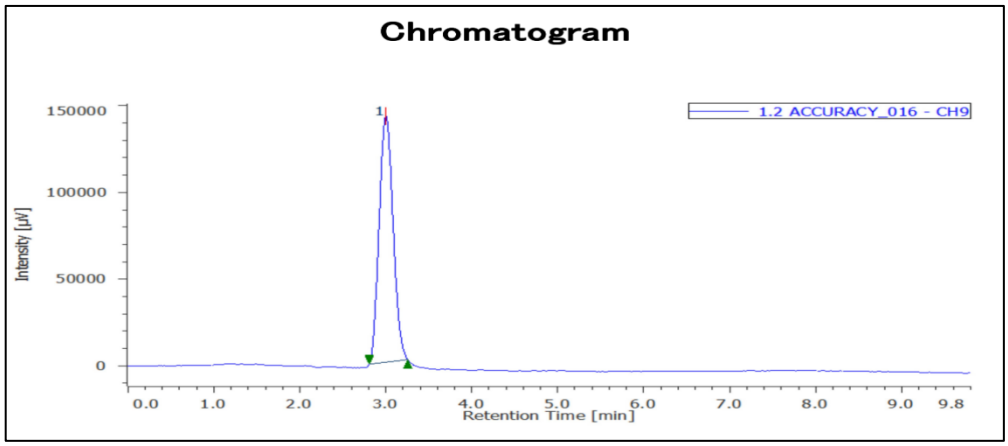

Figure 11. Chromatogram of Acalabrutinib for accuracy [120%]

Precision

Precision was assessed using intraday approach, which involves analyzing the performance of standard solution on the same day, & the interday method,which involves analyzing the standard solution on three differrent days. Precision study was done by injecting six times of standard solution. Results are expressed as %RSD.

Table X. Results of intraday precision by HPLC method

Sr no.	*Sample name*	*Area*	*Theoretical plate*	*Tailing factor*	*Retention time*
1	Test_1	2102013	2394	1.13	2.92
2	Test_2	2077087	2412	1.12	2.92
3	Test_3	2067171	2311	1.13	2.92
4	Test_4	2101604	2340	1.14	2.92
5	Test_5	2102592	2389	1.12	2.92
6	Test_6	2105023	2415	1.15	2.92
MEAN		2092581.67	2376.83	1.13	2.92
SD		16193.41	42.02	0.01	0.01
%RSD		0.77	1.77	0.48	0.26

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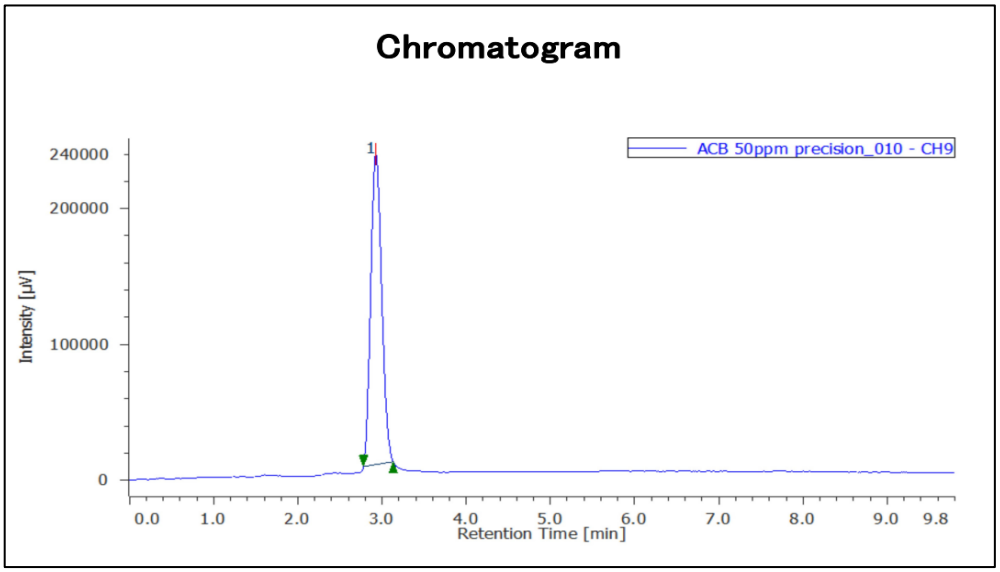

Figure 12. Chromatogram of Acalabrutinib for intraday precision

Table XI. Results of interday precision by HPLC method

Sr no.	Sample name	Area	Theoretical plate	Tailing factor	Retention time
1	Test_1	2110350	2586	1.16	2.91
2	Test_2	2029561	2576	1.16	2.91
3	Test_3	2116379	2569	1.14	2.91
4	Test_4	2053378	2503	1.15	2.91
5	Test_5	2078819	2512	1.14	2.91
6	Test_6	2071725	2600	1.13	2.92
MEAN		2076702	2557	1.2	2.91
SD		33172	40.33	0.01	0.00
%RSD		1.60	1.58	1.01	0.13

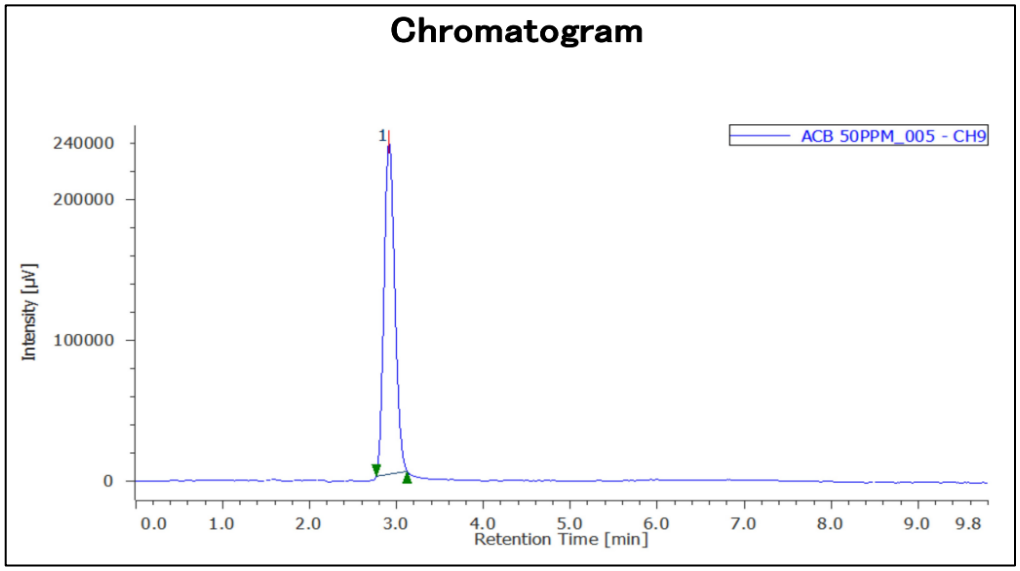

Figure 13. Chromatogram of Acalabrutinib for interday precision

Robustness

Each of the following parameters has been individually modified and their impact on the system suitability test has been examined.

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- Change in flowrate ±0.2 mL/min
- Change in temperature ±5°C
- Change in wavelength ±2 nm

Table XII. Robustness data of Acalabrutinib by HPLC

SR NO.			1	2	3	MEAN	SD	%RSD
FLOW RATE	1.2ml/min	AREA	358053	350966	353541	354187	3587.35	1.01
		RT	2.427	2.500	2.497	2	0.04	1.67
		NTP	2196	2185	2170	2184	13.05	0.60
	0.8ml/min	AREA	521579	513131	522425	519045	5139.11	0.99
		RT	3.62	3.62	3.62	4	0.00	0.10
		NTP	2331	2347	2354	2344	11.79	0.50
TEMP	20°C	AREA	425895	412788	421259	419981	6646.35	1.58
		RT	2.963	3.003	3	3	0.02	0.75
		NTP	2696	2640	2657	2664	28.71	1.08
	30°C	AREA	401036	412045	412980	408687	6642.43	1.63
		RT	2.927	2.903	2.900	3	0.01	0.51
		NTP	2775	2678	2755	2736	51.40	1.88
WAVELENGTH	226nm	AREA	405329	409536	415263	410043	4986.34	1.22
		RT	2.900	2.903	2.900	3	0.00	0.06
		NTP	2642	2584	2564	2597	40.51	1.56
	230nm	AREA	414395	409543	305333	410266	3819.62	0.93
		RT	2.970	2.897	2.9	3	0.04	1.41
		NTP	2609	2568	2545	2574	32.42	1.26

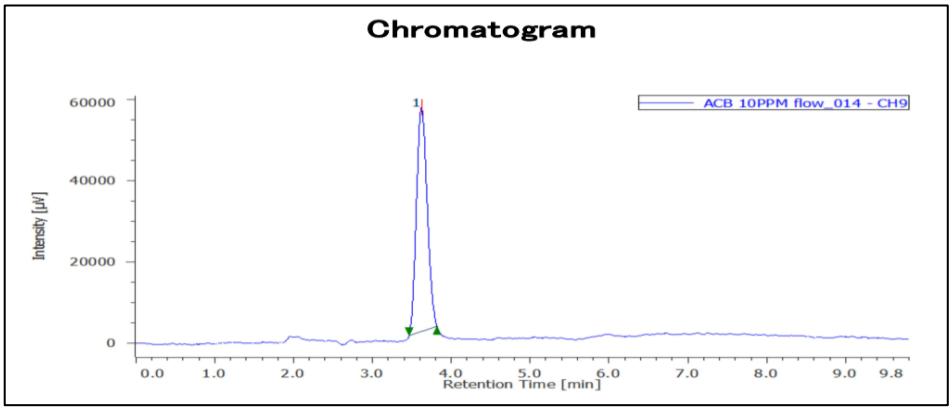

Figure 14. Chromatogram of Acalabrutinib for robustness (0.8 mL/min)

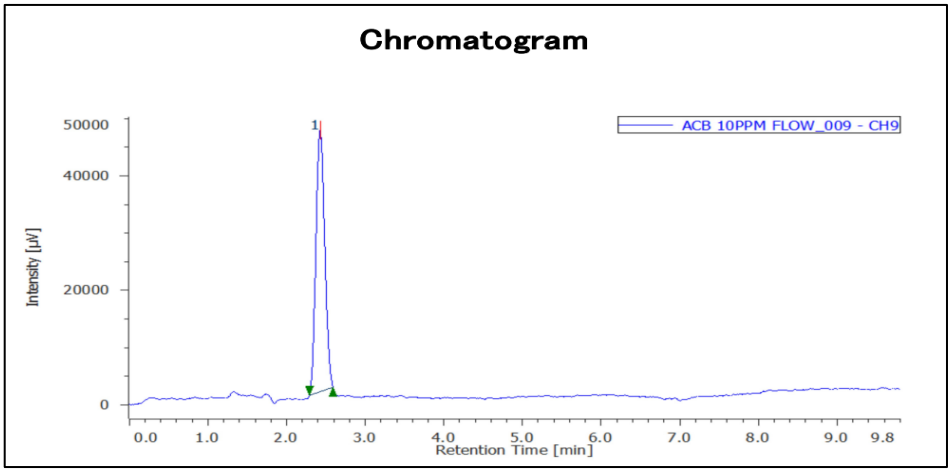

Figure 15. Chromatogram of Acalabrutinib for robustness (1.2 mL/min)

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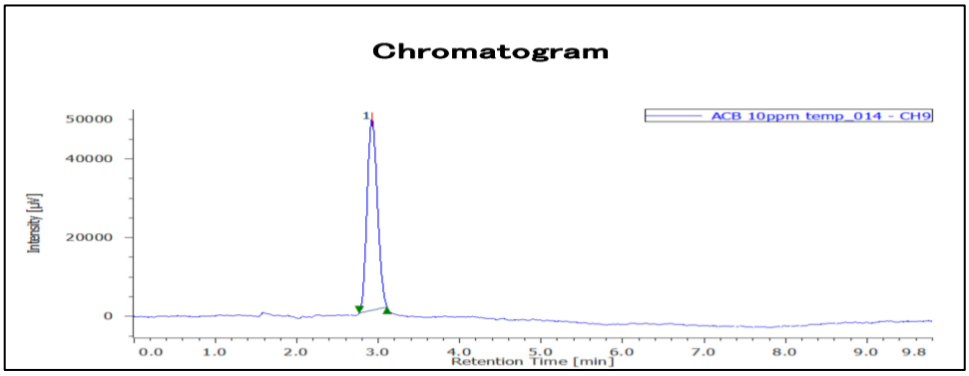

Figure 16. Chromatogram of Acalabrutinib for robustness (20°C)

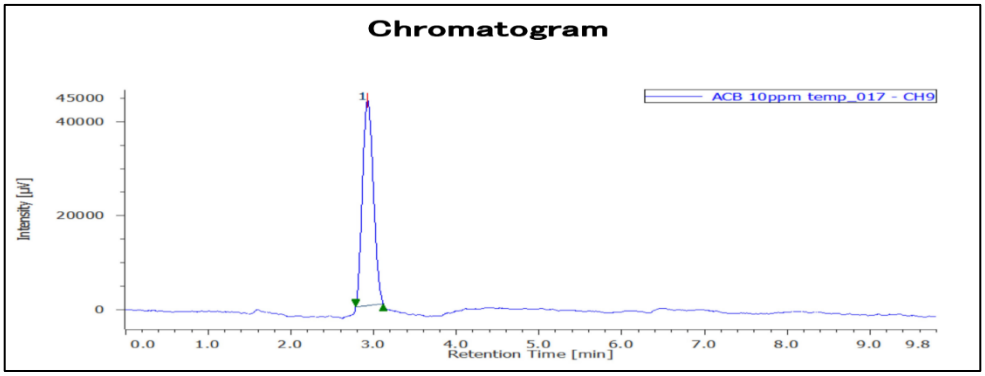

Figure 17. Chromatogram of Acalabrutinib for robustness (30°C)

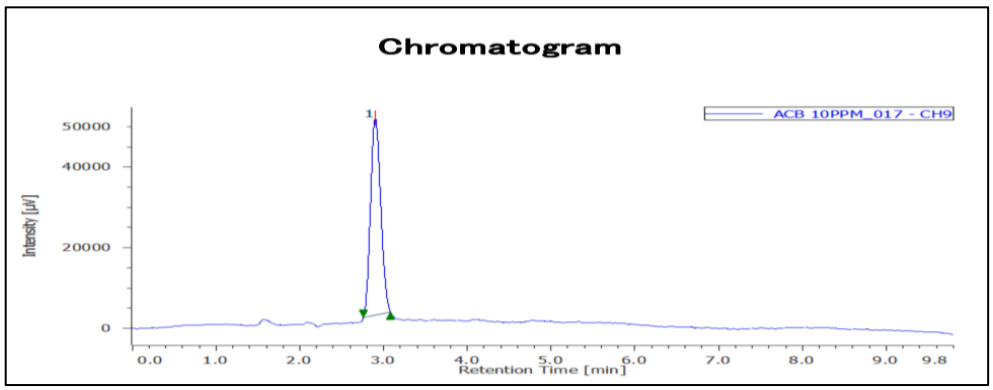

Figure 18. Chromatogram of Acalabrutinib for robustness (226 nm)

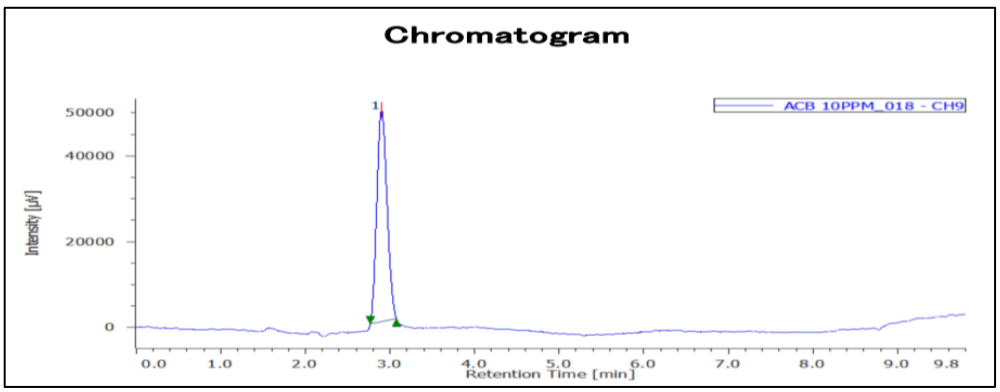

Figure 19. Chromatogram of Acalabrutinib for robustness (230 nm)

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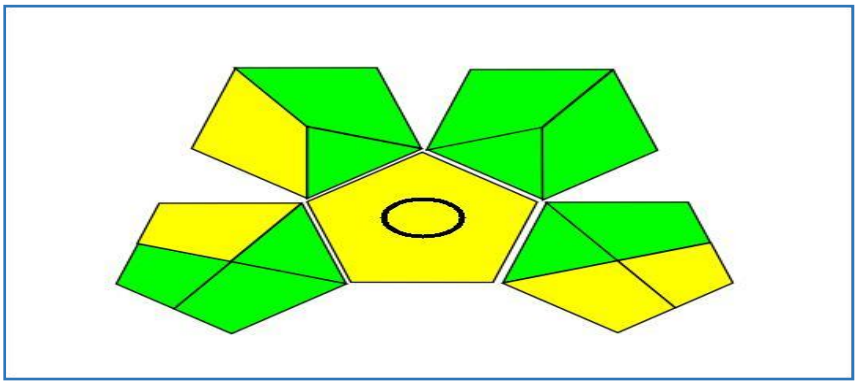

Figure 20. Pictogram of GAPI Assesment of current method

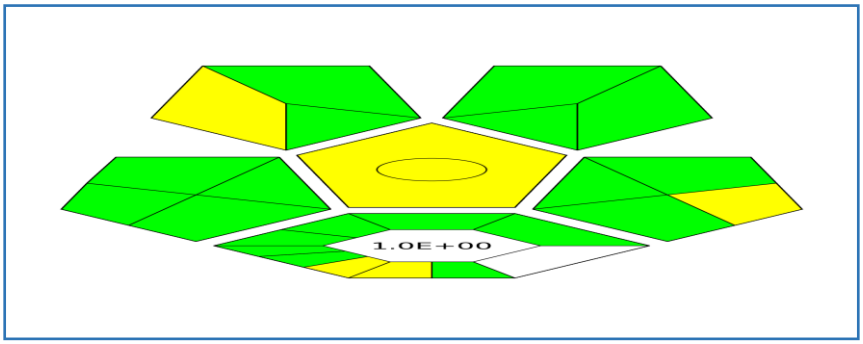

Figure 21. Pictogram of COMPLEX GAPI Assesment of current method

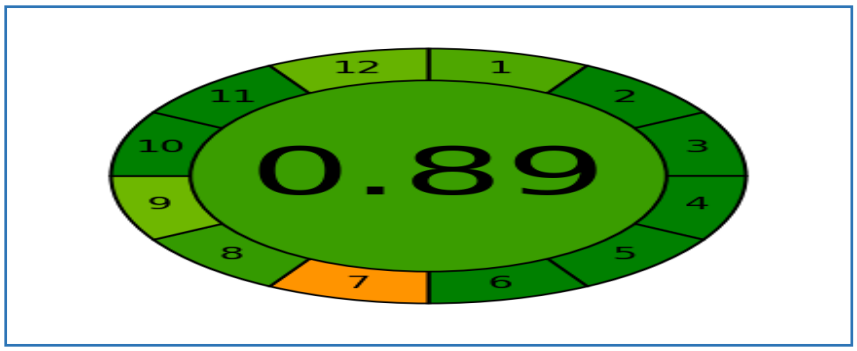

Figure 22. Pictogram of AGREE Assesment of current method

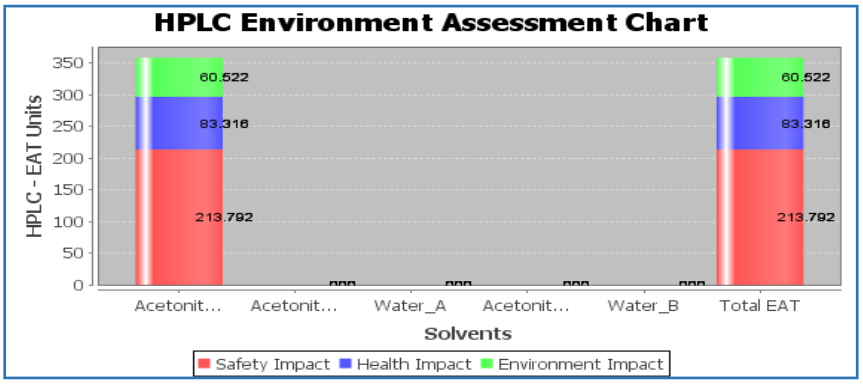

Figure 23.Pictogram of HPLC_EAT Assesment of current method

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III. Conclusions

A novel, simple, rapid, precise, accurate and reproducible UV spectroscopic & reverse phase liquid chromatography method was produced for the determination of Acalabrutinib in bulk & pharmaceutical dosage form. The devised method was verified for linearity, precision, accuracy, robustness, and limit of detection (LOD) and limit of quantification (LOQ). The developed method can be used for the rapid quantification of Acalabrutinib in its pharmaceutical dosage form**.** In a short amount of time, the analytical conditions were created with acceptable resolution. Less than 2% was discovered to be the upper limit of the percentage RSD for all metrics. This shows that the technique created is appropriate for measuring acalabrutinib in labs and for quality assurance needs.The greenness approach was developed for estimation of acalabrutinib method development and validation using the tools like HPLC_EAT,AGREE,GAPI & COMPLEX GAPI.So the suggested approach is green field of analysis that proven environment health beneficiary.

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V. Conflict of interest

The authors declare that they do not have any conflicts of interest. The authors are keenly responsible for the content and preparation of this article.

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