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A New Simple Method Development and Validation of Ibrutinib In Bulk and Pharmaceutical Dosage Form By RP-HPLC

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Abstract
A simple, rapid, precise, accurate and sensitive reverse phase liquid chromatographic method has been developed for the determination of Ibrutinibin bulk and pharmaceutical dosage form. The chromatographic method was standardized using Develosil ODS HG-5 RP C18, 5µm, 15cmx4.6mm i.d. column with UV detection at 287nm and mobile phase with the composition of 0.1% Orthophosphoric Acid: Methanol with 35:65 ratio at a flow rate of 1.0 ml/ mi. The proposed method was successfully applied to the determination of Ibrutinib in bulk and pharmaceutical dosage form. The method was linear over the range of 0-14µg/ml. The recovery was in the range of 98% to 102% and limit of detection was found to be 0.09 µg/ml and quantification was found to be 0.29 µg/ml. Different analytical performance parameters such as precision, accuracy, limit of detection, limit of quantification and robustness were determined according to International Conference on Harmonization (ICH) guidelines.

Keywords
RP-HPLC, Ibrutinib, Method development and validation, 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). As per the literature review, Ibrutinib was estimated individually by few methods like simple HPLC1, Ultra HPLC2, HPLC-MS 3method 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.[1].It is an orally administered, selective and covalent inhibitor of the enzyme Bruton's tyrosine kinase (BTK)[3].As per the literature review, Ibrutinib was estimated individually by few methods like simple HPLC1 , Ultra HPLC2 ,HPLC-MS 3method 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.

The IUPAC Name of Ibrutinib is 1-[(3R)-3-[4-Amino-3-(4-phenoxyphenyl)-1Hpyrazolo [3, 4-d] pyrimidin-1-yl] piperidin-1-yl] prop-2-en-1-one. [4]

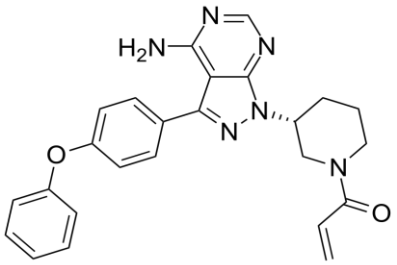


Fig 1: Chemical Structure of Ibrutinib

MATERIALS AND METHODS

HPLC Instrumentation & Conditions:

The HPLC system employed was HPLC with Empower2 Software with Isocratic with UV-Visible Detector.

Standard & sample preparation for UV-spectrophotometer analysis:

25 mg of Ibrutinibstandard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase.Further dilution was done by transferring 0.1 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase.The standard & sample stock solutions were prepared separately by dissolving standard & sample in a solvent in mobile phase diluting with the same solvent.(After optimization of all conditions) for UV analysis.Itscanned in the UV spectrum in the range of 200 to 400nm. This has been performed to know the maxima of Ibrutinib, so that the same wave number can be utilized in HPLC UV detector for estimating the Ibrutinib. While scanning the Ibrutinib solution we observed the maxima at 287nm. The UV spectrum has been recorded on ELICO SL-159 make UV – Vis spectrophotometer model UV-2450[5,6]. The scanned UV spectrum is attached in the following page.

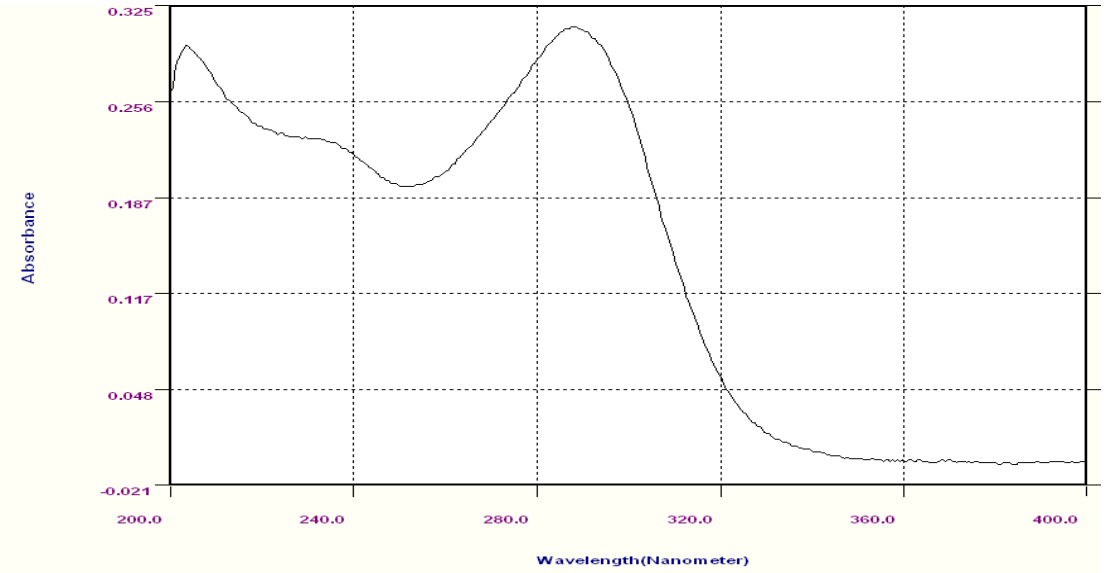


Fig 2: UV spectrum

Optimized Chromatographic Conditions:

Column: Waters ODS (C18) RP Column, 250 mm x 4.6 mm. 5µm.
Mobile Phase :0.1% Orthophosphoric Acid: Methanol in the ratio 35:65(v/v)
Flow Rate: 1.0ml/minute
Wave length :287nm
Injection volume :20µl
Run time :09mins.
Column temperature: Ambient
Sampler cooler: Ambient

MOBILE PHASE PREPARATION

Mobile phase was prepared by taking Orthophosphoric Acid: Methanol (35:65v/v). Mobile phase was filtered through 0.45 µm membrane filter and degassed under ultrasonic bath prior to use. The mobile phase was pumped through the column at a flow rate of 1.0 ml/min.

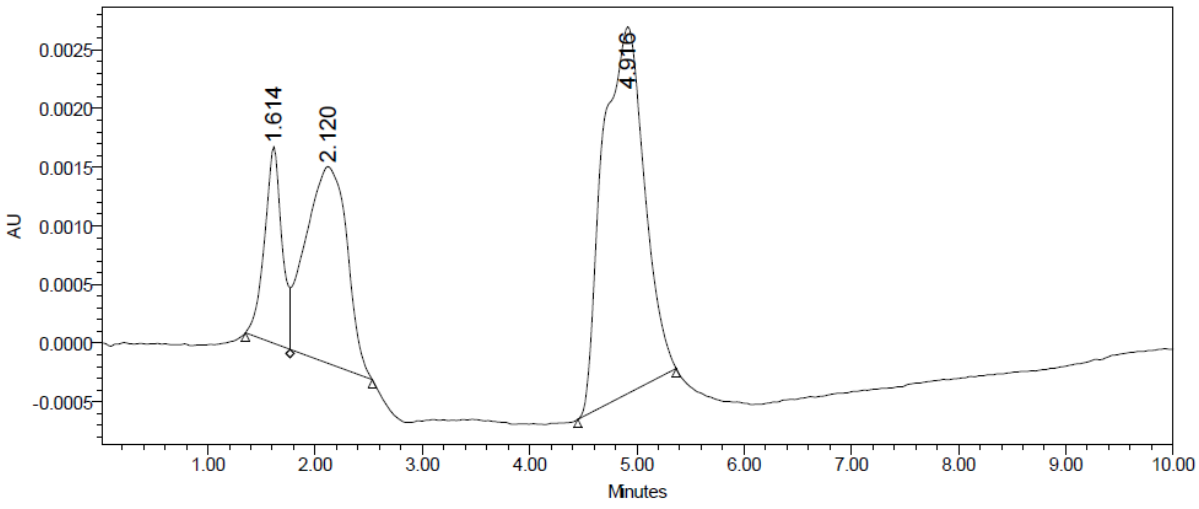
SAMPLE & STANDARD PREPARATION FOR THEANALYSIS

25 mg of Ibrutinib standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution was done by transferring 0.1 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase[7].

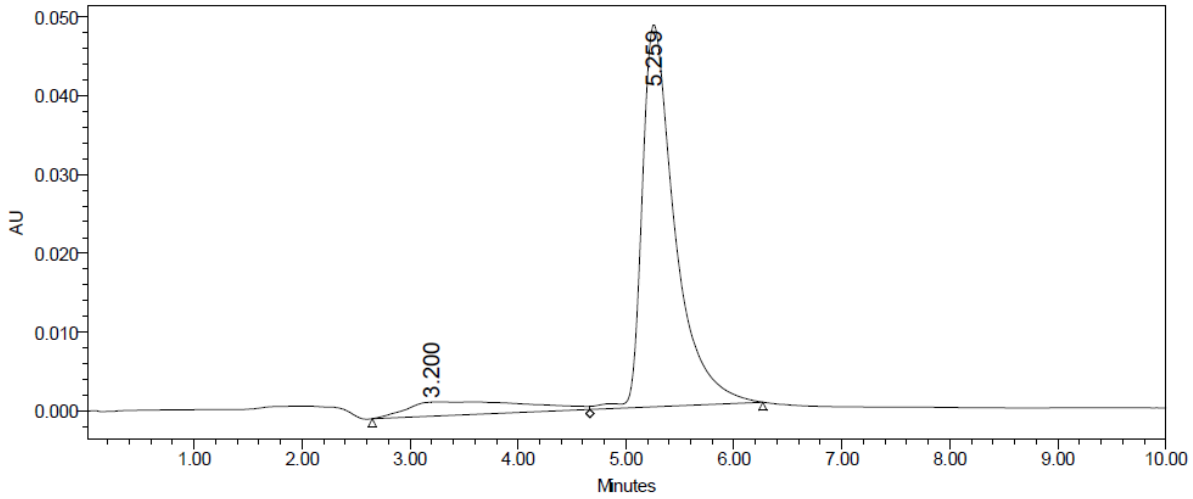
RESULT AND DISCUSSION:

Table-1: Trials for method development

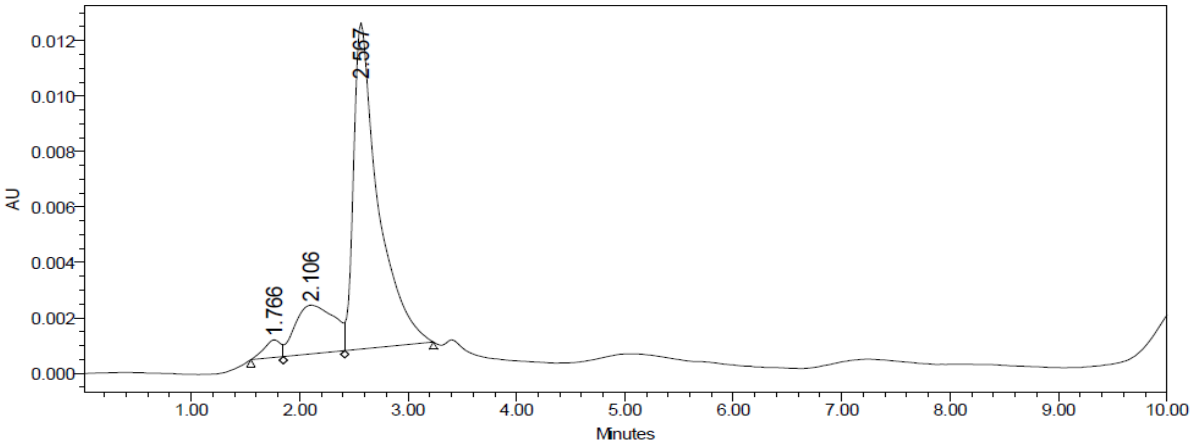
Column Used	Mobile Phase	Flow Rate	Wave length	Observation	Result
Phenomenex Luna C_{18}, 100A, 5µm, 250mmx4.6mm i.d.	Acetonitrile: Water = 70 :30	0.8 ml/min	287nm	Peak broken at the end	Method rejected
Phenomenex Luna C_{18}, 100A, 5µm, 250mmx4.6mm i.d.	Methanol: Water = 60 :40	1.0 ml/min	287nm	Tailing Peaks	Method rejected
Phenomenex Luna C_{18}, 100A, 5µm, 250mmx4.6mm i.d.	Acetonitrile: Methanol = 70:30	1.0 ml/ min	287nm	Tailing and Frontings	Method rejected
Phenomenex Luna C_{18}, 100A, 5µm, 250mmx4.6mm i.d.	Acetonitrile: Methanol = 50:50	1.0 ml/ min	287nm	Tailing Peak	Method rejected
Phenomenex Luna C_{18}, 100A, 5µm, 250mmx4.6mm i.d.	1% Orthophosphoric Acid: Methanol = 20:80	1.0 ml/ min	287nm	Broad Peak	Method rejected
Phenomenex Luna C_{18}, 100A, 5µm, 250mmx4.6mm i.d.	0.1% Orthophosphoric Acid: Methanol = 35:65	1.0 ml/ min	287nm	Good Peak	Method Accepted



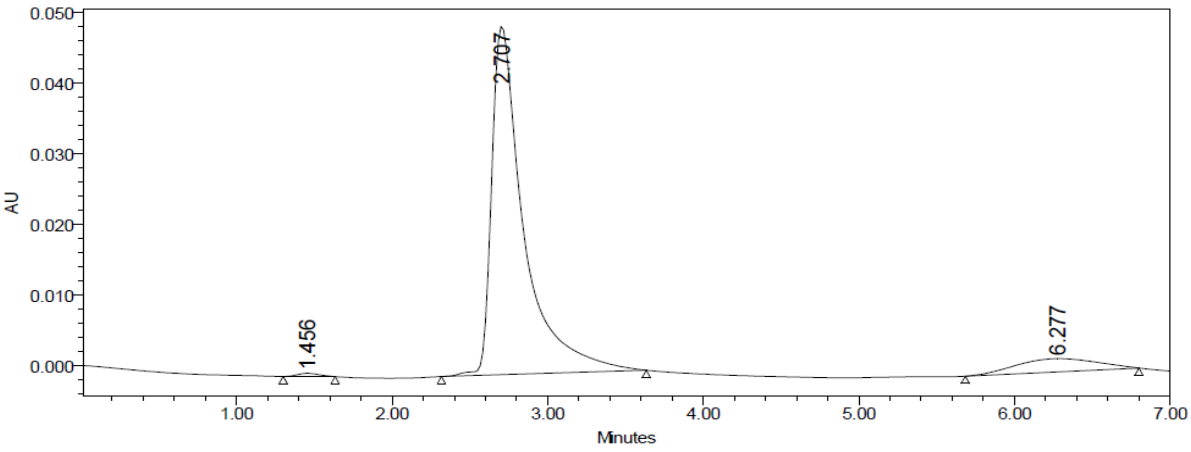
Trial-1



Trial-2



Trial-3



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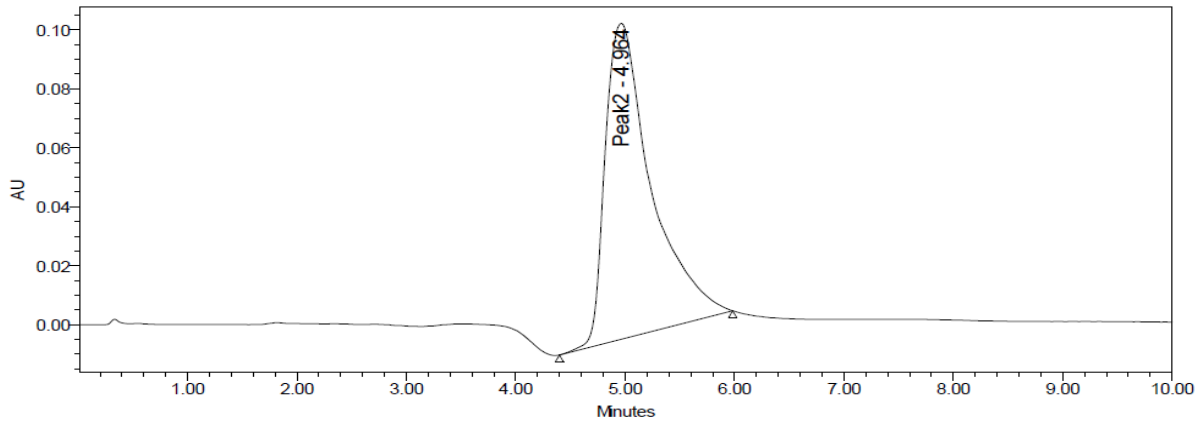

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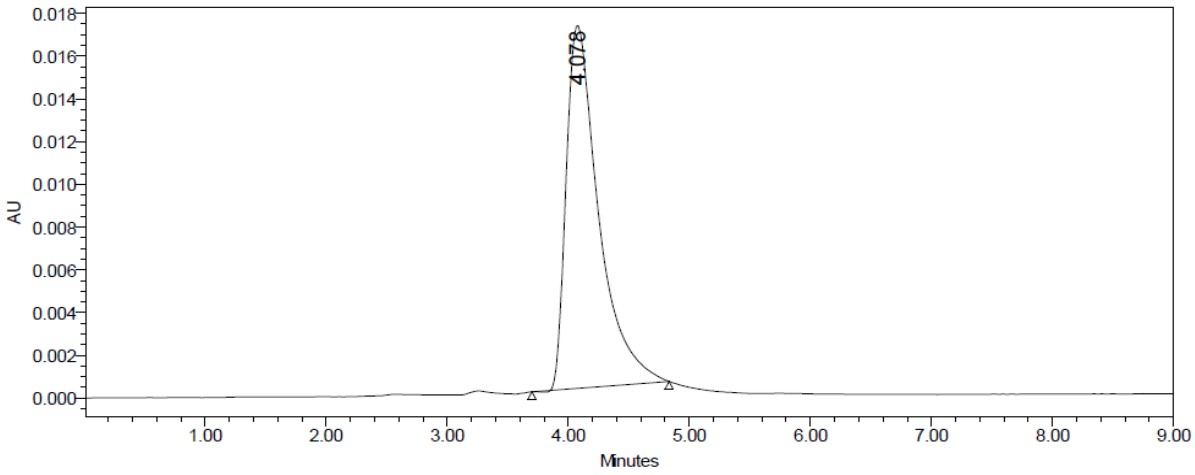

Trial-6

Table 2: Peak results

Rt	Peak Area	Theoretical Plates	Tailing Factor
4.078	327368	4687	1.29

METHOD VALIDATION:

Accuracy: ***Recovery study:***

To determine the accuracy of the projected technique, recovery studies were distributed by adding totally different amounts (80%, 100%, and 120%) of pure drug of Ibrutinib were taken and side to the pre-analysed formulation of concentration 10µg/ml [8,9]. From that proportion recovery values were calculated.

Table-3: Accuracy Readings

Sample ID	Concentration (µg/ml)			%Recovery of Pure drug	Statistical Analysis
	Conc. Injected	Conc. Recovered	Peak Area		
S_1: 80 %	8	7.991	269458	99.887	Mean= 100.4953 S.D. = 0.701041 % R.S.D.=0.697586
S_2: 80 %	8	8.101	273103	101.262	
S_3: 80 %	8	8.027	270641	100.337	
S_4: 100 %	10	9.914	332711	99.14	Mean= 99.22667 S.D. = 0.185831 % R.S.D.= 0.18728
S_5: 100 %	10	9.910	332567	99.10	
S_6: 100 %	10	9.949	333854	99.44	
S_7: 120 %	12	11.939	399277	99.491	Mean= 99.894 S.D. = 0.786211 % R.S.D.=0.787045
S_8: 120 %	12	11.927	398886	99.391	
S_9: 120 %	12	12.096	404441	100.80	

Precision:

Repeatability

The precision of each method was ascertained separately from the peak areas & retention times obtained by actual determination of six replicates of a fixed amount of drug. Ibrutinib (API) the percent relative standard deviations were calculated for Ibrutinib [10].

Table-4: Repeatability Results of Precision

HPLC Injection Replicates of Leucovorin	Retention Time	Peak Area
Replicate – 1	4.399	1067796
Replicate – 2	4.399	1073916
Replicate – 3	4.398	1077381
Replicate – 4	4.392	1064998
Replicate – 5	4.392	1060633
Replicate –6	4.393	1061645
Average		**1067728**
Standard Deviation		**6727.127**
% RSD		**0.630041**

Intra day & Inter day: The intra & inter day variation of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Ibrutinib revealed that the proposed method is precise [11,12].

Table-5: Results of Intra day&Inter day

Conc. Of Ibrutinib (API) (µg/ml)	Observed Conc. OfIbrutinib (µg/ml) by the proposed method			
	Intra day		Inter day	
	Mean (n=6)	% RSD	Mean (n=6)	% RSD
8	7.96	1.09	8.06	1.06
10	10.09	0.95	9.86	0.92
12	12.03	0.96	11.96	0.99

Linearity and Range

The calibration curve showed good linearity in the range of 0-14µg/ml, for Ibrutinib (API) with correlation coefficient (r^2) of 0.998 (Fig-37). A typical calibration curve has the regression equation of y = 32880x + 6712 for Ibrutinib [13].

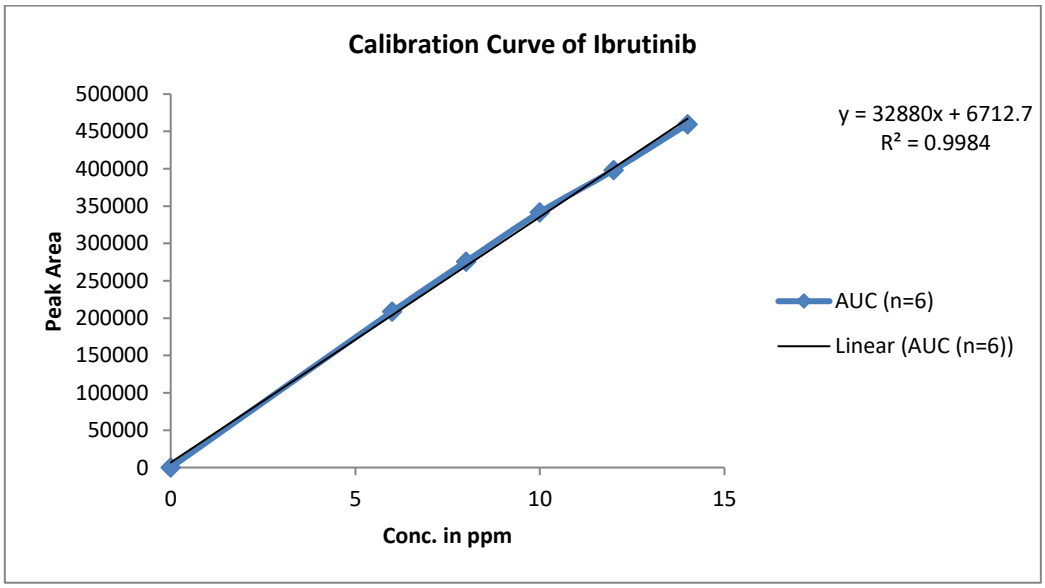

Fig-3: Calibration curve of Ibrutinib (API)

Table-6: Linearity Results of Ibrutinib

CONC.	AUC (n=6)
0	0
6	208757
8	275909
10	341782
12	398192
14	459633

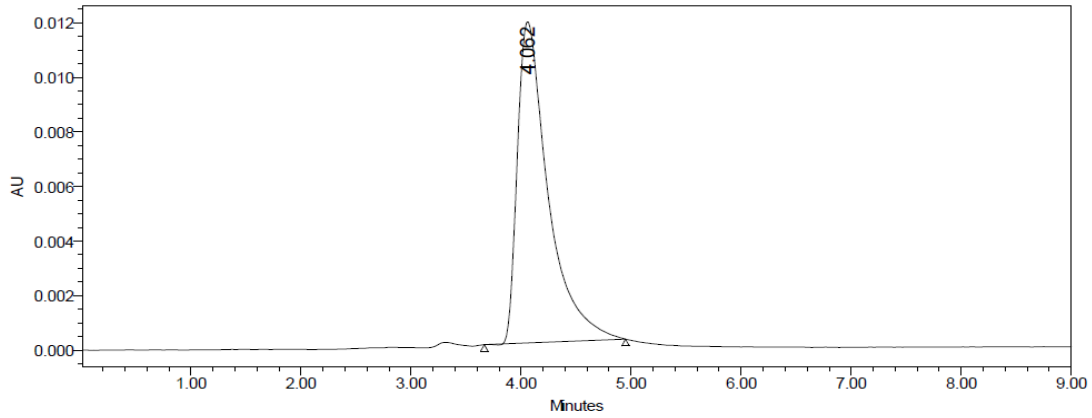

Fig 4: Calibration of Ibrutinib concentration in 6 ppm

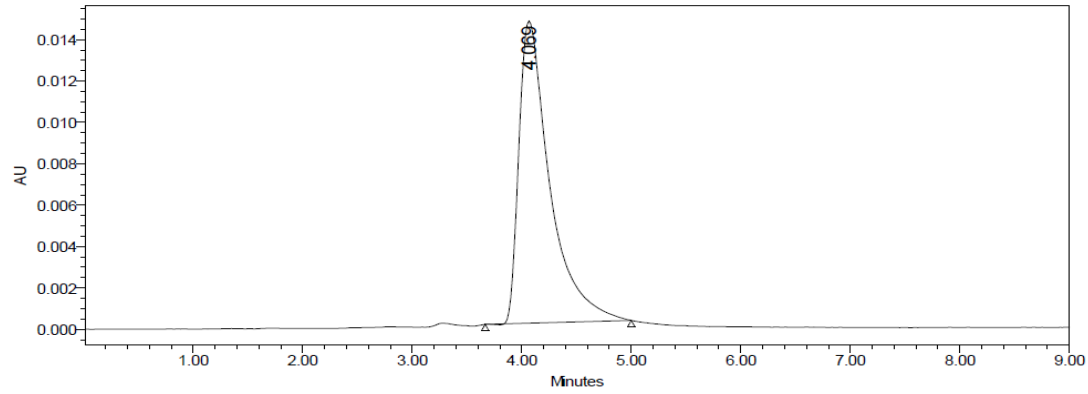

Fig 5: Calibration of Ibrutinib concentration in 8 ppm

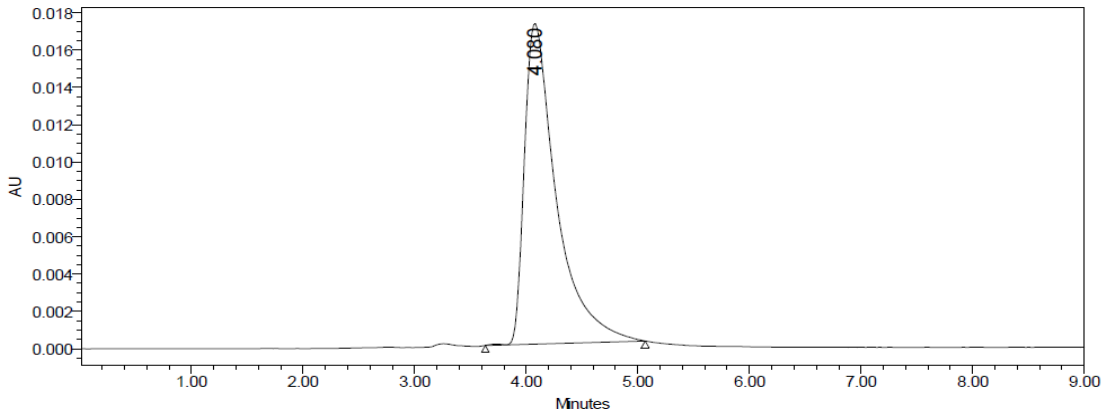

Fig 6: Calibration of Ibrutinib concentration in 10 ppm

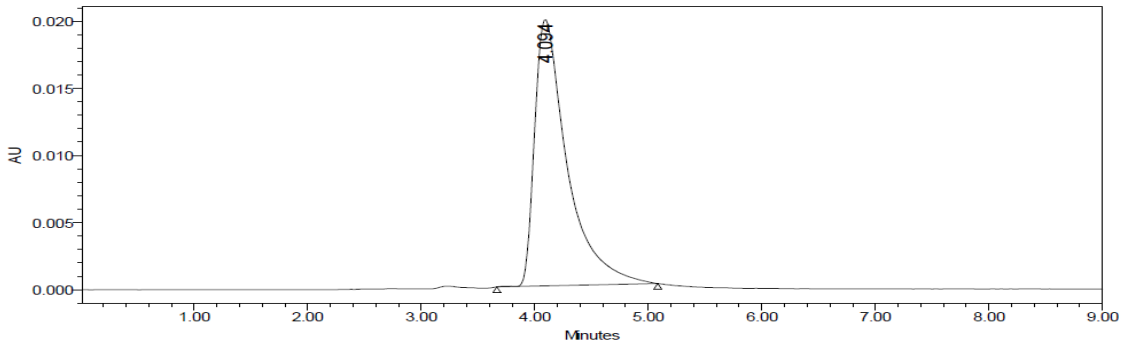

Fig 7: Calibration of Ibrutinib concentration in 12 ppm

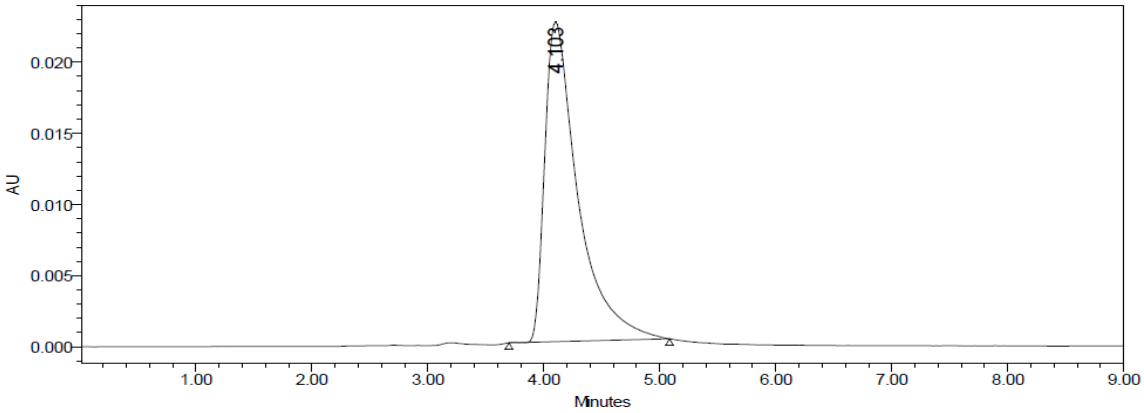

Fig 8: Calibration of Ibrutinib concentration in 14 ppm

LOD & LOQ: The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified (LOQ) were found to be 0.09 & 0.29 µg/ml respectively.

System Suitability Parameter

System quality testing is Associate in nursing integral a part of several analytical procedures. The tests area unit supported the construct that the instrumentation, physics, Associate in Nursingalytical operations and samples to be analysed represent an integral system that may be evaluated intrinsically [14]. Following system quality take a look at parameters were established.

Table-7: Data of System Suitability Parameter

S.No.	Parameter	Limit	Result
1	Resolution	Rs > 2	8.64
2	Asymmetry	T ≤ 2	Ibrutinib=0.87
3	Theoretical plate	N > 2000	Ibrutinib=4689
4	Tailing Factor	T<2	Ibrutinib=1.29

FORCED DEGRADATION STUDIES:

1. Acid Degradation:

A precisely measured 10 mg of unadulterated medication was exchanged to a clean and dry round base cup. 30 ml of 0.1 N HCl was added to it and it was refluxed in a water shower at 600C for 4 hours. Permitted to cool to room temperature. The sample was then neutralized using dilute NaOH solution & final volume of the sample was made up to 100ml with water to prepare 100 µg/ml solution. It was injected into the HPLC system against a blank of mobile phase (after optimizing the mobile phase compositions). This experiment was repeated several times using same concentration of HCl (0.1N) and observed its degradation profile. The typical chromatogram shown below is the degradation profile of Minocycline in 0.1N HCl.

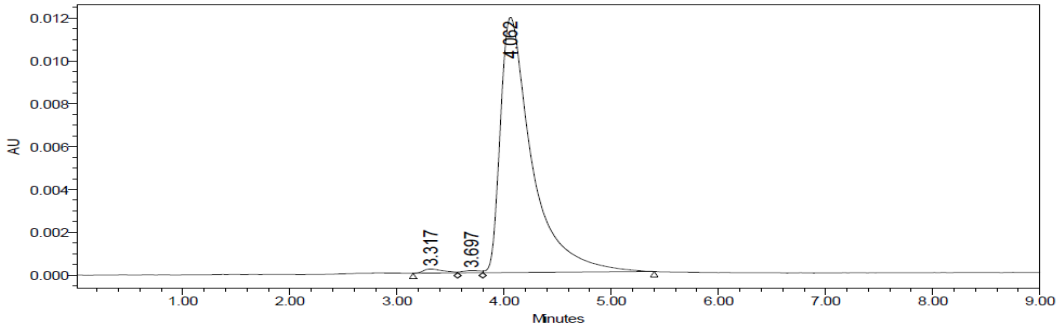

Fig-9: Chromatogram showing degradation for Ibrutinib in 0.1 N HCl

2. Basic Degradation:

An accurately weighed 10 mg of pure drug was transferred to a clean & dry round bottom flask. 30 ml of 0.1N NaOH was added to it. & it was refluxed in a water bath at 60^{0}C for 4 hours. Allowed to cool to room temperature. The sample was than neutralized using 2N HCl solution & final volume of the sample was made up to 100ml to prepare 100 µg/ml solution. It was injected into the HPLC system against a blank of mobile phase after optimizing the mobile phase compositions. This experiment was repeated several times using same concentration of NaOH such as 0.1N to observe its degradation profile. The chromatogram shown below is the degradation profile of Minocycline in 0.1N NaOH.

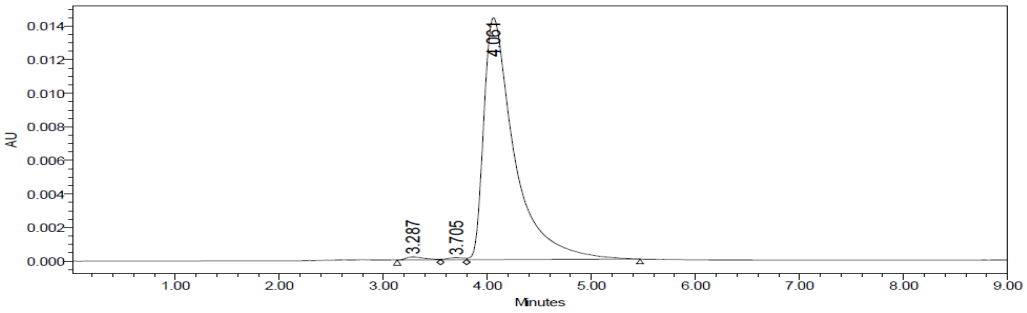

Fig-10: Chromatogram showing degradation related impurity in 0.1 N NaOH

3. Thermal Degradation:

Accurately weighed 10 mg of pure drug was transferred to a clean & dry round bottom flask. 30 ml of HPLC water was added to it. Then, it was refluxed in a water bath at 60^0 c for 6 hours uninterruptedly. After the reflux was over, the drug became soluble and the mixture of drug & water was allowed to cool to room temperature. Final volume was made up to 100 ml with HPLC water to prepare 100 µg/ml solution. It was injected into the HPLC system against a blank of mobile phase.

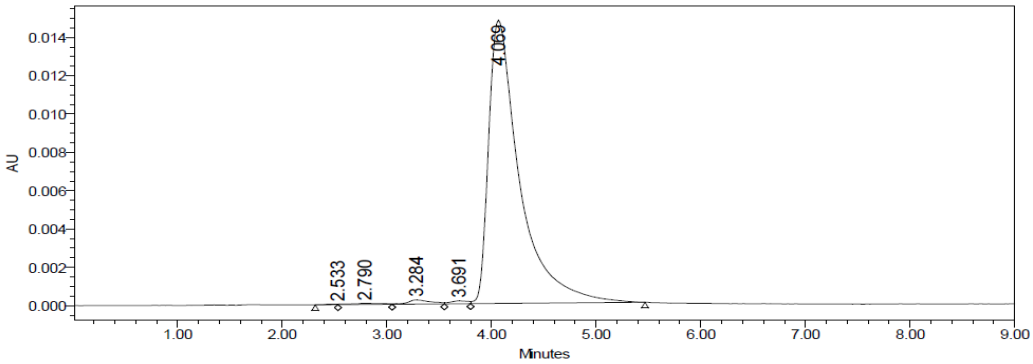

Fig-11: Chromatogram showing thermal degradation studies

4. Photolytic Degradation:

Approximately 10 mg of pure drug was taken in a clean & dry Petri dish. It was kept in a UV cabinet at 254 nm wavelength for 24 hours without interruption. Accurately weighed 1 mg of the UV exposed drug was transferred to a clean & dry 10 ml volumetric flask. First the UV exposed drug was dissolved in methanol & made up to the mark with mobile phase to get 100 µg/ml solution. Finally, this solution was injected into the HPLC system against a blank of mobile phase and chromatogram was obtained.

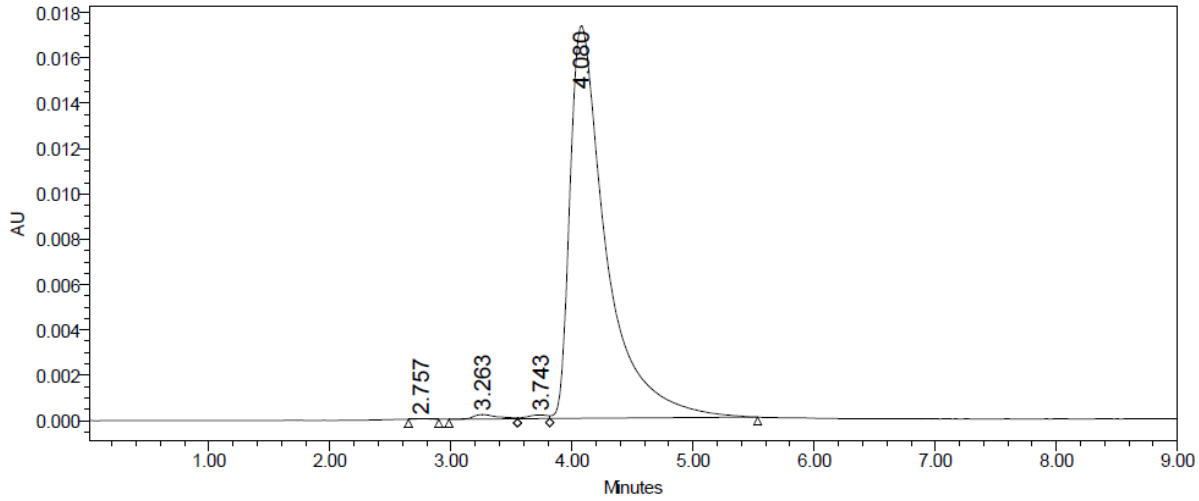

Fig-12: Chromatogram showing photolytic degradation.

5. Oxidation with (3%) H_2O_2:

Accurately weighed 10 mg. of pure drug was taken in a clean & dry 100 ml volumetric flask. 30 ml of 3% H_2O_2 and a little methanol was added to it to make it soluble & then kept as such in dark for 24 hours. Final volume was made up to 100 ml. using water to prepare 100 µg/ml solution. The above sample was injected into the HPLC system [15].

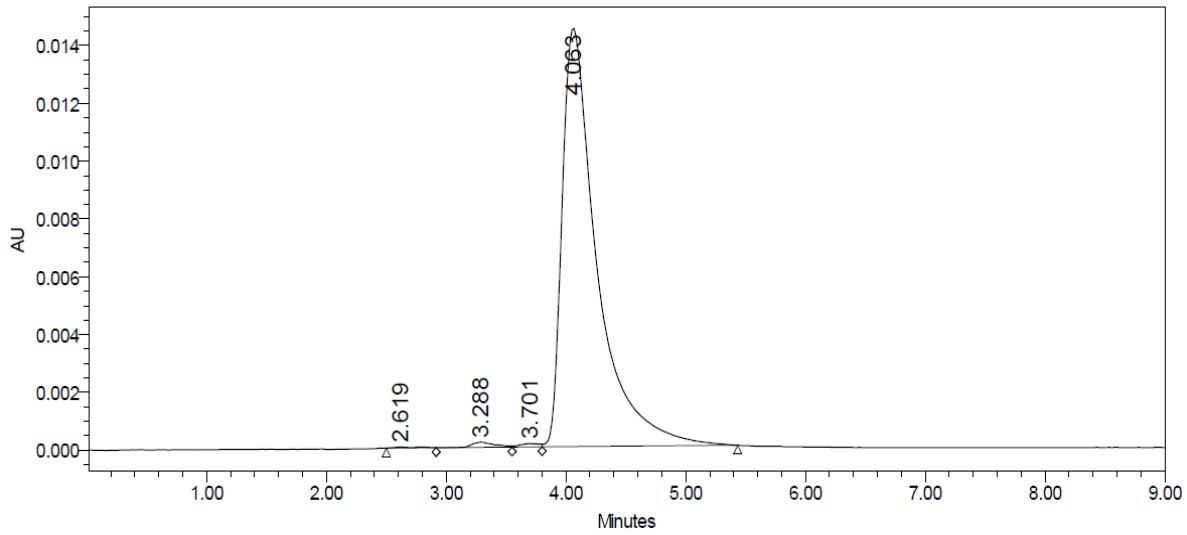

Fig-13: Chromatogram showing oxidative degradation.

Table-8: Results of forced degradation Studies of Ibrutinib.

Stress condition	Time	Assay of active substance	Assay of degraded products	Mass Balance (%)
Standard	--	100	--	100
Acid Hydrolysis (0.1 M HCl)	24Hrs.	88.32	11.68	100
Basic Hydrolysis (0.I M NaOH)	24Hrs.	90.04	9.96	100
Thermal Degradation (50 ^{0}C)	24Hrs.	91.02	8.98	100
UV (254nm)	24Hrs.	97.25	2.75	100
3 % Hydrogen peroxide	24Hrs.	90.84	9.16	100

CONCLUSION

A sensitive& selective RP-HPLC method has been developed & validated for the analysis of Ibrutinib. Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility. The result shows the developed method is yet another suitable method for assay, purity & stability which can help in the analysis of Ibrutinib in different formulations.

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