ijlpr 2021; doi 10.22376/ijpbs/lpr.2021.11.5.P41-50

International Journal of Life science and Pharma Research

Research Article

Pharmaceutical Analysis For Novel Drug Formulation

Quantification and Stability Indicating UPLC Method Development and Validation of Acalabrutinib in Bulk and Pharmaceutical Dosage Form

Mohan Goud V* and Pooja Singh N

Department of Pharmaceutical Analysis, Joginpally B. R Pharmacy College, Yenkapally, Moinabad, R.R. Dist., Telangana, 500075

Abstract: Acalabrutinib (ABN) is a Bruton tyrosine kinase inhibitor used to treat mantle cell lymphoma, chronic lymphocytic leukemia and small lymphocytic lymphoma. It has been used in the treatment of Hodgkin lymphoma, multiple myeloma and ovarian cancer. It is available in capsule dosage form, usually prescribed twice a day. The objective of the present study describes the ultra-performance liquid chromatography (UPLC) method development and validation for the estimation of the ABN in Capsule dosage form by following ICH guidelines because no methods were reported in this category. The column and Mobile phase were selected based on trial and error methods. For the estimation, the chromatogram BEH C_{18} (50 mm x 2.1 mm, 1.7µm) column was run through with Mobile phase 0.01N KH_2PO_4: Methanol in the ratio of 50:50 at a flow rate of 0.3 ml/min. Temperature was maintained at 30°C. Optimized wavelength selected for the separation was 234nm. Retention time was achieved 1.148minute in optimized chromatogram. Theoretical plate count and tailing factor were obtained "as per recommendations of ICH limits". The % RSD precision obtained was 0.7% and Intermediate precision obtained was 0.7% as the limit of Precision was less than 2. %Recovery obtained for marketed formulation was 99.23%. LOD, LOQ values obtained from the regression equation (y=9128.5x + 14854) of ABN were 0.18µg/ml and 0.55µg/ml respectively. Proposed method was linear over the concentration range25-150µg/ml. Different degradation studies (acid, alkali, oxidation, thermal, UV, water) were performed and all these samples passed the limits of degradation. Retention time and run time was decreased, so the method developed was simple and economical that can be adopted in regular quality control tests in Industries.

Keywords: *Reverse Phase-UPLC, Acalabrutinib (ABN), Validation, ICH Guidelines.*

***Corresponding Author**

Mohan Goud V , Department of Pharmaceutical Analysis, Joginpally B. R Pharmacy College, Yenkapally, Moinabad, R.R. Dist., Telangana, 500075

Received On 26 June 2021
Revised On 06 September 2021
Accepted On 09 September 2021
Published On 28 September 2021

Funding This research did not receive any specific grant from any funding agencies in the public, commercial or not for profit sectors.

Citation Mohan Goud V and Pooja Singh N , Quantification and Stability Indicating UPLC Method Development and Validation of Acalabrutinib in Bulk and Pharmaceutical Dosage Form.(2021).Int. J. Life Sci. Pharma Res.11(5), P41-50 http://dx.doi.org/10.22376/ijpbs/lpr.2021.11.5.P41-50

1. INTRODUCTION

Acalabrutinib ABN Chemical name is 4-[8-amino-3-[(2*S*)-1-but-2-ynoylpyrrolidin-2-yl]imidazo[1,5-a]pyrazin-1-yl]-*N*-pyridin-2-ylbenzamide.[1] It was approved by [2] FDA in 2017 for the treatment of mantle cell lymphoma and chronic lymphocytic leukemia. [3] It is an orally available Bruton's tyrosine kinase inhibitor with potential antineoplastic activity which prevents the activation of the B-cell antigen receptor signaling pathway. This prevents both B-cell activation and Bruton's tyrosine kinase mediated activation of downstream survival pathways leads to an inhibition of the growth of malignant B cells.[4-11]Chronic lymphocytic leukemia (CLL), the most common leukemia is characterized by the accumulation of mature, CD5+CD23+ monoclonal B lymphocytes in the blood, secondary lymphatic tissues, and the bone marrow.[12] Proliferating CLL cells, which account for approximately 0.1% to 1% of the CLL clone, [13] are typically found within microanatomical structures called proliferation centers or pseudofollicles,[14] where Chronic lymphocytic leukemia (CLL), cells interact with accessory cells (ie, stromal cells or T cells), thereby receiving survival and growth signals. [15] Such external signals from the leukemia microenvironment can supplement intrinsic oncogenic lesions, thereby promoting maintenance and expansion of the Chronic lymphocytic leukemia (CLL) clone. [14, 16-17] In reported Spectrofluorometric method, [18] observed fluorescence data resulting from the acalabrutinib (ACLB) -human serum albumin (HSA) interaction presented binding constants in the range of $6.65\text{-}7.54\times10^{4}M^{-1}$ with the studied temperatures. Those constants showed steady decline with the rising temperatures that further signifies static interaction of the HSA and ACLB. Binding energetics was also interpreted using the fluorescence-recorded results that exhibited a spontaneous exothermic binding reaction with a negative change in Gibbs free energy as well as negative enthalpy and positive entropy changes. Those results suggested the involvement of electrostatic forces as discovered by further computational investigation. Those docking results verified that ACLB binds to domain IIA (site I) of the HSA as demonstrated experimentally by site markers displacement binding studies. Circular dichroism studies along with the synchronous and 3D fluorescence observations showed that ACL binding does not alter the HSA conformation. In the reported RP-HPLC [19] method the separation was done by using Zodiasil C18 (150 x 4.6mm, 5µ) column with mobile phase (Water and methanol 60:40%v/v) at 230nm. The retention time achieved in this method was 2.76 ± 0.1 min and the method was linear over the concentration range of 25-150 µg/ml(r2- 0.9993). In recovery studies, %RSD from reproducibility was found to be below 2%. LOD and LOQ were 0.03 µg/ml and 0.08µg/ml respectively. In another reported RP-HPLC [20] method the separation was done by using kromosilC_{18} column (250×4.6 mm 5µ) with mobile phase 0.1% O-Phosphoric acid and methanol in ratio of 50:50 at wavelength of 236 nm. The method was linear over the concentration range of 25-150µg/ml with coefficient of correlation 0.999. Literature reviewdiscloses that very few different methods were reported for the analysis of ABN in bulk and formulations by fluorescence detection method [18] and RP-HPLC. [19-20]After detailed studies no method was reported to estimate ABN by Ultra Performance Liquid Chromatography (UPLC); hence our present plan is to develop a new, sensitive, economical method for its analysis in bulk and formulation and validated as per ICH norms. [23-24]

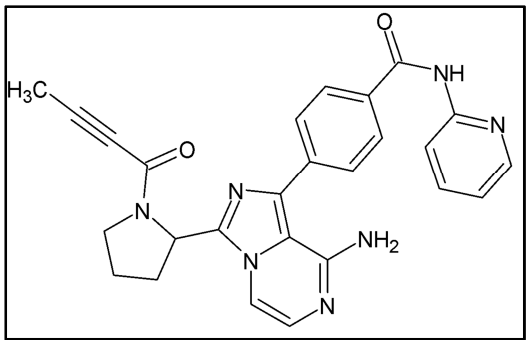

Fig 1. Structure of Acalabrutinib

2. MATERIALS AND METHODS

2.1. Materials

ABN standard active pharmaceutical ingredient (API) obtained from Spectrum lab Private Ltd., Calquence (AstraZeneca Pharmaceuticals) capsule dosage forms, distilled water (milli-Q), Acetonitrile, phosphate buffer and potassium dihydrogen phosphate buffer. All chemicals were HPLC grade and were purchased from local distributors.

2.2. Instruments

The instrument employed for the research was WATERS UPLC 2965 SYSTEM with Auto Injector and Acquity TUV detector. Software used is Empower 2. UV-VIS spectrophotometer PG Instruments T60 with special bandwidth of 2mm and 10mm and matched quartz was used for measuring absorbance of ABN. Sonicator (Ultrasonic sonicator), pH meter (Thermo scientific), Micro balance (Sartorius), Vacuum filter pump (Welch) are the other instruments used for this study.

2.3. Analytical methodology

2.3.1. Preparation of buffer(0.01N KH_2PO_4 Buffer)[8]

Accurately weighing 1.36gm of Potassium dihydrogen Orthophosphate in a 1000ml of volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water.

2.3.2. Standard/ Working solution preparation

2.3.3. Preparation of Standard stock solutions:

Accurately weighed 25mg of ABN transferred 25ml and volumetric flasks, 3/4th of diluents was added and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution (1000µg/ml of ABN).

2.3.4 *Preparation of Standard working solutions (100% solution)*

1ml of ABN from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (100µg/ml of ABN).

2.3.5 *Preparation of Sample stock solutions:*

5 capsules were weighed and the average weight of each capsule was calculated, then the weight equivalent to 1 capsule was transferred into a 100 ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by UPLC filters (1000 µg/ml of ABN).

2.3.6 *Preparation of Sample working solutions (100% solution)*

5ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (100µg/ml of ABN)

2.3.7 *Diluent*

Based up on the solubility of the drugs, diluent was selected,Water and Methanol were selectedin the ratio of 50:50

2.3.8 Linearity

Linearity solutions are prepared by withdrawing 0.25, 0.5, 0.75, 1, 1.25 and 1.5ml from the Stock solutions of ABN in to 6 different volumetric flasks and diluted to 10ml with diluents to get 25, 50, 75, 100, 125 and 150 µg respectively.

2.3.9 Precision

5 Capsules were weighed and the average weight of each capsule was calculated, then the weight equivalent to 1 capsule was transferred into a 100ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters(1000µg/ml of ABN) 5ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (100µg/ml of ABN)

2.3.10 Accuracy

Accurately weighed 5 mg of ABN, transferred to individual 25 ml volumetric flasks separately. 3/4th of diluents was added to these flasks and sonicated for 10 min. Flasks were made up with diluents and labeled as Standard stock solution (1000µg/ml of ABN). From this solution 0.5, 1.0 and 1.5ml was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent to produce 50, 100, 150% of spiked solution respectively.

2.4 Validation Procedure [24]

The analytical method was validated as per ICH Q2(R1) guidelines for the parameters like system suitability, specificity, accuracy, precision, linearity, robustness, limit of detection (LOD), limit of quantitation (LOQ) and forced degradation.

2.4.1. System Suitability [24]

System suitability parameters were measured to verify the system performance. The parameters including USP plate count, USP tailing and % RSD are calculated and found to be within the limits. [24]

2.4.2. Accuracy [24]

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found.[24]It was assessed by the recovery studies at three different concentration levels (i.e., 50, 100 and 150 %). In each level, a minimum of three injections were given and the amount of the drug present, percentage of recovery and related standard deviation were calculated. [24]

2.4.3. Precision [24]

The precision of an analytical procedure expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. [24]The precision of the present method was assessed in terms of repeatability, intra-day and inter-day variations. It was checked by analyzing the samples at different time intervals of the same day as well as on different days. [24]

2.4.4. Linearity and range [24]

The linearity of an analytical procedure is its ability to obtain test results which are directly proportional to the concentration of analyte in the sample within a given range. The six series of standard solutions were injected for assessing linearity range. The calibration curve was plotted using peak area with concentration of the standard solution and the regression equations were calculated. [24]

2.4.5. LOD and LOQ [24]

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. LOD and LOQ were separately determined based on the calibration curve. The LOD and LOQ of ABN are determined by injecting progressively low concentrations of standard solutions by using the developed method. The LOD and LOQ were calculated as 3.3S/N and 10S/N respectively as per ICH guidelines, where S/N indicates signal-to-noise ratio. [24]

2.4.6. Robustness [24]

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate

variations in method parameters and provides an indication of its reliability during normal usage. Robustness study was performed by injecting standard solution into the UPLC system and altered chromatographic conditions such as Flow minus, Flow plus, mobile phase minus, mobile phase plus, temperature minus and temperature plus. The separation factor, retention time and peak asymmetry were calculated by determining the effect of the modified parameters. [24]

2.5. Stress degradation and procedure [25- 26]

Stress degradation should be no interference between the peaks obtained for the chromatogram of forced degradation preparations. Stress degradation studies were performed as per ICH guidelines Q1A (R2).The degradation peak purity of the principal peaks shall pass. The degradation peak purity of the principle peaks shall pass. Forced degradation studies were performed by different types of stress conditions (acid, alkali, oxidation, thermal, UV, water) to obtain the degradation of about 20%w/v. [25-26]

2.5.1. *Hydrolytic conditions*

Hydrolysis is a chemical process that includes decomposition of a chemical compound by reaction in presence of water at different pH levels. Hydrolysis can be done by Sulphuric acid and hydrochloric acid at 0.1–1M strength for acids and NaOH or KOH at 0.1–1M strength for bases are recommended as suitable reagents. Co-solvents can also employ in case of poor in water soluble compounds which are using for stress testing. The selection of co-solvent is depends on the drug structure. Stress testing trial is normally started at room temperature, and if there is no degradation reaction, it is refluxed in elevated temperature at 50–70°C for 30 minutes. [25- 26]For Hydrolytic conditions like Acid and Alkali Degradation Studies, to 1 ml of stock solution of ABN, 1ml of 2N Hydrochloric acid, and 1 ml of 2 N sodium hydroxide were added separately, and refluxed for 30mins at 60^0c. The resultant solutions were diluted to obtain (100µg/ml) solutions respectively, and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of the samples. [25-26]

2.5.2. *Oxidation conditions*

Peroxides like Hydrogen peroxide; perbenzoicacid is commonly used solvents at strength of 0.1–3% for oxidation of drug substances in stability degradation studies. [25- 26] To 1 ml of stock solution of ABN 1 ml of 20% hydrogen peroxide (H_2O_2) was added separately. The solutions were kept for 30 min at 60^0c. For UPLC study, the resultant solution was diluted to obtain (100µg/ml) solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample. [25-26]

2.5.3. *Photolytic conditions*

The photo stability studies must be carried out to explain that a light exposure does not result in unacceptable change of drug substances. Photo stability studies are conducted to generate primary degraded drug substance by exposure to UV or fluorescent light conditions. [25- 26]

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

2.5.4. Thermal conditions

Thermal degradation study is carried out at 40–80°C. The standard drug, ABN solution was placed in oven at 105^0c for 6 h to study dry heat degradation. For UPLC 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. [25- 26]

2.5.5. *Neutral Degradation Studies*

Stress testing under neutral conditions was studied by refluxing the drug, ABN in water for 6hrs at a temperature of 60°c. For UPLC study, the resultant solution was diluted to (100µg/ml) solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample. [25]

3. STATISTICAL ANALYSIS [27-28]

Statistical analysis of data obtained during a method validation should be performed to demonstrate validity of the analytical method. The statistics required for the interpretation of analytical method validation results are the calculation of the mean, standard deviation (SD), relative standard deviation (RSD), and regression analysis (r2). These calculations are typically performed using statistical software packages such as Excel, regression analysis, Minitab, etc. The purpose of statistical analysis is to summarize a collection of data that provides an understanding of the examined method characteristic. The acceptance criteria for each validation characteristic are typically around the individual values as well as the mean and relative standard deviation. The statistical analysis explained in this paper is based on assumption of normal distribution. [27] The data obtained were analysed by Graph pad prism software version 9. The data is subjected to regression analysis to obtain the line of equations in linearity studies.

4 RESULTS AND DISCUSSION

4.1 Method Development

4.2 Optimized method

Trials were performed for the method development by using different columns like CHS C_{18}, BEH C_{18}, SB C_8, HSS C_{18}, Hibar C_{18} etc., and the best peak with least fronting factor was found with R_t= 1.148 min for ABN. Optimized chromatographic conditions were shown in Table 1 and optimized chromatogram was shown in Figure 2.

Table 1. Chromatographic conditions	
Mobile phase	0.01N KH_2PO_4: Methanol (50:50)
Flow rate	0.3 ml/min
Column	BEH C_{18} 50mm x 2.1 mm, 1.7µm
Detector wavelength	Acquity TUV 234 nm
Column temperature	30°C
Injection volume	1.00µL
Run time	3 min
Diluent	Water and Methanol in the ratio of 50:50

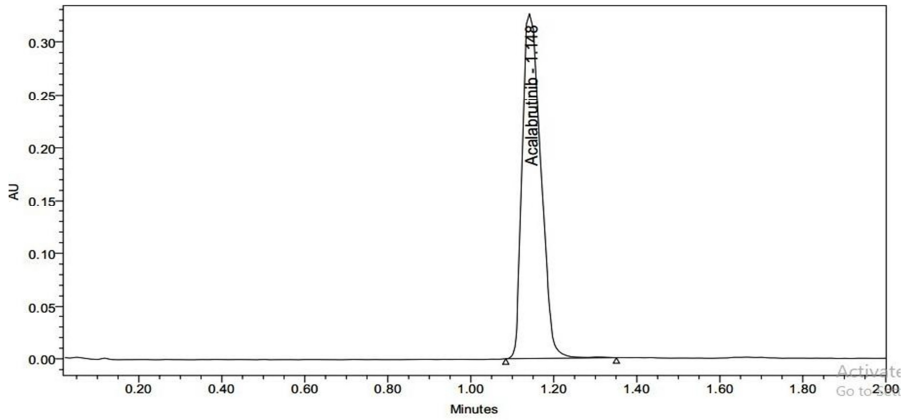

Fig2.Optimized chromatogram

4.3 System suitability

According to ICH guidelines plate count should be more than 2000, tailing factor should be less than 2 and resolution must be more than 2. All the system suitable parameters were passed and were within the limits. System suitability parameters were shown in Table 2 and chromatogram was shown in Figure 3.

Table 2. System suitability parameters of ABN				
Injection S. No.	**RT(min)**	**Peak area**	**USP Plate Count**	**Tailing**
1	1.154	1051370	3139	1.36
2	1.154	1060857	2822	1.34
3	1.156	1047409	2924	1.36
4	1.157	1063500	3095	1.38
5	1.158	1044630	3243	1.39
6	1.160	1050622	2859	1.21
Mean	1.16	1053065	3013.67	1.34
S.D	0.002	7504.5	169.48	0.07
%RSD	0.2	0.7	5.6	4.9

S.D=standard deviation (n=6) %RSD=relative standard deviation

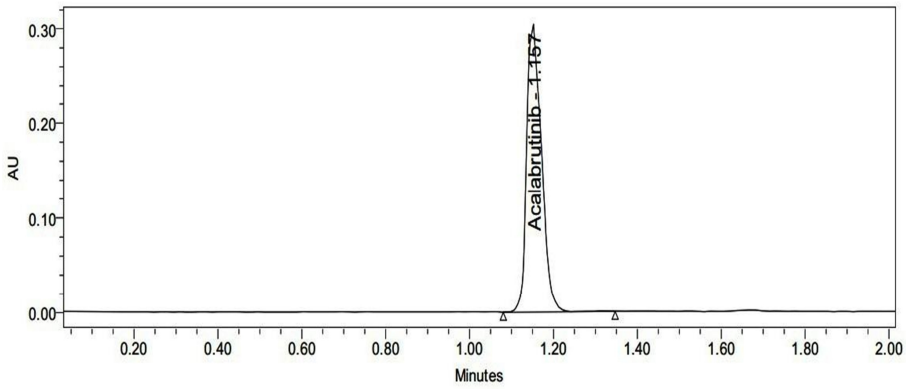

Fig 3. System suitability Chromatogram

5 Methods for Validation

5.1 Linearity

To demonstrate the linearity of the assay method, injected 6 standard solutions with concentrations of about 25-150µg/mlof ABN and plotted a graph(y = 9128.5x + 14854) to concentration versus peak area. Slope obtained was 14854 Y-Intercept was 9128.5and the Correlation Coefficient was found to be r2=0.9993. Calibration curve was depicted in Figure 4 and the data's of linearity studies are enlisted in Table 3.

Table 3. Linearity table of ABN

Linearity Level (%)	Concentration (µg/ml)	Peak area		
		Set 1	Set 2	Mean±S.D
0	0	0	0	0
25	25	232191	271193	251692±27578.6
50	50	491049	494705	492877±2585.2
75	75	682284	694990	688637±8984.5
100	100	903699	936897	920298±23474.5
125	125	1137872	1187873	1162873±35356.0
150	150	1338107	1421997	1380052±59319.2

S.D=standard deviation (n=2)

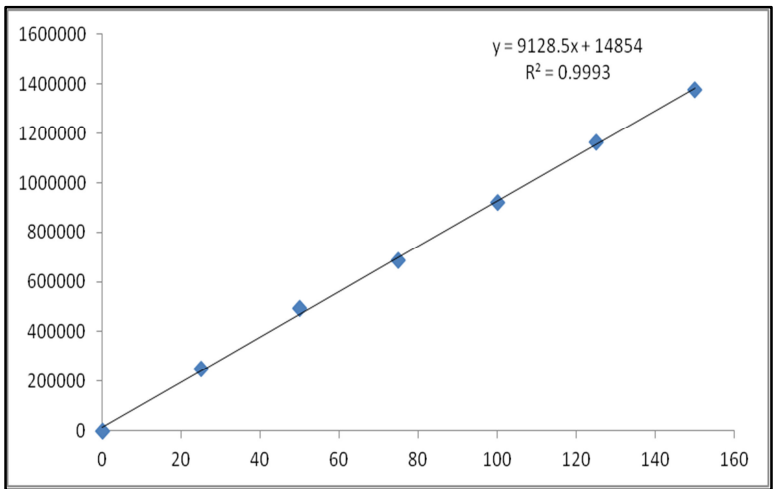

Fig 4. Calibration curve of ABN

5.2 Precision

From a single volumetric flask of working standard solution, six injections were given and the obtained chromatographic peak areas were mentioned below. The Average peak area, standard deviation and % RSD were calculated. The Average peak area precision obtained was 1047099 and Intermediate precision peak area obtained was 1014024. The % RSD precision obtained as 0.7% and Intermediate precision obtained as 0.7% as the limit of Precision was less than "2" the system precision was passed in this method. System precision values were shown in Table 4.

Table 4. System precision table of ABN

S. No	Precision peak area	Intermediate precision Peak area
1.	1041118	1003963
2.	1044211	1024542
3.	1058608	1014156
4.	1047104	1009888
5.	1040360	1016819
6.	1051190	1014773
Mean	1047099	1014024
S.D	6909.7	6889.8
%RSD	0.7	0.7

Where S.D=standard deviation (n=6) %RSD=relative standard deviation

5.3 Accuracy

Three levels of Accuracy samples were prepared by standard addition method. Triplicate injections were given for each level of accuracy and mean %Recovery was obtained as 100.24%. Recovery studyvalues were shown in Table 5.

Table 5.Accuracy studies of ABN

%Concentration	Amount recovered (µg/mL)			% Recovery		
	50%	100%	150%	50%	100%	150%
Trail-I	50.04	99.86	150.51	100.09	99.86	100.34
Trail-II	50.21	99.65	152.23	100.41	99.65	101.48
Trail-III	49.66	99.73	151.95	99.32	99.73	101.30
AVG(%Recovery)±S.D				99.9±0.56	99.75±0.11	101.04±0.61
%RSD				0.56	0.11	0.61
Mean %Recovery±S.D				100.24±0.74		
Mean %RSD				0.73		

Where S.D=standard deviation (n=3) %RSD=relative standard deviation, AVG=average

5.4 Robustness

Robustness conditions like Flow minus (0.2ml/min), Flow plus (0.4ml/min), mobile phase minus (55W:45M), mobile phase plus (45W:55M), temperature minus (25°C) and temperature plus (35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the acceptance criteria. Robustness data were shown in Table 6.

Table 6. Robustness data of ABN

S. No.	Condition	%RSD of ABN
1	Flow rate (-) 0.2ml/min	0.5
2	Flow rate (+) 0.4 ml/min	0.3
3	Mobile phase (-)55W:45M	0.4
4	Mobile phase (+)45W:55M	0.2
5	Temperature (-) 25°C	0.2
6	Temperature (+) 35°C	0.2

5.5 LOD and LOQ

LOD and LOQ were estimated from the signal-to-noise ratio. The LOD of ABN was found to be 0.18µg/ml and the LOQ was 0.55µg/ml respectively. LOD and LOQ values were shown in Table 7. LOD and LOQ Chromatograms were shown in Figure 5 & 6 respectively.

Table 7. Sensitivity table of ABN

Molecule	ABN (µg/ml)
LOD	0.18
LOQ	0.55

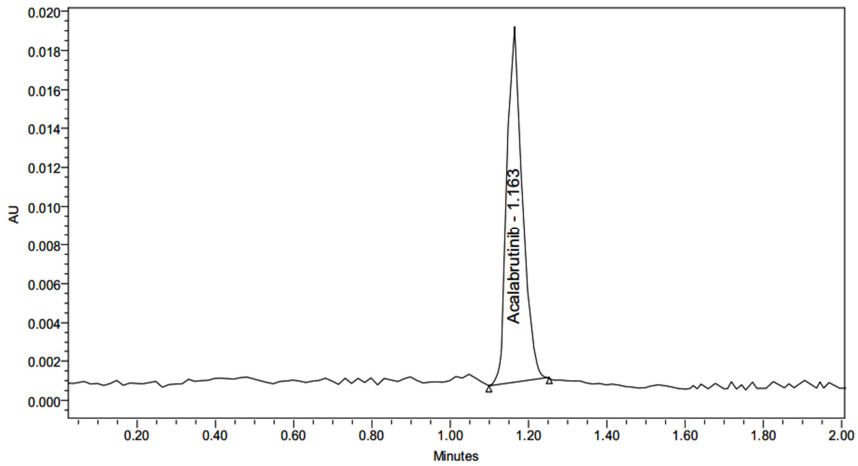

Fig 5.LOD Chromatogram ofABN

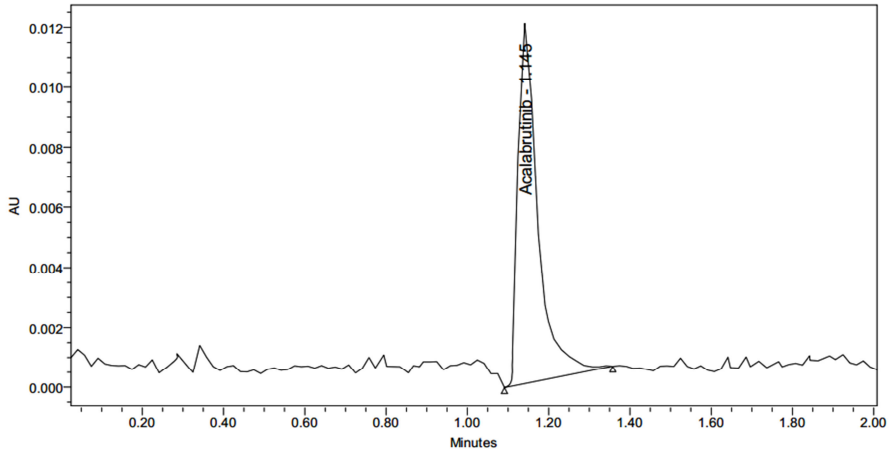

Fig 6.LOQ Chromatogram of ofABN

5.6 Degradation Data

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 values were shown in Table 8.

Table 8. Degradation data of ABN

S. No	Degradation Condition	Standard Drug	Analyte Assay	% Drug Undegraded	% Drug Degraded
1	Acid	1051370	1035257	98.11	1.89
2	Alkali	1060857	1025617	97.20	2.80
3	Oxidation	1047409	1019630	96.63	3.37
4	Thermal	1063500	1034818	98.07	1.93
5	UV	1044630	1013345	96.04	3.96
6	Water	1050622	1021640	96.04	3.96
AVG±S.D		1053065±7504.5			
%RSD		0.7			

Where S.D=standard deviation, %RSD=relative standard deviation, AVG=average, n=6

5.7 Assay of Marketed formulation

AstraZeneca Pharmaceuticals, Calquence capsule bearing the label claim Acalabrutinib 100mg. Assay was performed with the above formulation. Average % Assay obtained was 99.23%. Assay Data of Marketed Formulation were shown in Table 9.

Table 9. Assay Data of Marketed Formulation of ABN

Sample No	% Assay
1	98.67
2	98.96
3	100.33
4	99.24
5	98.60
6	99.62
Mean	99.23
S.D	0.65
%RSD	0.7

Where S.D=standard deviation, %RSD=relative standard deviation

6. DISCUSSION

Literature review depicts that no UPLC analytical method reported for ABN. Up-to-date only three methods were reported including one spectrofluorometric method [18] another two were RP-HPLC methods. [19, 20] In our studies, the aim and objectives was to develop a new UPLC method for rapid, simple and simultaneous quantification, validation and stability studies of ABN. The present method was developed with trials and error methods by using different mobile phases and differentcolumns like CHS C_{18}, BEH C_{18}, SB C_8, HSS C_{18}, Hibar C_{18} etc.,The mobile phase with 0.01N KH_2PO_4: Methanol in the ratio 50:50 produced the optimized separation chromatogram (Figure 2) using BEH C_{18}(50mm x 2.1 mm, 1.7µm) column. The developed method

was validated as per ICH guidelines. The validation parameters[24] such as specificity, Theoretical plates (3013.67), Tailing factor (1.34), linearity (r2=0.9993), recision (0.7% RSD), accuracy(100.24%), robustness and system suitability results were achieved and were within the ICH guidelines [22-24] for ABN. The retention time shown in this proposed method was 1.148 min. where in reported methods [19, 20] it was above 2 min. The calibration curve was linear over the concentration range of 25-150µg/ml. The LOD of ABN was found to be 0.18µg/ml and the LOQ was 0.55µg/ml respectively. For the assay of marketed formulation, the average % Assay obtained was 99.23±0.65 w/v is under the limits. The high percentage of recovery and low percentage coefficient of variance confirm the suitability of the method. Hence it was concluded that the RP-UPLC method developed was very much suited for routine analysis. System suitability and validation parameters of the developed method of ABN summary data showninTable10.

Table 10. Summary data of ABN

Parameter	ABN Observed value
Theoretical plates (>2000)	3013.67
Tailing factor (>2)	1.34
Retention time (min)	1.148 min.
Linearity range (µg/ml)	25-150 µg/mL
Regression equation y=mx+c	y = 9128.5x + 14854
Slope (m) & Intercept (c)	9128.5 & 14854
Correlation coefficient(R^2 not less than 0.99)	R^2=0.9993
Precision repeatability (n=6, RSD within 2%)	0.7
Intermediate precision (n=6, RSD within 2%)	0.7
LOD (S/N=3)	0.18
LOQ (S/N=10)	0.55

7. CONCLUSION

In the present investigation, from the above experimental results it was concluded that the newly developed RP-UPLC method was simple, specific, accurate and precise. The method was effectively validated in terms of system suitability, linearity, precision, accuracy, range, LOD, LOQ and robustness and stability indicating studies according to ICH guidelines. Hence the developed method can be used for estimation of ABN in quality control departments of pharmaceutical industries and testing laboratories.

8. ACKNOWLEDGMENTS

The authors are thankful to Spectrum lab Private Ltd., for providing gift sample of the API. The authors are also grateful to Smt. Shri. J. Gayatri Ma'am, Joint Secretary ,Jogin pally B. R Educational Society, for her continuous support and providing all the facilities to carry out research work.

9. AUTHORS CONTRIBUTION STATEMENT

Mohan Goud V involved in planning and supervised the work, processed the experimental data, drafted the manuscript and supervised the findings of this work. Pooja Singh N designed and performed the experiments and analyzed the data.

10. CONFLICTS OF INTEREST

Conflict for interest declared of none.

11. REFERENCES

1. AcalabrutinibChemical-Structure ID 36764951, Available from: http://www.chemspider.com
2. Acalabrutinib Monograph for Professionals. Drugs.com. Retrieved16 March 2019.
3. Calquence, EPAR. European Medicines Agency, 20 July 2020. Retrieved 11 November 2020.
4. Podoll T, Pearson PG, Evarts J, Ingallinera T, Bibikova E, Sun H, et al. Bioavailability, Biotransformation, and Excretion of the Covalent Bruton Tyrosine Kinase Inhibitor Acalabrutinib in Rats, Dogs, and Humans. Drug MetabDispos. 2019 Feb;47(2):145-154.
5. Ponader S, Chen SS, Buggy JJ, Balakrishnan K, Gandhi V, Wierda WG, et al. The bruton tyrosine kinase inhibitor PCI-32765 thwarts chronic lymphocytic leukemia cell survival and tissue homing in vitro and in vivo. Blood, 2012 Feb 2;119(5):1182-9. doi: 10.1182/blood-2011-10-386417.
6. Yazdy MS, Mato AR, Roeker LE. Toxicities and outcomes of acalabrutinib-treated patients with chronic lymphocytic leukemia: a retrospective analysis of real world patients. Blood. 2019;134 (Supplement_1):4311.
7. Acalabrutinib,DrugBank Accession Number DB11703,Available from: https://go.drugbank.com.
8. British Pharmacopeia, The BritishPharmacopoeial Commission, the stationary office, UK, London, 2011;1408-1409: 2.
9. Byrd JC, Harrington B,O'Brien S, Jones JA, Schuh A, Devereux S, et al. Acalabrutinib (ACP-196) in relapsed chronic lymphocytic leukemia. N Engl J Med. 2016;374(4):323–332.
10. Barf T, Covey T, Izumi R, Kar BVD, Gulrajani M, Lith BV, et al. Acalabrutinib (ACP-196): a covalent bruton tyrosine kinase inhibitor with a differentiated selectivity and in vivo potency profile. J PharmacolExpTher. 2017;363(2):240-252.
11. Barf T, Covey T, Izumi R, Kar BVD , Gulrajani M , Lith BV, et al. Acalabrutinib (ACP-196): A Covalent Bruton Tyrosine Kinase Inhibitor with a Differentiated Selectivity and In Vivo Potency Profile. J PharmacolExpTher. 2017 Nov;363(2):240-252. doi: 10.1124/jpet.117.242909.
12. Chiorazzi N, Rai KR, Ferrarini M. Chronic lymphocytic leukemia. N Engl J Med. 2005;352(8):804–815.

13. Messmer BT, Messmer D, Allen SL,Kolitz JE, Kudalkar P, Cesar D, et al. In vivo measurements document the dynamic cellular kinetics of chronic lymphocytic leukemia B cells. J Clin Invest. 2005;115(3):755–764.
14. Burger JA, Ghia P, Rosenwald A, Caligaris-Cappio F. The microenvironment in mature B-cell malignancies: a target for new treatment strategies. Blood. 2009;114(16):3367–3375.
15. Patten PE, Buggins AG, Richards J,Wotherspoon A, Salisbury J, Mufti GJ,et al. CD38 expression in chronic lymphocytic leukemia is regulated by the tumor microenvironment. Blood. 2008;111(10):5173–5181.
16. Ghia P, Caligaris-Cappio F. The indispensable role of microenvironment in the natural history of low-grade B-cell neoplasms. Adv Cancer Res. 2000;79:157–173.
17. Herishanu Y, Perez-Galan P, Liu D, Biancotto A, Pittaluga S, Vire B, et al. The lymph node microenvironment promotes B-cell receptor signaling, NF-kappaB activation, and tumor proliferation in chroniclymphocytic leukemia. Blood.2011;117(2):563–574.
18. Abdelhameed AS, Alanazi AM, Bakheit AH, Hassan ES, Herqash RN, Almutairi FM, Novel BTK inhibitor acalabrutinib (ACP-196) tightly binds to site I of the human serum albumin as observed by spectroscopic and computational studies. Int J BiolMacromol, 2019Apr 15;127:536-543.
19. Anusha A, PushpaLatha E. Stability indicating RP-HPLC method development and validation for the determination of Acalabrutinib in bulk drug and capsule dosage form. Int. j. bio-pharma res. 2019;8(8): 2758-2762.
20. Priyanka P, Shyamala, Mounika D, Nadeemuddin,Moyeez A. Development and validation of RP-HPLC method for determination of new anticancer agent Acalabrutinib in bulk and its pharmaceutical formulation. European j. biomed. pharm.2019;6(4):465-470.
21. Mohan Goud V, Roja Y, SharmaJVC, Gupta AVSSS. Stability indicating method development and validation for the estimation of Daunorubicin and Cytarabine in bulk and pharmaceutical dosage form by ultra performance liquid chromatography. Int J Life SciPharma Res. ISSN 2250 – 0480; SP-09; "Current Trends in Pharmacology Research – Preclinical Trials" 2020.
22. ICH Harmonised Tripartite Guideline. Validation of analytical procedures, Text and methodology, Q1 R2. International Conference on Harmonization, 2005, 1-13.
23. ICH Harmonised Tripartite Guideline, Stability Testing of New Drug Substances and Products, Q1A (R2). International Conference on Harmonization, 2003, 1-18.
24. ICH Harmonised Tripartite Guideline. Validation of analytical procedures, Text and methodology, Q2 R1. International Conference on Harmonization, 2005, 1-17.
25. Singh S, Bakshi M. Guidance on conduct of stress tests to determine inherent stability of drugs, Pharm. Technol. 2000;24:1–14.
26. Bakshi M, Singh S. Development of validated stability-indicating assay methods—critical review, J. Pharm. Biomed. Anal. 2002;28(6):1011–1040.
27. Belouafa S, Habti F, Benhar S, Belafkih B, Tayane S, Hamdouch S, et al, Statistical tools and approaches to validate analytical methods: methodology and practical examples. Int. J. Metrol. Qual. Eng. 2017;(8)9:1-10.
28. Eugenie Webster (Khlebnikova). Statistical Analysis in Analytical Method Validation. J. Valid. Technol. 2013Dec;19(4):1-8.