Analytical Method Development and Validation for Determination of Acalabrutinib by Using RP-HPLC

Oindrila Mazumder and Raja Sundararajan*

Department of Pharmaceutical Analysis, GITAM Institute of Pharmacy, GITAM (Deemed to be University), Visakhapatnam, Pincode:530 045, Andhra Pradesh (State), India

***Corresponding author:**

Raja Sundararajan

GITAM Institute of Pharmacy

GITAM (Deemed to be University)

Visakhapatnam, Pincode: 530 045,

Andhra Pradesh (State), India

Mobile No. +91 9160508261

E mail: sraja61@gmail.com

ABSTRACT

A unique, selective, and precise RP-HPLC technique for quantifying acalabrutinib in pure and pharmaceutical dose form was developed and validated. Acalabrutinib is an oral kinase inhibitor with an antineoplastic effect and an apoptosis inducer. It is used for the treatment of adult patients with mantle cell lymphoma (MCL). The isolation was obtained on the BDS C18 column (150 x 4.6mm) with a 5µ particle size. At a flow rate of 1ml/min, an optimised mobile phase of 0.1 percent potassium di hydrogen ortho phosphate and acetonitrile (70:30 v/v) was utilised. The wavelength was chosen to be 294nm. Acalabrutinib had a retention time of 2.585 minutes. Acalabrutinib linearity was found to be 12.5-75 g/ml. The linearity equations for acalabrutinib were y = 71296x + 49305, with a correlation coefficient of 0.999. The precision percent RSD was found to be less than 2%. Acalabrutinib recovered at a rate of 99.81 percent. The LOD and LOQ for acalabrutinib were obtained as 0.408 µg/ml and 1.236 µg/ml respectively. The projected technique was recognized to be precise, accurate and ideal for use in QC laboratories for quantitative analysis of both individual and mixed dosage forms of pharmaceuticals.

Keywords: *RP-HPLC, Method Development and Validation*

Introduction

Acalabrutinib ($C_{26}H_{23}N_7O_2$) is chemically 4-{8-amino-3-[(2S)-1-(but-2-ynoyl) pyrrolidin-2-yl] imidazo[1,5-a] pyrazin-1-yl}-N-(pyridin-2-yl) benzamide. The molecular weight of acalabrutinib is 465.517 g/mol. It is a Bruton tyrosine kinase inhibitor that is used in the treatment of mantle cell lymphoma, chronic lymphocytic leukaemia, and small lymphocytic lymphoma. Bruton Tyrosine Kinase (BTK) is a B-cell antigen receptor and cytokine receptor signalling protein [1]. BTK signalling activates pathways required for B-cell proliferation, trafficking, chemotaxis, and adhesion. Acalabrutinib and its active metabolite, ACP-5862, form a covalent link with a cysteine residue (Cys481) in the BTK active site, resulting in BTK enzymatic activity suppression [2]. Acalabrutinib is orally bioavailable. It is administered every 12 hours and can result in atrial fibrillation, malignancies, cytopenia, bleeding, and infection. The chemical structure of acalabrutinib was shown in Figure 1. According to the literature survey, LC-MS/MS methods were reported for the determination of acalabrutinib [3-4]. The aim of this study was to establish novel, specific and precise reverse-phase high-performance liquid chromatography method for the quantification of acalabrutinib in pure and its capsule dosage form.

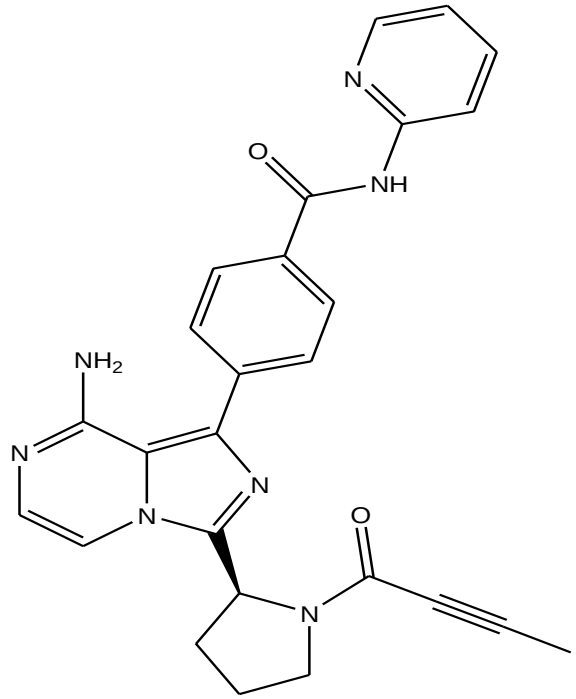

Figure 1. Structure of acalabrutinib

Experimental

Instruments

Waters 2695 HPLC system equipped with quaternary pumps and photodiode array detector was used. The pH of the solutions was calculated by a pH meter (BVK enterprises, India). All analytical measurements were done on analytical balance (Denver).

Chemicals and Reagents

Spectrum pharma research solutions (Hyderabad) provided a free sample of acalabrutinib. Ortho-phosphoric acid, potassium dihydrogen ortho phosphate (KH_2PO_4) buffer, acetonitrile and methanol were purchased from Rankem Laboratories Pvt.Ltd. Millipore Milli Q water was prepared in the laboratory.

Preparation of Diluents

Potassium dihydrogen ortho phosphate (0.1%) and acetonitrile (70:30 v/v) were taken as a diluent.

Preparation of Standard stock solutions

Acalabrutinib (25 mg) was weighed precisely and transferred to 50 ml volumetric flask. The volume was then made up with diluent to obtain a drug standard stock solution of 500 µg/ml concentration.

Preparation of Standard working solutions

Drug standard stock solution (1 ml) was transferred to volumetric flasks (10 ml). The volume was then made up with diluent and drug concentrations ranging from 12.5-75 µg/ml were prepared.

Preparation of Sample stock solutions

Ten capsules were weighed and the average weight of each capsule was determined. Then weight equivalent one capsule was taken into a volumetric flask (100 ml) and diluent (50 ml) was added. For 25 mins, the flask was sonicated. The solution was filtered by HPLC filters and the volume was made with diluent (1000µg/ml of acalabrutinib).

Preparation of Sample working solutions

Filtered sample stock solution (0.5 ml) was taken into a volumetric flask (10 ml) and made up to volume with diluent (50 µg/ml of acalabrutinib).

Chromatographic Conditions

HPLC method development and validation was carried out on an BDS C18 (150 x 4.6 mm, 5µm) column, using a mobile phase of with a 0.01N KH_2PO_4: acetonitrile (70:30 v/v) and flow rate of 1.0 ml/min. The column temperature was 30°C. The sample injection volume was 10 µl. From the UV spectrum of acalabrutinib, 294nm was chosen which displayed the isosbestic wavelength. The eluted compounds were monitored at 294 nm. The chromatographic conditions were illustrated in Table 1 and the optimized chromatogram was represented in Figure 2.

Method Validation

System Suitability Parameters

System suitability was determined by introducing six replicate injections of the drug standard solution (50µg/ml). Parameters like tailing factor, plate count and column efficiency were noted [5].

Specificity

The specificity was evaluated by comparing the placebo solution with the drug solution by introducing samples into the HPLC system. The resulting chromatograms were seen for the interference of placebo response with a drug peak response [6].

Linearity

Dissimilar drug standard solutions were made to evaluate the linearity by diluting the drug stock solutions with diluents in different concentrations of acalabrutinib ranging from 12.5 to 75 µg/ml. The linearity plot of the calibration curve was assessed by linear regression analysis [7].

Sensitivity

The lowest limit of detection and limit of quantification were determined by means of the subsequent equations based on the slope of the calibration and the SD of the responses using different concentrations of the standard stock solution [8].

Limit of detection= 3.3 × standard deviation of the response/ slope of calibration curve of the analyte.

Limit of quantification=10 × standard deviation of the response/ slope of calibration curve of the analyte.

Accuracy

Accuracy was determined at 50%, 100% and 150% by adding an acknowledged amount of sample stock solution of acalabrutinib (0.5, 1, 1.5 µg/ml) to the standard stock solution. The percentages of recoveries were calculated [9].

Precision

Precision was studied as system precision, intraday and inter day. Intraday precision was determined by injecting six different concentrations of standard solutions in the same day. The peak area was measured, and the percent RSD was computed. Inter-day precision was measured by injecting six different concentrations of standard solutions three times a week for three days. The peak area was measured, and the percent RSD was computed [10].

Robustness

Robustness was estimated varying the flow rate, temperature, ratio of the mobile phase. Samples were injected into the system and the %RSD was determined [11].

Forced Degradation Studies

Oxidation

One ml of twenty percent hydrogen peroxide (H_2O_2) was added to one ml of acalabrutinib stock solution. The solution was heated for 30 minutes at 60ºC on a water bath. Then the solution was cooled and made up to the mark using diluent. 10µl of this solution was introduced into the HPLC system [12].

Acid Degradation

One millilitre of 2N hydrochloric acid (HCl) was added to a drug stock solution. For 30 minutes, the solution was heated at 60ºC on a water bath. Then the solution was cooled and 1ml 2N sodium hydroxide was added. The solution was made up to the mark using diluent.10µl of the solution was introduced into the system.

Alkali Degradation

One ml of 2N sodium hydroxide (NaOH) was added to the drug solution. The solution was heated for 30 minutes at 60ºC on a water bath. Then the solution was cooled and 1ml 2N HCl was added. The solution made up with diluent. Ten µl of this solution was introduced into the system.

Dry Heat Degradation

The standard drug solution was placed in oven at 105°C for 6hours. The solution was made up with diluent.10µl of the solution was introduced into the system.

Photolytic Degradation

1000µg/ml acalabrutinib solution was exposed to UV light by placing a beaker in UV chamber for 200 Watt-hours/m^2 or 7 days in a photo stability chamber. Then the solution was diluted and made up to volume with the diluent.10µl of this solution was introduced into the HPLC system [13].

Neutral Degradation

Neutral degradation was determined by refluxing the drug in the water at 60ºC temperature for 6hrs. The solution was made up to volume with the diluent. 10µl of this solution was introduced into the HPLC system [14].

Table 1. Optimized chromatographic conditions

Parameter	Chromatographic conditions
Stationary phase	BDS C18 150 x 4.6 mm, 5µm
Mobile phase	0.01N KH_2PO_4: Acetonitrile (70:30 v/v)
Column temperature	30°C
Injection volume	10 µL
Total Run Time	5 min
Detector	Photodiode Array detector
Elution	Isocratic mode
Flow rate	1.0ml/min
λ_{max}	294nm

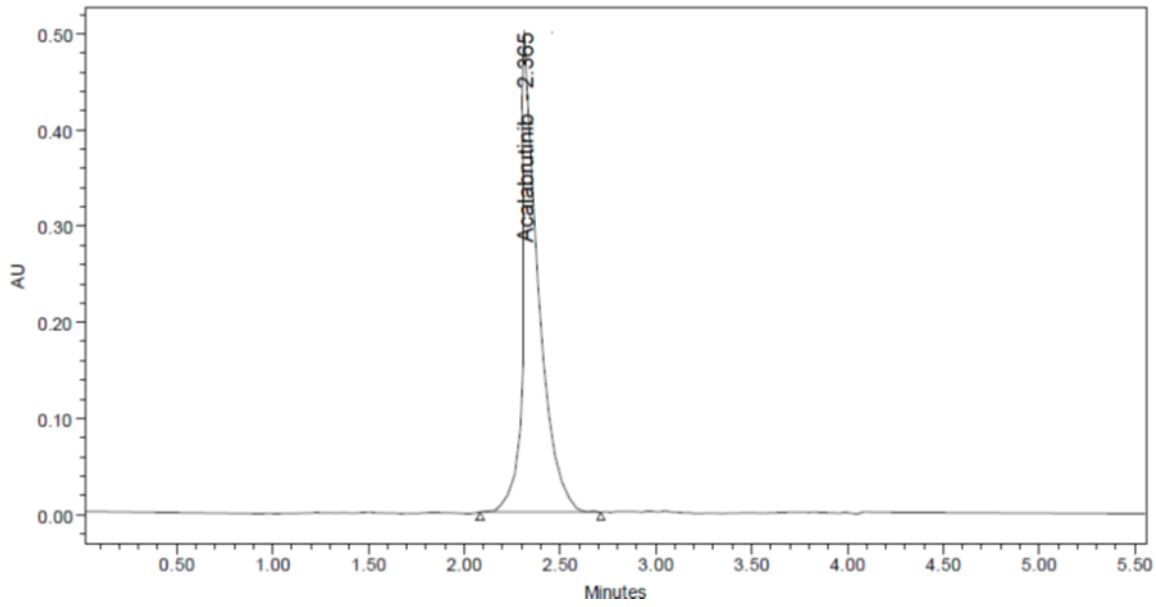

Figure 2. Optimized chromatogram of acalabrutinib

RESULTS AND DISCUSSION

System Suitability Parameters

A system suitability test was an essential part of the method development to confirm that the system was satisfactory for the analysis of acalabrutinib. Prior to the analysis of samples of each day, establishment of a proper procedure were performed to check the capability of the HPLC instrument to perform methods that could generate results of acceptable accuracy and precision. The parameters for the acalabrutinib revealed that the theoretical plates were >2000 and the tailing factor was <2. System suitability data was represented in Table 2.

Table 2. System suitability parameters

Parameter	Acalabrutinib
Retention time	2.365 min
Theoretical plates	4368
Tailing factor	1.5

Linearity

Linearity was obtained at a concentration range of 12.5-75µg/ml through proposed HPLC method. Linearity equation for acalabrutinib was found to be y = 71296x + 49305 with a correlation coefficient of 0. 999. The correlation coefficient was found within the limits which indicated good linearity. The linearity data was illustrated in Table 3 and the calibration plot was graphically depicted in Figure 3.

Table 3. Linearity data of acalabrutinib

Linearity level (%)	Conc (µg/ml)	Peak area
25	12.5	944293
50	25	1855793
75	37.5	2828638
100	50	3533298
125	62.5	4530098
150	75	5368120

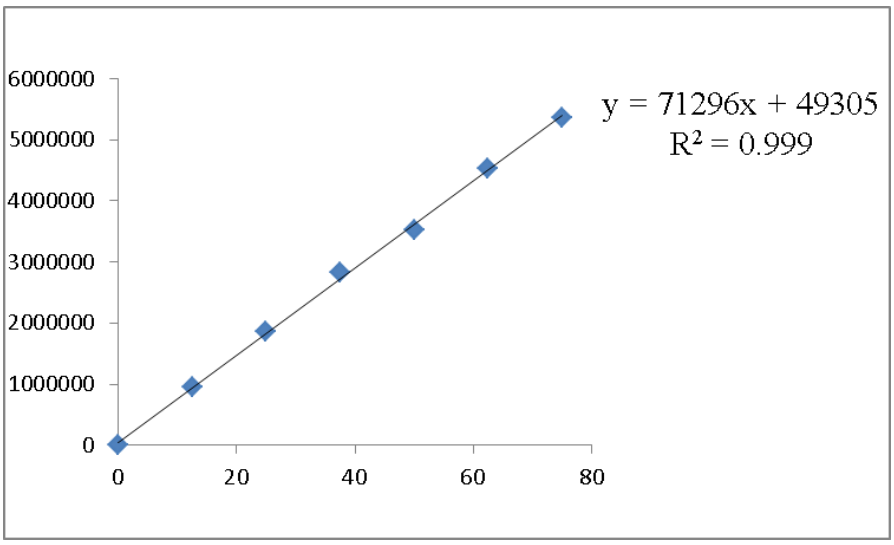

Figure 3. Calibration curve of acalabrutinib

Accuracy

Three levels (50%, 100% and 150%) of accuracy samples were made using the standard addition method. The % recovery was obtained in the range of 98.73%- 100.66%. High recovery results obtained from the method showed that the suggested method can be utilised for QC analysis of capsule dosage forms. The accuracy results were enumerated in Table 4.

System Precision

Chromatogram data for system precision revealed that %RSD was found to be 0.9. Chromatogram data for system precision was found within the limit specified (%RSD NMT 2.0%). Hence it proved that the method was found to be precise. System precision data was represented in Table 5.

Table 4. Accuracy data of acalabrutinib

% Level	Amount spiked (µg/ml)	Amount recovered (µg/ml)	% Recovery	Mean % Recovery
50%	25	24.81	99.26	99.81%
	25	24.68	98.73	
	25	24.93	99.71	
100%	50	50.20	100.39	
	50	49.64	99.28	
	50	50.15	100.31	
150%	75	75.26	100.35	
	75	75.49	100.66	
	75	74.71	99.61	

Table 5. System precision data of acalabrutinib

S.No.	Area of acalabrutinib
1	2376304
2	2425718
3	2402289
4	2392470
5	2362687
6	2392667
Mean	2392023
SD	21675.1
%RSD	0.9

Repeatability

Chromatogram data for repeatability revealed that %RSD was found to be 0.7. Chromatogram data for repeatability was found within the limit specified (%RSD NMT 2.0%). Hence it proved that the method was found to be precise. Results for repeatability were illustrated in Table 6.

Table 6. Repeatability data of acalabrutinib

S. No	Area of acalabrutinib
1	2401963
2	2383306
3	2404426
4	2403607
5	2360365
6	2383619
Mean	2389548
S. D	17314.2
%RSD	0.7

Intermediate Precision

Chromatogram data for intermediate precision revealed that %RSD was found to be 1.2. Chromatogram data for intermediate precision was found within the limit specified (%RSD NMT 2.0%). Hence it proved that the method was found to be precise. The results were represented in Table 7.

Table 7. Intermediate precision table of acalabrutinib

S. No	Area of acalabrutinib
1	2361973
2	2350595
3	2403607
4	2362289
5	2342687
6	2333619
Mean	2358559
S. D	27297.4
%RSD	1.2

Robustness

The robustness was assessed by introducing little, intentional variations in the chromatographic conditions, which comprise the flow rate of mobile phase (± 0.1ml/min), % of acetonitrile in the mobile phase (25 & 35%) and temperature (±5). Robustness was carried out using 10µg/ml of acalabrutinib and the %RSD was found to be 0.2-1.4. The results were illustrated in Table 8.

Table 8. Robustness data for acalabrutinib

S. No.	Condition	%RSD of Acalabrutinib
1	Flow rate (-) 0.9ml/min	1.0
2	Flow rate (+) 1.1ml/min	0.6
3	Mobile phase (-) 75B:25A	0.2
4	Mobile phase (+) 65B:35A	1.4
5	Temperature (-) 27ºC	1.3
6	Temperature (+) 33ºC	0.8

Specificity

Specificity was performed by introducing a blank and placebo solution which indicate that there was no interference in the placebo and blank sample at the retention time of the standard acalabrutinib sample. There was no interference in the placebo and blank sample at the retention time of the standard acalabrutinib sample. Therefore, it can be stated that the method was specific. The specificity data was represented in Table 9 and the chromatograms of blank and placebo were illustrated in Figure 4 and 5 respectively.

Table 9. Specificity data of acalabrutinib

S. No.	Sample details	Retention time (min)
1	Placebo solution	Interference is not detected
2	Blank solution	Interference is not detected
3	Acalabrutinib	2.383 min

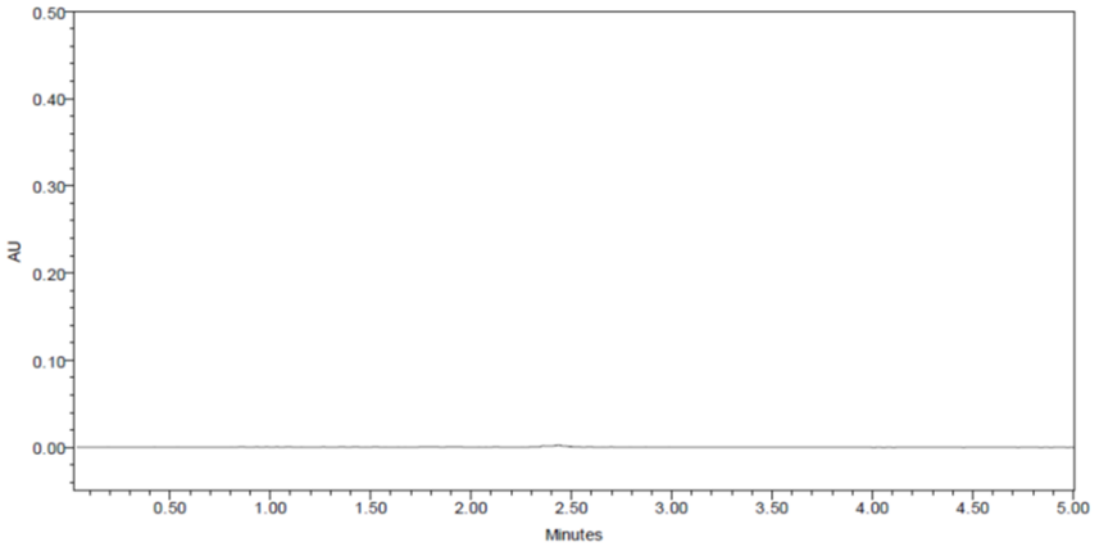

Figure 4. Chromatogram of blank sample

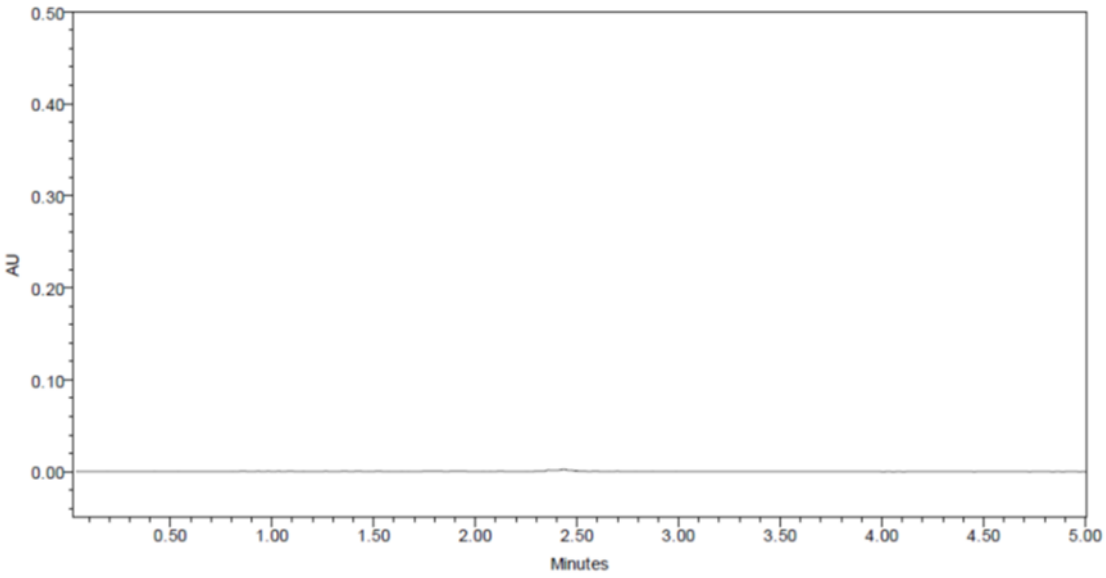

Figure 5. Chromatogram of placebo

Sensitivity

For determination of LOD and LOQ, signal-to-noise (s/n) ratio was taken into consideration, with LOD defined as approximate s/n ~3 and LOQ as the lowest validated concentrations with (%) RSD and (%) error ≤20%. LOD of acalabrutinib was detected at 0.408 µg/ml. LOQ of acalabrutinib was detected at 1.236 µg/ml.

Assay

Acalabrutinib drug (100mg) was used to study the assay. The percentage purity was found between 98.58-100.42 in pharmaceutical formulations. The percentage purity was found within the range of 90-110%. Acalabrutinib was eluted at 2.383 min. The assay data was demonstrated in Table 10. The chromatograms for pure and formulated drug were represented in the Figure 6 and 7, respectively.

Table 10. Assay data of acalabrutinib

S.No.	Standard area	Sample area	% Recovery
1	2376304	2401963	100.32
2	2425718	2383306	99.54
3	2402289	2404426	100.42
4	2392470	2403607	100.38
5	2362687	2360365	98.58
6	2392667	2383619	99.55
Avg	2392023	2389548	99.80
Std dev	21675.1	17314.2	0.7231
%RSD	0.9	0.7	0.72

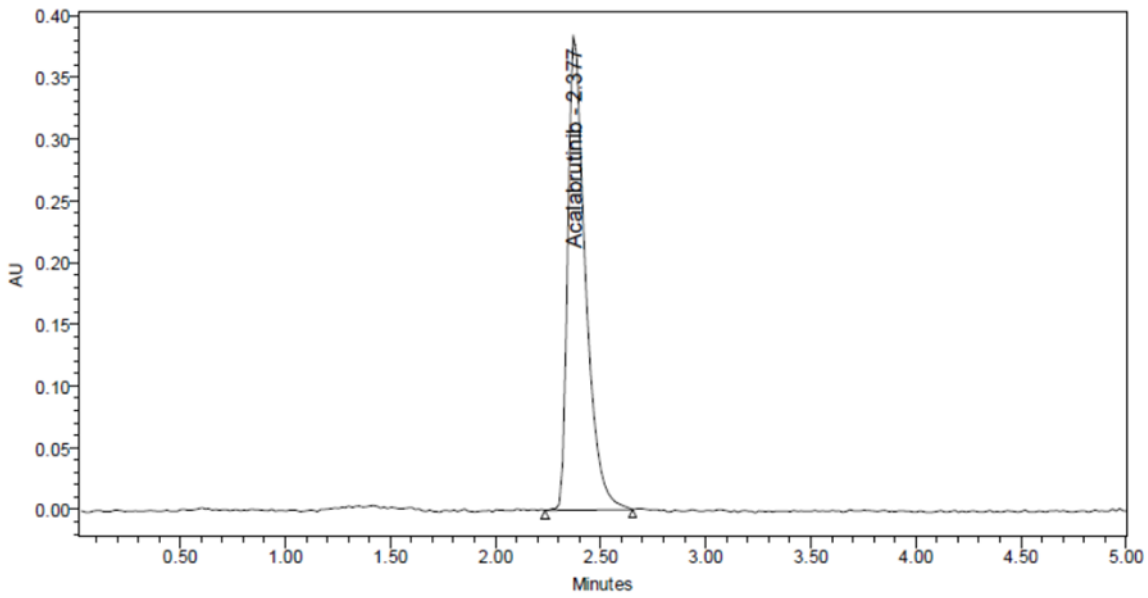

Figure 6. Chromatogram of acalabrutinib pure sample

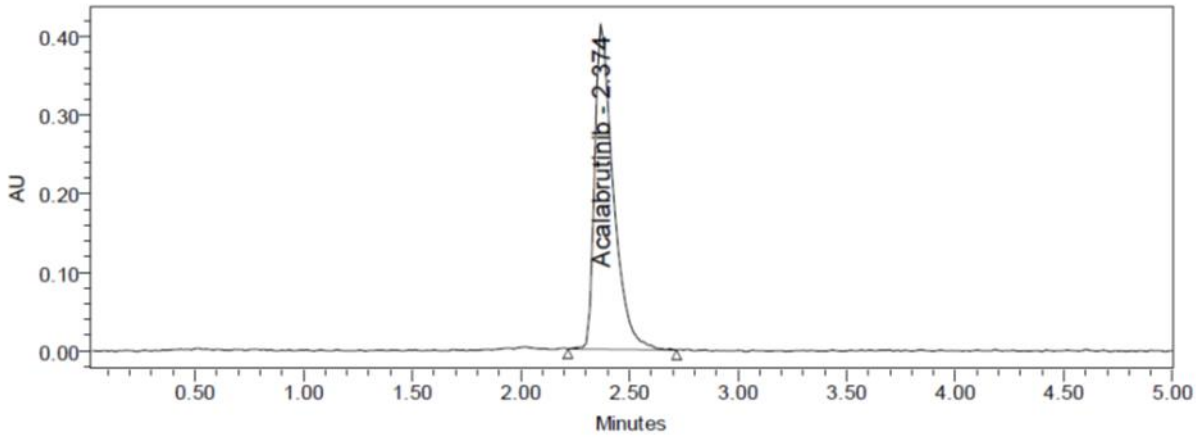

Figure 7. Chromatogram of calquence (label claim 100mg)

Forced Degradation Studies

Acalabrutinib was subjected to acid degradation (6.21%), alkali degradation (4.15%), oxidation (4.77%), photolytic degradation (1.80%), thermal degradation (1.20%) and neutral degradation (0.92%). The amount of degraded drug samples were 6.21%, 4.15%, 4.77%, 1.80%, 1.20%, 0.92%, respectively. The results were less than 10% indicating that acalabrutinib was more resistant towards all forced degradation conditions applied. During basic and oxidative degradation an extra peak was observed at 2.193 min and 2.614 min along with the drug peak. The system suitability parameters were also within limits. The results were demonstrated in Table 11. The chromatograms for acid degradation, basic degradation, oxidation, thermal degradation, photolytic degradation and neutral degradation were enumerated in Figures 8-13. The summary of the developed and validated method was tabulated in Table 12.

Table 11. Degradation data of Acalabrutinib

S. No.	Degradation Condition	% Drug degraded	%Drug undegraded	Retention time	Peak area
1	Acid	6.21	93.79	2.352	2245803
2	Alkali	4.15	95.85	2.383	625156
3	Oxidation	4.77	95.23	2.374	2280251
4	Thermal	1.20	98.80	2.380	2365803
5	Photo stability	1.80	98.20	2.356	2351303
6	Neutral	0.92	99.08	2.359	2372492

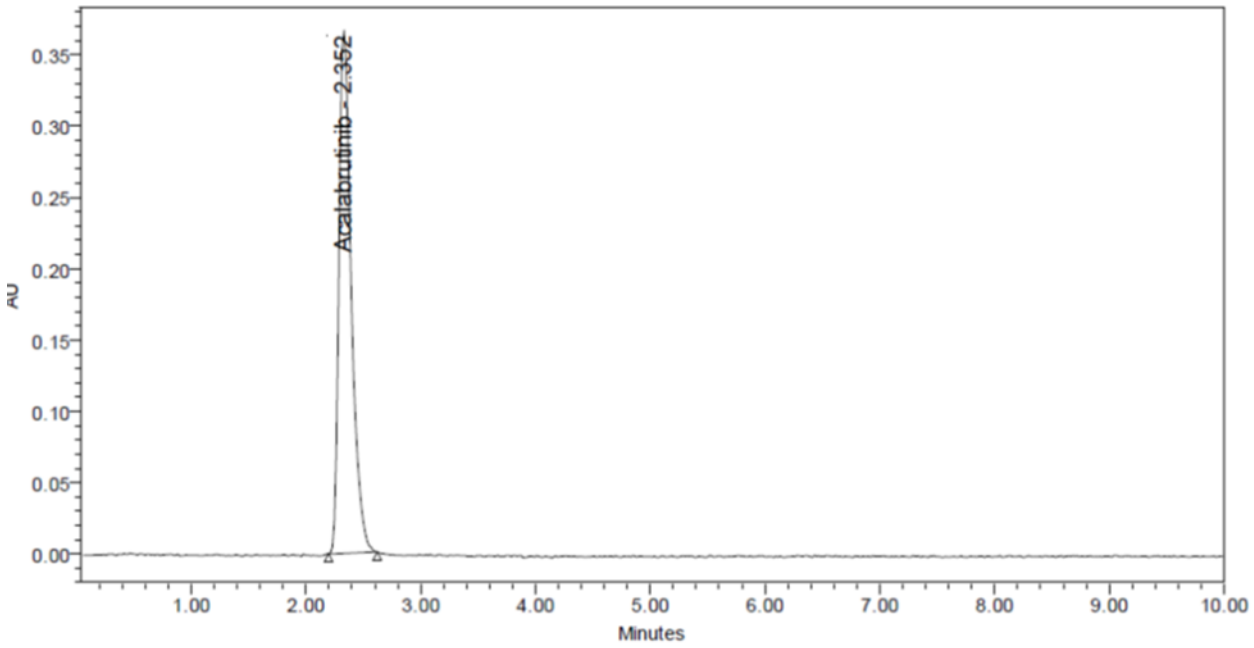

Figure 8. Chromatogram of acidic degradation of acalabrutinib

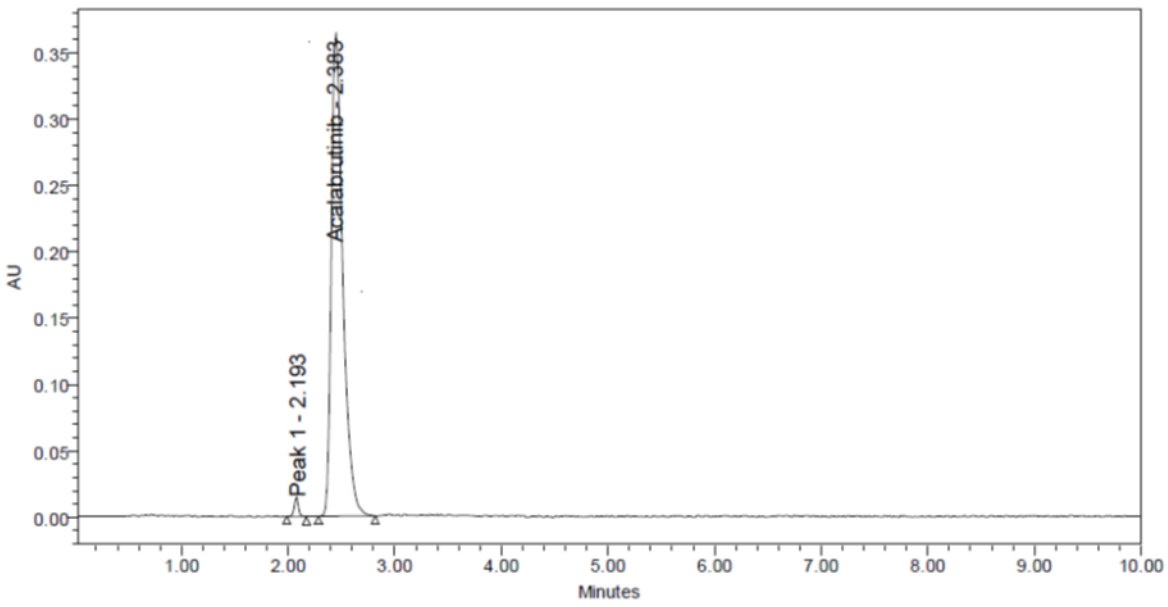

Figure 9. Chromatogram of basic degradation of acalabrutinib

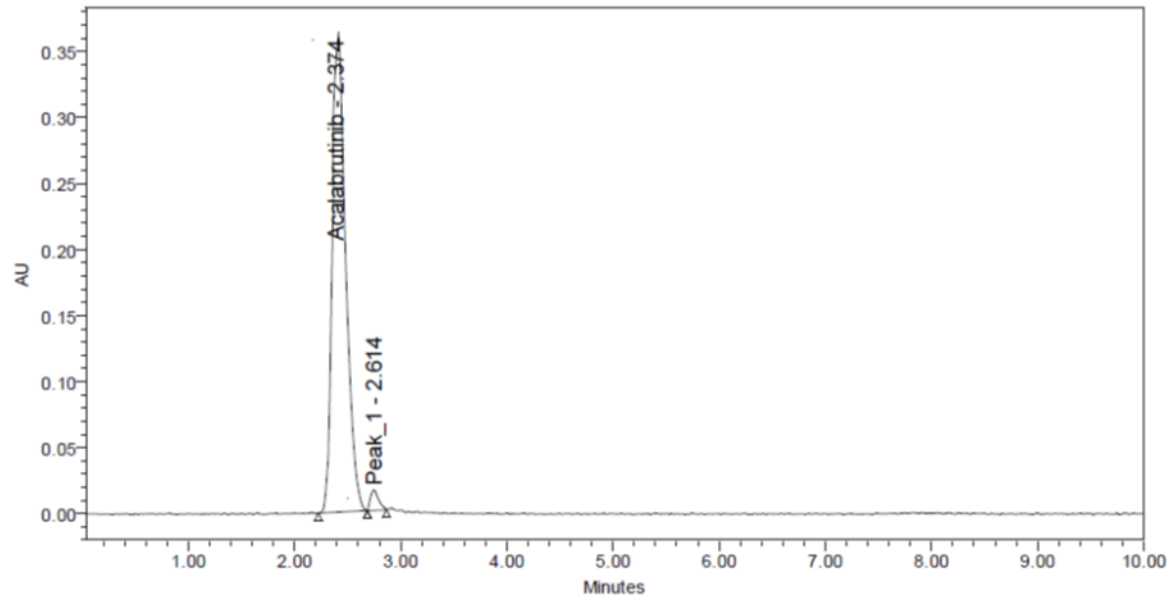

Figure 10. Chromatogram of oxidative degradation of acalabrutinib

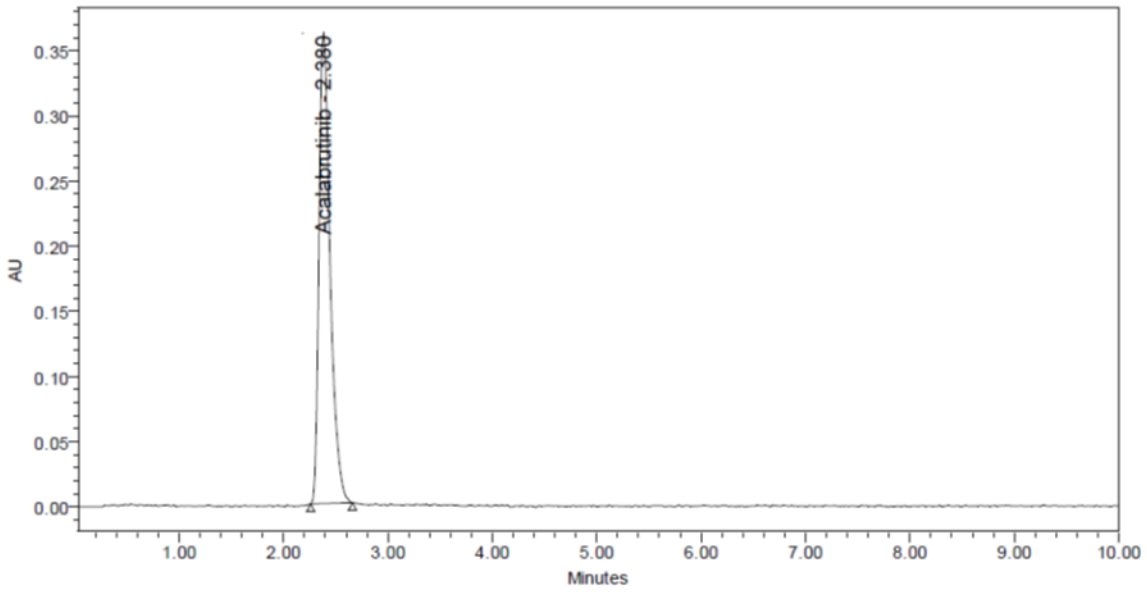

Figure 11. Chromatogram of thermal degradation of acalabrutinib

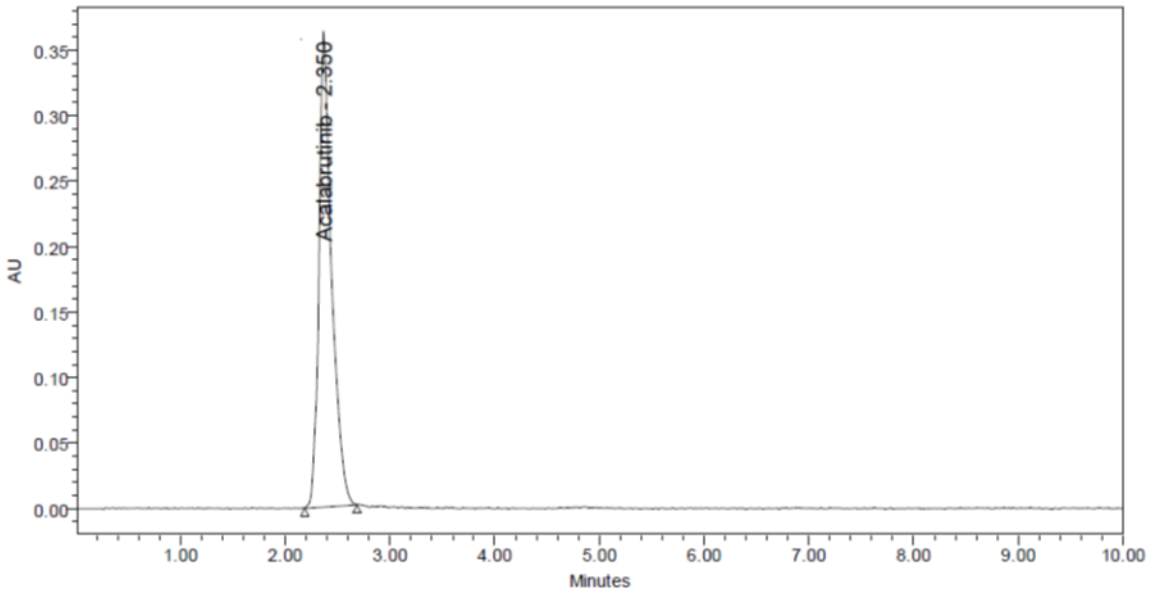

Figure 12. Chromatogram of photo stability degradation of acalabrutinib

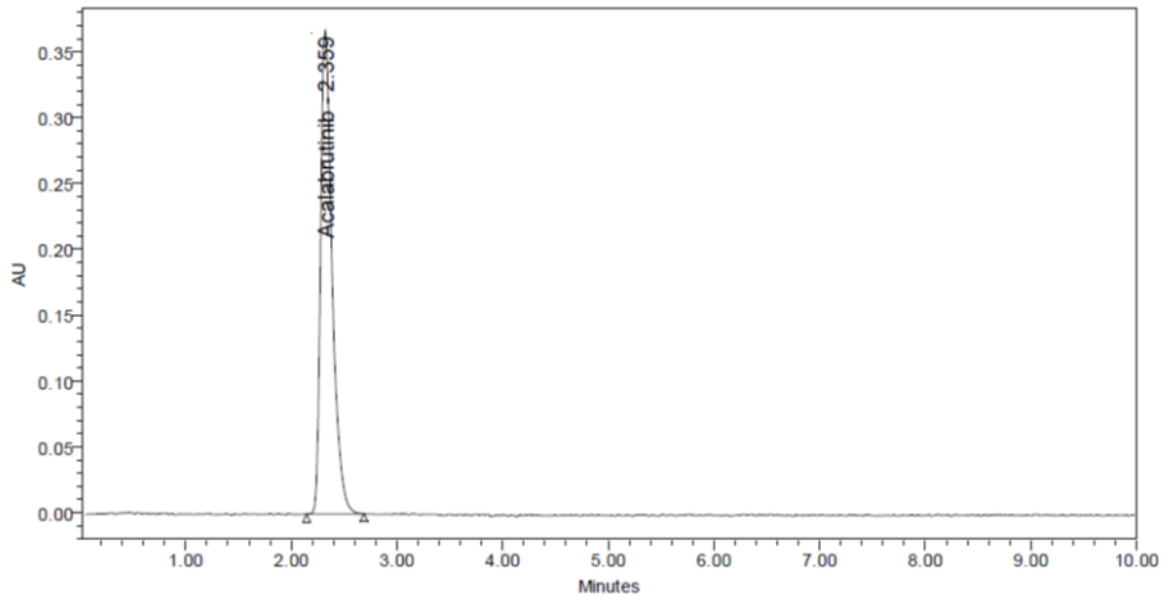

Figure 13. Chromatogram of neutral degradation of acalabrutinib

Table 12. Summary

Parameters		Values	Limit
Linearity(µg/ml)		12.5-75(µg/ml)	R<1
Regression coefficient		0.999	
Slope(m)		71296	
Intercept(c)		49305	
Regression equation (y= mx +c)		y =71296x+49305	
Assay (% mean assay)		99.80%	90-110%
Specificity		Specific	No interference of any peak
System precision (%RSD)		0.9	NMT 2.0%
Intermediate precision (%RSD)		1.2	
Repeatability (%RSD)		0.7	
LOD		0.408	NMT 3
LOQ		1.236	NMT 10
Robustness	Flow rate (-) 0.9ml/min	1.0	%RSD NMT 2.0
	Flow rate (+) 1.1ml/min	0.6	
	Mobile phase (-) 75B:25A	0.2	
	Mobile phase (+) 65B:35A	1.4	
	Temperature (-)27ºC	1.3	
	Temperature (+)33ºC	0.8	

Conclusion:

A validated RP-HPLC method was established for the quantification of acalabrutinib in pure and its capsule dosage forms. The developed method was found to be precise, accurate and specific. Hence, the method can be utilised in the quality control analysis of acalabrutinib.

Conflicts of Interest

The authors declare there are no conflicts of interest regarding the research or authorship of this manuscript.

Acknowledgements

The authors are thankful to the management of GITAM (Deemed to be University), Visakhapatnam, Andhra Pradesh, India, for providing necessary facilities to carry out the research work

References

1. *Furman RR, Byrd JC, Owen RG, O'Brien SM, Brown JR, Hillmen P, Stephens DM, Chernyukhin N, Lezhava T, Hamdy AM, Izumi R. Pooled analysis of safety data from clinical trials evaluating acalabrutinib monotherapy in mature B-cell malignancies. Leukemia.* ***(2021)*** *35(11):3201-3211.*

2. *Barf T, Covey T, Izumi R, van de Kar B, Gulrajani M, van Lith B, van Hoek M, de Zwart E, Mittag D, Demont D, Verkaik S. Acalabrutinib (ACP-196): a covalent Bruton tyrosine kinase inhibitor with a differentiated selectivity and in vivo potency profile. Journal of Pharmacology and Experimental Therapeutics.* ***(2017)*** *363(2):240-252.*

3. *Surendran S, Paul. D, Pokharkar S, Choulwar S, Deshpande A, Giri S, Satheeshkumar N. Novel Bruton tyrosine kinase inhibitor acalabrutinib quantification by validated LC-MS/MS method: An application to pharmacokinetic study in Sprague Dawley rats. Journal of Pharmaceutical and Biomedical Analysis.* ***(2019)*** *164:509-513.*

4. *Valluri VR, Katari NK, Khatri C, Kasar P, Polagani SR, Jonnalagadda SB. A novel LC-MS/MS method for simultaneous estimation of acalabrutinib and its active metabolite acalabrutinib M 27 in human plasma and application to a human pharmacokinetic study. RSC Advances.* ***(2022)*** *12(11):6631-6639.*

5. *Anjaneyulu Reddy B, Radhakrishnan P, Irshad Alam M, Kiran R. A Validated Stability Indicating RP-HPLC Method Development for Anti-Cancer Drug Enzalutamide in Bulk and Pharmaceuticals. International Journal of Pharmaceutical Sciences and Drug Research.* ***(2019)*** *13(3): 85-90.*

6. *Latha ST, Thangadurai SA, Jambulingam M, Sereya K, Kamalakannan D, Anil kumar M. Development and validation of RP-HPLC method for the estimation of Erlotinib in pharmaceutical formulation. Journal of Pharmaceutical Research,* ***(2011)*** *4(3):637-638.*

7. *Palyam B, Raja S, Peethala P, Annapurna MM. A new stability indicating RP-UFLC method for determination of luliconazole in bulk and pharmaceutical formulation. Research Journal of Pharmacy and Technology,* ***(2020)*** *13(6):2859-2863.*

8. *Szepesi G, Gazdag M, Mihályfi K. Selection of high-performance liquid chromatographic methods in pharmaceutical analysis, III. Method validation. Journal of Chromatography.* ***(1989)*** *464(2):265-278.*

9. *Jahani M, Fazly Bazzaz BS, Akaberi M, Rajabi O, Hadizadeh F. Recent Progresses in Analytical Perspectives of Degradation Studies and Impurity Profiling in Pharmaceutical Developments: An Updated Review. Critical Reviews in Analytical Chemistry.* ***(2021)*** *1-22.*

10. *Virani P, Jain P, Raj H, Jain V. Updated review: validation and method validation parameter. Pharma Tutor.* ***(2014)*** *2(10): 27-37.*

11. *Gujju HB and Mukthinuthalapati MA. A sensitive stability indicating RP-HPLC method for the determination of ibrutinib - an anti-cancer drug. Research Journal of Pharmacy and Technology,* ***(2018)*** *11(10): 4587-4591.*

12. Alagar RM, Anusha S, Banji D, Rao KN, Kuamar DS. Analytical method development and validation of anticancer drugs (Imatinib and Capecitabine) by RP-HPLC method. *Asian Journal of Research in Chemistry and Pharmaceutical Sciences.* ***(2015)*** *3(2): 51-65.*

13. *Djurdjevic P, Ciric A, Djurdjevic A, Stankov MJ. Optimization of separation and determination of moxifloxacin and its related substances by RP-HPLC. Journal of Pharmacy and Biomedical Analysis.* ***(2009)*** *50(2): 117-126.*

14. *Manikandan K, and Lakshmi KS. Method development and validation of risperidone by RP-HPLC. International Journal of Research in Pharmaceutical and Nano Sciences.* ***(2012)*** *1(1).*