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VALIDATED STABILITY-INDICATING RP-HPLC METHOD FOR DETERMINATION OF IBRUTINIB

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Abstract:
A simple, Precised, Accurate method was developed for the estimation of Ibrutinib by RP-HPLC technique. Chromatographic conditions used are stationary phase Kromosil150mm x 4.6 mm, 5µ ,Mobile phase0.1% OPA: Acetonitrile in the ratio of 40:60and flow rate was maintained at 1ml/min, detection wave length was 296nm, column temperature was set to 30oC and diluent was mobile phase Conditions were finalized as optimized method. System suitability parameters were studied by injecting the standard six times and results were well under the acceptance criteria. Linearity study was carried out between 25% to150 % levels, R2 value was found to be as 0.999. Precision was found to be 1.01 for repeatability and 1.50 for intermediate precision. LOD and LOQ are 0.394µg/ml and 1.194µg/ml respectively. By using above method assay of marketed formulation was carried out 100.55% was present. Degradation studies of ibrutinib were done, in all conditions purity threshold was more than purity angle and within the acceptable range.
Key Words: *HPLC, Ibrutinib, Method development. ICH Guidelines.*

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INTRODUCTION:

Ibrutinib is a small molecule anti-cancer drug that targets B-cell malignancies. In November 2013 Ibrutinib was approved by the FDA for the treatment of mantle cell lymphoma, and later in February 2014 for the treatment of chronic lymphocytic leukemia. Ibrutinib is also indicated for the treatment of patients with Waldenström's Macroglobulinemia (WM). Ibrutinib is marketed under the brand Imbruvica® by Janssen Biotech, Inc., but was first designed and synthesized at CeleraGenomics in 2007.the chemical structure of Ibrutinib was given in Fig.1.

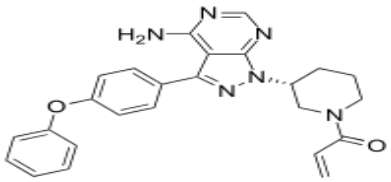

Fig1: Structure of Ibrutinib

As per the literature review, Ibrutinib was estimated individually by few methods like simple HPLC[1], Ultra HPLC[2],HPLC-MS [3]method validation of ibrutinib. The objective of the work is to develop RP-HPLC method for estimation of ibrutinib in tablet dosage form with simple , rapid, accurate and economical methods and validated for system suitability, linearity, accuracy, precision, robustness and stability of sample solution as per ICH guidelines.

MATERIALS AND METHODS:

Chemicals and Reagents:

Ibrutinib was obtained as gift sample from Spectrum Laboratories, Hyderabad. Acetonitrile and water used were of HPLC grade.

Instrumentation:

A waters HPLC system with empower 2 software, fitted with a PDA detector and a kromosil column was used for the analysis.

Chromatographic Conditions:

An HPLC system (make: waters, model 2996) which is operated using software, Empower 2, fitted with BDS column and PDA detector (at 296nm) was used for the analysis. Isocratic run with a flow rate 1ml/min was preferred for resolving the drug.

Preparation of Mobile Phase:

A mixture (40:60) of 0.1% Ortho phosphoric acid and Acetonitrile was used as mobile phase.

Standard preparation:

Accurately Weighed and transferred 14mg Ibrutinib working Standard into a 10 ml clean dry volumetric flask, add 7ml of diluent, sonicated for 30 minutes and make up to the final volume with diluents .From the above stock solution, 1 ml was pipeted out in to a 10ml Volumetric flask and then make up to the final volume with diluent. The chromatogram of standard Ibrutinib solution was shown in Fig. 2. And the average retention time was found to be 3.11min

Validation:

System Suitability:

5 Capsules were weighed and calculate the average weight of each Capsule then the weight equivalent to 1 Capsule was transferred into a 100 ml volumetricflask, 5ml of diluent added and sonicated for 30 min, further the volume made up with diluent and filtered. From the filtered solution 1ml was pipette out into a 10 ml volumetric flask and made up to 10ml with diluent.

Linearity:

To demonstrate the linearity of assay method, inject 5 standard solutions with concentrations of about 25ppm to 150ppm of Ibrutinib. Plot a graph to concentration versus peak area. Slope obtained was 29160 Y-Intercept was 1140 and Correlation Co-efficient was found to be 0.999 and Linearity plot was shown in Table2.

Accuracy:

Three Concentrations of 50%, 100%, 150% are Injected in a triplicate manner and %Recovery was calculated as 100.36. and chromatograms were shown in table 3.

Precision:

Repeatability:

Six working sample solutions of 100ppm are injected and the % Amount found was calculated and %RSD was found to be 1.01 and chromatogram was shown in Table 4.

Intermediate Precision:

Five working sample solutions of 100ppm are injected on the next day of the preparation of samples and the % Amount found was calculated and %RSD was found to be 1.50 and chromatogram was shown in Table 5.

Robustness:

Small Deliberate change in the method is made like Flow minus, flow plus, Mobile phase minus, Mobile phase plus, Temperature minus, Temperature Plus. %RSD of the above conditions are calculated and shown in Table 5.

Limit of Detection (LOD):

LOD is the lowest level of concentration of analyte in the sample that can be detected, though not necessarily quantitated. It is calculated to be 0.394µg.ml using the formula,

$LOD = 3.3\sigma/S$

Where,

σ= Standard deviation of the response,

S= Slope of calibration curve.

Limit of Quantitation (LOQ):

LOQ is the lowest concentration of analyte in a sample that may be determined with acceptable

accuracy and precision when the required procedure is applied. It was calculated to be 1.194µg/ml using the formula,

LOQ=10σ/S

Where,

σ = Standard deviation of the response,

S = Slope of calibration curve.

Degradation Studies:

Oxidation:

To 1 ml of stock solution of Ibrutinibe, 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The solutions were kept for 30 min at 60^0c. For HPLC study, the resultant solution was diluted to obtain 140µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Acid Degradation Studies:

To 1 ml of stock ssolution of Ibrutinibe, 1 ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60^0c .The resultant solution was diluted to obtain 140µg/ml solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.

Alkali Degradation Studies:

To 1 ml of stock solution Ibrutinibe, 1 ml of 2N sodium hydroxide was added and refluxed for 30mins at 60^0c. The resultant solution was diluted to obtain 140µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Dry Heat Degradation Studies:

The standard drug solution was placed in oven at 105^0c for 6 h to study dry heat degradation. For HPLC study, the resultant solution was diluted to 140µg/ml solution and10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

Photo Stability Studies:

The photochemical stability of the drug was also studied by exposing the 1400µg/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 HPLC study, the resultant solution was diluted to obtain 140µg/ml solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.

Neutral Degradation Studies:

Stress testing under neutral conditions was studied by refluxing the drug in water for 6hrs at a temperature of 60º. For HPLC study, the resultant solution was diluted to 140µg/ml solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of the samples.

RESULTS:

Table 1: System Suitability Parameters and their Recommended Limits.

S no	PeakName	RT	Area	USP Plate Count	USP Tailing
1	Ibrutinib	3.052	1144018	7143	1.31
2	Ibrutinib	3.052	1157129	6997	1.33
3	Ibrutinib	3.053	1134468	7135	1.34
4	Ibrutinib	3.055	1146943	7042	1.32
5	Ibrutinib	3.055	1132425	6936	1.30
6	Ibrutinib	3.056	1153109	6843	1.32
Mean			1144682		
Std.Dev.			9859.56		
%RSD			0.86		

Table 2: Linearity Concentration and Response

Linearity Level (%)	Concentration (ppm)	Area
0	0	0
25	35	276279
50	70	570114
75	105	862544
100	140	1179328
125	175	1416511
150	210	1700873

Table 3: Accuracy Data

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	70	71.66	102.4	100.36%
	70	70.55	100.8	
	70	69.82	99.7	
100%	140	140.52	100.37	
	140	140.26	100.18	
	140	140.59	100.42	
150%	210	210.41	100.20	
	210	208.53	99.30	
	210	209.80	99.91	

Table 4: Repeatability Data

S.No	Peak Area
1	1161542
2	1168399
3	1139374
4	1140998
5	1158685
6	1157806
AVG	1154467
STDEV	11681.7
%RSD	1.01

Table 5: Intermediate Precision Data

S.No	Peak Area
1	1135518
2	1155202
3	1172667
4	1173988
5	1178878
AVG	1166121
STDEV	17488.4
%RSD	1.50

Table 6: Robustness Data

Parameter	%RSD
Flow Minus	0.0
Flow Plus	0.3
Mobile phase Minus	0.1
Mobile phase Plus	0.1
Temperature minus	0.3
Temperature plus	0.2

Table 7: Degradation Data of Ibrutinib

S.NO	Degradation condition	%Drug degraded	Purity Angle	Purity Threshold
1	Acid	3.512	0.18	0.245
2	Alkali	2.564	0.182	0.248
3	Oxidation	4.246	0.211	0.252
4	Thermal	1.231	0.182	0.252
5	UV	0.499	0.179	0.251
6	Water	0.573	0.187	0.247

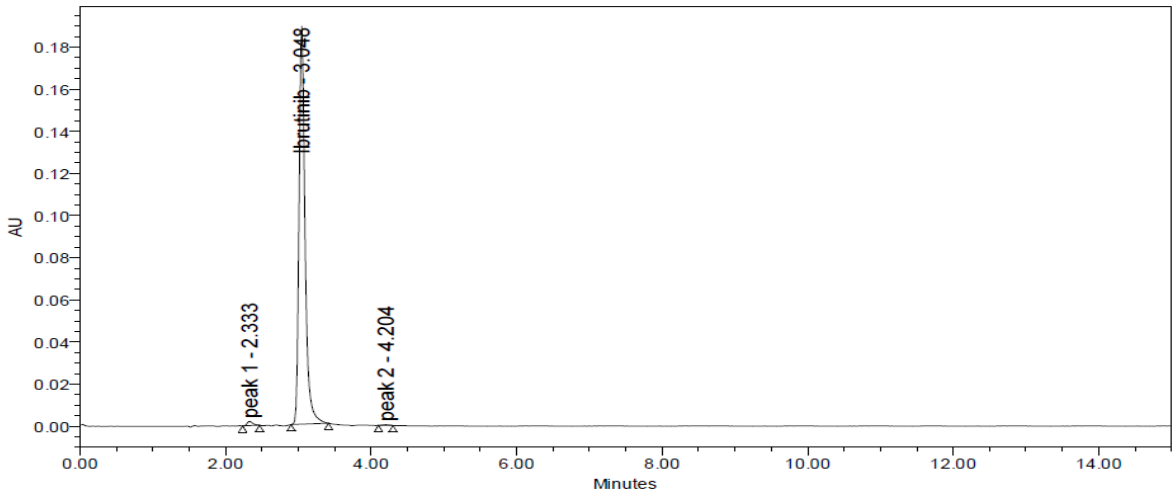

Fig 2: System Suitability Chromatogram

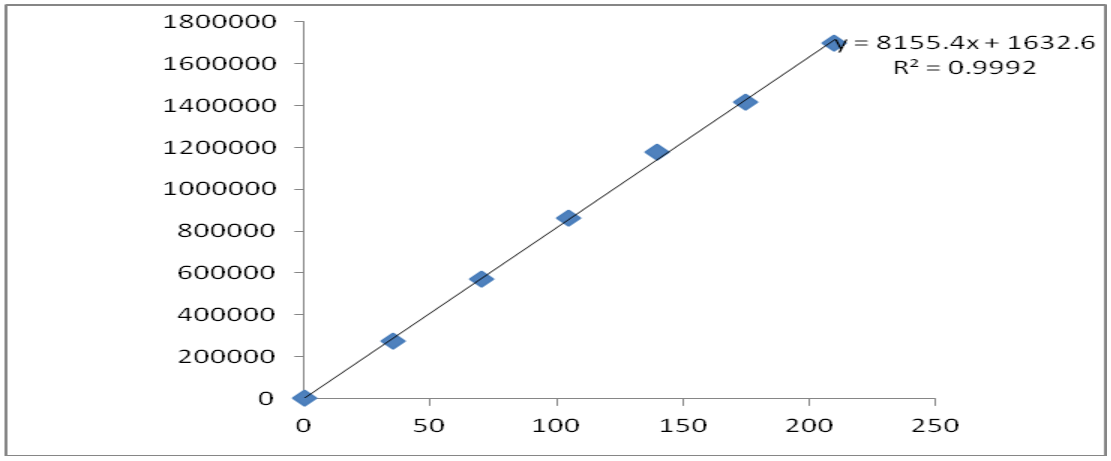

Fig 3: Acid Degradation Chromatogram

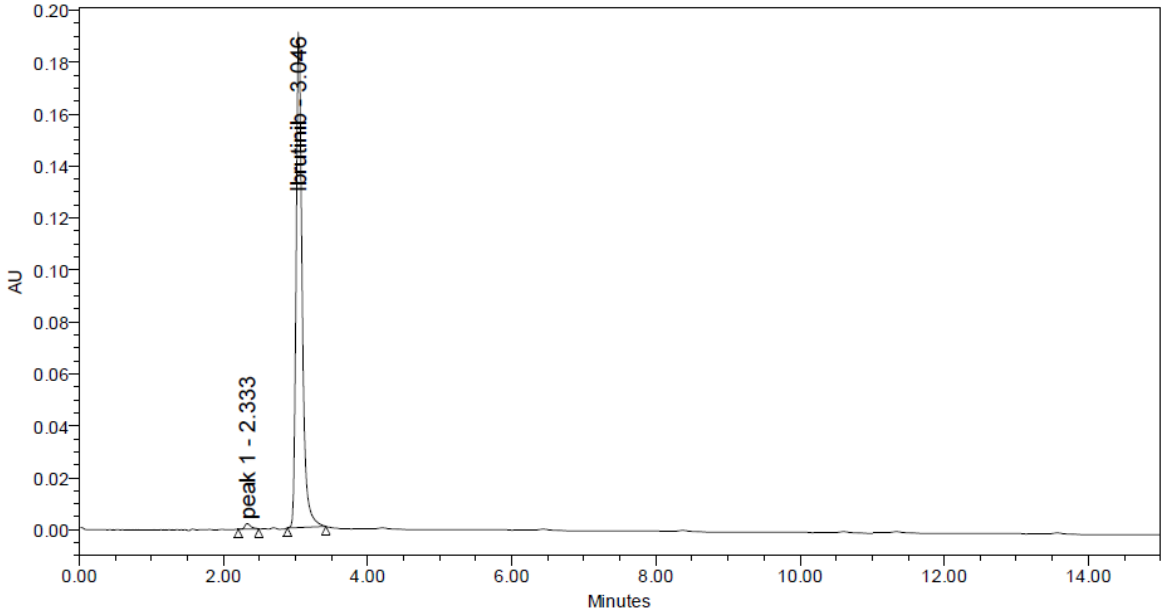

Fig 4: Base Degradation Chromatogram

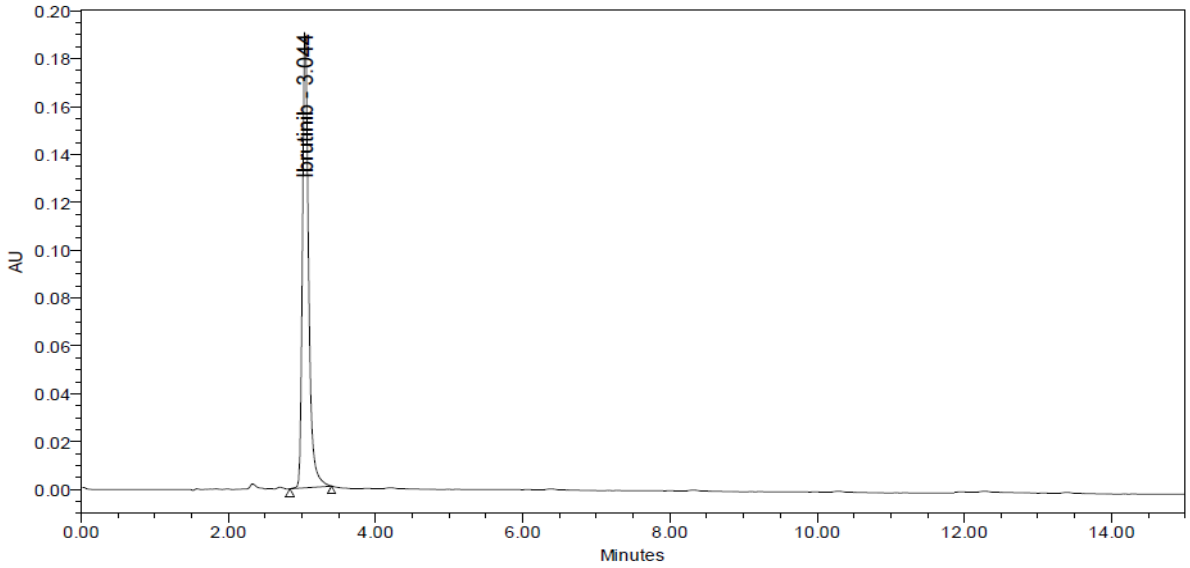

Fig 5: Peroxide Degradation Chromatogram

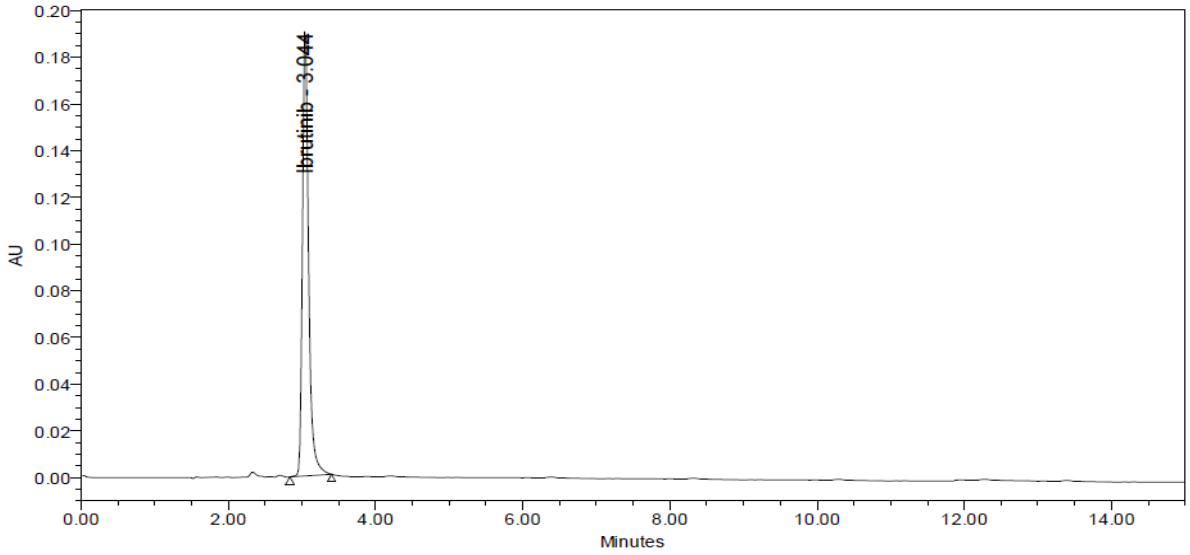

Fig 6: Thermal Degradation Chromatogram

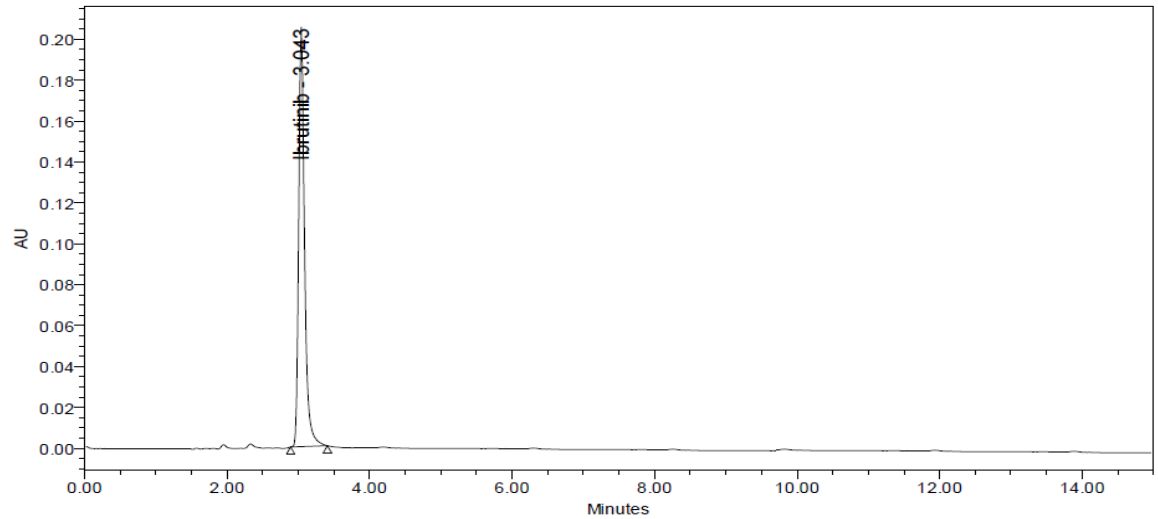

Fig 7: UV Degradation Chromatogram

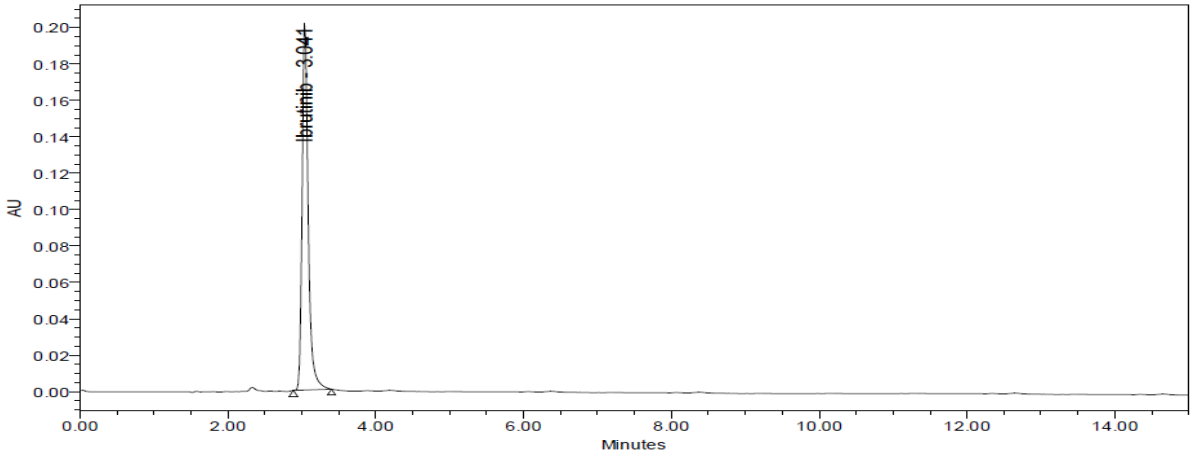

Fig 8: Water Degradation Chromatogram

CONCLUSION:

Chromatographic conditions used are stationary phase Kromosil (150mm*4.6mm), Mobile phase 0.1% OPA: Acetonitrile in the ratio of 40:60 and flow rate was maintained at 1ml/min, detection wave length was 296nm, column temperature was set to 30ºC and diluent was mobile phase Conditions were finalized as optimized method.

System suitability parameters were studied by injecting the standard six times and results were well under the acceptance criteria.

Linearity study was carried out between 25% to150 % levels, R^2 value was found to be as 0.999.

Precision was found to be 1.01 for repeatability and 1.50 for intermediate precision.

LOD and LOQ are 0.394µg/ml and 1.194µg/ml respectively

By using above method assay of marketed formulation was carried out 100.55% was present. Degradation studies of Ibrutinib were done, in all conditions purity threshold was more than purity angle and within the acceptable range. Full length method was not performed; if it is done this method can be used for routine analysis of Ibrutinib.

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