Scholars Academic Journal of Pharmacy (SAJP)
Abbreviated Key Title: Sch. Acad. J. Pharm.
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A Unit of Scholars Academic and Scientific Society, India
www.saspublisher.com

ISSN 2347-9531 (Print)
ISSN 2320-4206 (Online)

Pharmaceutical Analysis

Development of a New RP-HPLC Method for the Estimation of IBRUTINIB

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Original Research Article

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Article History
Received: 02.12.2017
Accepted: 10.12.2017
Published:30.12.2017

DOI:
10.21276/sajp.2017.6.12.3

Abstract: A simple, Precised, Accurate method was developed for the estimation of Ibrutinib by RP-HPLC technique. Chromatographic conditions used are stationary phase Agilent 150mm x 4.6 mm, 5µ., Mobile phase buffer: acetonitrile in the ratio of 70:30 and flow rate was maintained at 1ml/min, detection wave length was 290nm, column temperature was set to 30^{o}C and diluent was mobile phase Conditions were finalized as optimized method. System suitability parameters were studied by injecting the standard five 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 0.89 for repeatability and 0.66 for intermediate precision. LOD and LOQ are 0.13µg/ml and 0.39µg/ml respectively. By using above method assay of marketed formulation was carried out 101.21% was present. Degradation studies of Ibrutinib were done, in all conditions purity threshold was more than purity angle and within the acceptable range.

Keywords: HPLC Ibrutinib, Method development. ICH Guidelines.

INTRODUCTION

Pharmaceutical Analysis is that core branch of pharmacy education and research, which is advancing very fast. It can be categorized as synthesis of new drugs molecules and pharmaceutical analysis. Analytical chemistry is the science of making quantitative and qualitative measurements.

Qualitative Inorganic Analysis seeks to establish the presence of a given element or inorganic compound in a sample. Qualitative Organic Analysis seeks to establish the presence of a given functional group or organic compound in a sample. Quantitative analysis seeks to establish the amount of a given element or compound in a sample. There are various techniques used for analysis of mixtures [1-5]**.** Spectroscopy measures the interaction of the molecules with electromagnetic radiation. Chromatography is the collective term for a family of laboratory techniques for the separation of mixtures. It involves passing a mixture dissolved in a "mobile phase" through a stationary phase, which separates the analyte to be measured from other molecules in the mixture and allows it to be isolated. Analytical Chromatography is used to determine the existence and possibly also the concentration of analyte(s) in a sample. Analytical chemistry has played critical roles in the understanding of basic science to a variety of practical applications, such as biomedical applications, environmental monitoring, quality control of industrial manufacturing, forensic science and so on [6-10].

The phenomenal growth in chromatography is largely due to the introduction of the versatile technique called high-pressure liquid chromatography, which is frequently called high-performance liquid chromatography. Both terms can be abbreviated as HPLC. High-pressure liquid-solid chromatography (HPLC) is rapidly becoming the method of choice for separations and analysis in many fields. Almost anything that can be dissolved can be separated on some type of HPLC column.

Characteristics of HPLC method

Efficient, highly selective, widely applicable, only small sample required, may be nondestructive of sample, readily adapted to quantitative analysis, High resolving power [11-15].

Modes of HPLC

Normal phase chromatography

In normal phase mode, the nature of stationary phase is polar and the mobile phase is non-polar. In this technique, non-polar compounds travel faster and are eluted first because of the lower affinity between the non-polar compounds and stationary phase. Polar compounds are retained for longer time and take more time to elute because if their higher affinity with the stationary phase. Normal phase mode of separation is, therefore, not generally used pharmaceutical applications because most of the drug molecules are polar in nature and hence take longer time to elute.

Reversed phase chromatography

Reversed phase mode is the most popular mode for analytical and preparative separations of compounds of interest in chemical, biological, pharmaceutical, food and biomedical sciences. In this mode, the stationary phase is non-polar hydrophobic packing with octyl and octadecyl functional group bonded to silica gel and the mobile phase is a polar solvent, often a partially or fully aqueous mobile phase. Polar substances prefer the mobile phase and elute first. As the hydrophobic character of the solutes increases, retention increases. Generally, the lower the polarity of the mobile phase, the higher is its eluent strength. The elution order of the classes of compounds is reversed (thus the name reverse-phase chromatography) [16].

Method Devlopment on HPLC

Method development and optimization in liquid chromatography is still an attractive field of research for theoreticians (researchers) and attracts also a lot of interest from practical analysts. Among all, the liquid chromatographic methods, the reversed phase systems based on modified silica offers the highest probability of successful results. However, a large number of (system) variables (parameters) affect the selectivity and the resolution [17].

Type of analytical procedures to be validated

Validation of analytical procedures is directed to the four most common types of analytical procedures. Identification test [15-18]

Quantitative test for impurities content

Limit test for the control of impurities

Quantitative test of the active moiety in samples of drug substance on drug product on other selected components in the drug product.

Reasons for method validation

There are two important reasons for validating assays in the pharmaceutical industry. The first, and by for the most important, is that assay validation is an integral part of the quality control system. The second is that current good manufacturing practice regulation requires assay validation [19-23].

Performance characteristics examined when carrying out method validation

Specificity, Linearity, Range, Accuracy, Precision (Repeatability and Ruggdness), Detection and Quantitation limit, Robustness, System suitability [23].

MATERIALS AND METHODS OF PREPARATION

Materials

Ibrutinib is the gifted sample from Sree Srinivasa Scientifics, Methanol HPLC Grade (RANKEM), Acetonitrile, HPLC Grade (RANKEM), Glacial Acetic acid and HPLC grade Water (RANKEM) are procured from Chaitanya private limited.

Methods

Based on drug solubility and P^{ka} Value following conditions has been used to develop the method estimation of Ibrutinib**.**

Chromatographic Conditions

Optimized Chromatographic Conditions

Column : Std Agilent (150*4.6 µm)
Mobile phase : buffer (Kh2po4): Acetonitrile (70:30)
Flow rate : 1.0 ml/min
Detector : PDA 290nm
Temperature : 30^0C
Injection Volume: 10µL

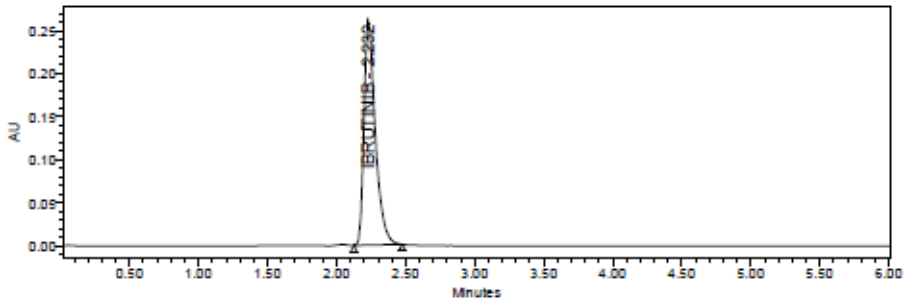

Fig-1: optimised chromatogram

Observations

Ibrutinib eluted with good peak shape and retention time and tailing was passed

Sample Preparation

5 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 5 tablets was transferred into a 100 ml volumetric flask, 50ml 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.

Buffer

kh2po4 1.36 gms of potassium dihydrogen phosphate was dissolved in 1000ml HPLC grade water

and sonicated for 30mins then pH adjusted to 4.0 with dil. Ortho phosphoric acid.

Mobile phase

Buffer and Acetonitrile taken in the ratio 70:30.

RESULTS AND DISCUSSIONS

Repeatability

Six working sample solutions of 140ppm are injected and the % Amount found was calculated and %RSD was found to be 0.89 and chromatogram was shown in fig 2.

Table-1: Repeatability data

Sl. No	Peak Area
1	1506709
2	1504587
3	1499591
4	1473355
5	1505766
6	1509548
AVG	1499926
STDEV	13420.5
%RSD	0.89

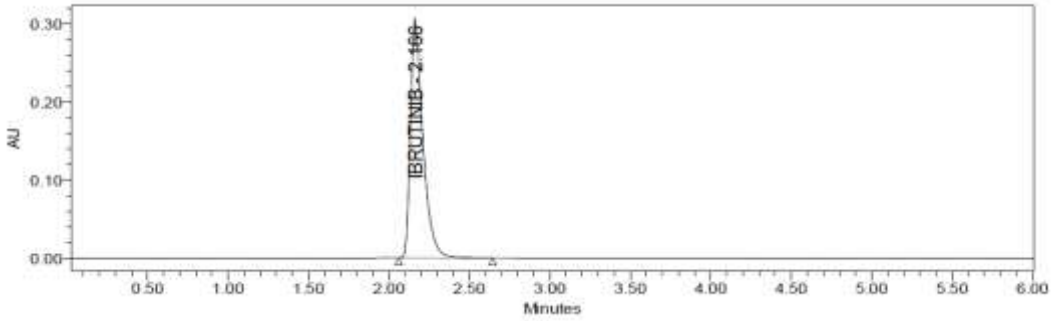

Fig-2: Repeatability Chromatogram

Intermediate precision

Six working sample solutions of 140ppm are injected on the next day of the preparation of samples and the % Amount found was calculated and %RSD was found to be 0.66 and chromatogram was shown in fig 3.

Table-2: Intermediate precision data

S.No	Peak Area
1	1488747
2	1515144
3	1505825
4	1510460
5	1505005
6	1494159
AVG	1503223
STDEV	9961.3
%RSD	0.66

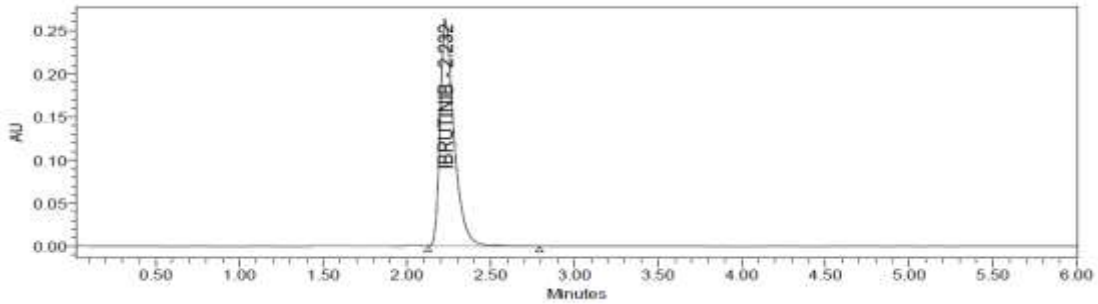

Fig-3: Intermediate precision Chromatogram

LINEARITY

To demonstrate the linearity of assay method, inject 5 standard solutions with concentrations of about 35 ppm to 210 ppm of Ibrutinib. Plot a graph to concentration versus peak area. Slope obtained was 8289.4 Y-Intercept was 448.69 and Correlation Co-efficient was found to be 0.999 and Linearity plot was shown in Fig.10

Table-3: Linearity Concentration and Response

Linearity Level (%)	Concentration (ppm)	Area
0	0	0
25	35	311439
50	70	553083
75	105	872469
100	140	1158427
125	175	1460135
150	210	1740287

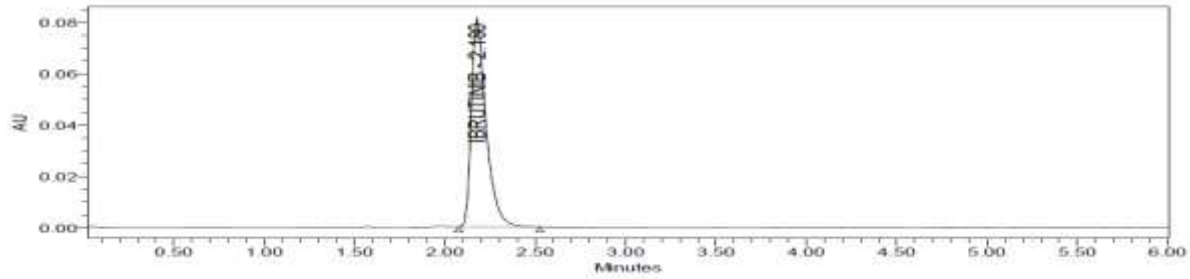

Fig-4: Linearity 25% Chromatogram

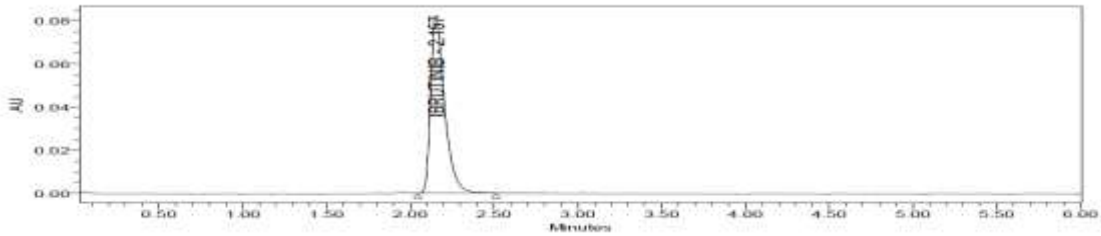

Fig-5: Linearity 50% Chromatogram

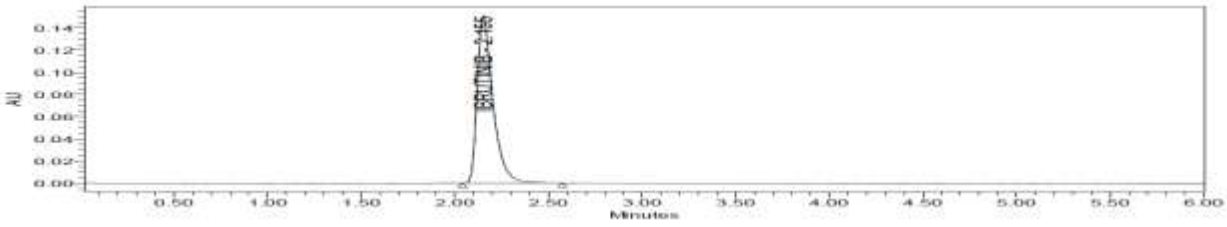

Fig-6: Linearity 75% Chromatogram

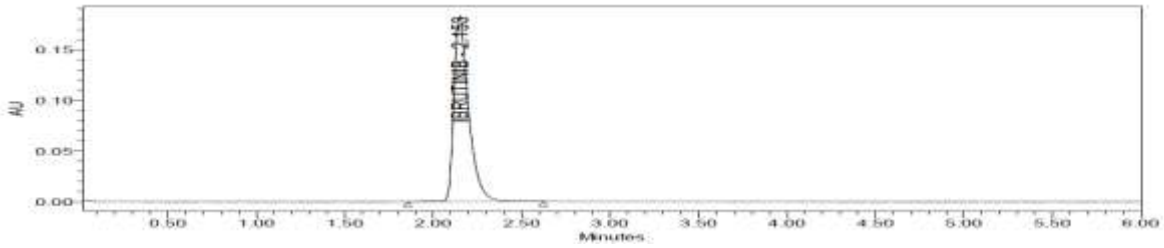

Fig-7: Linearity 100% Chromatogram

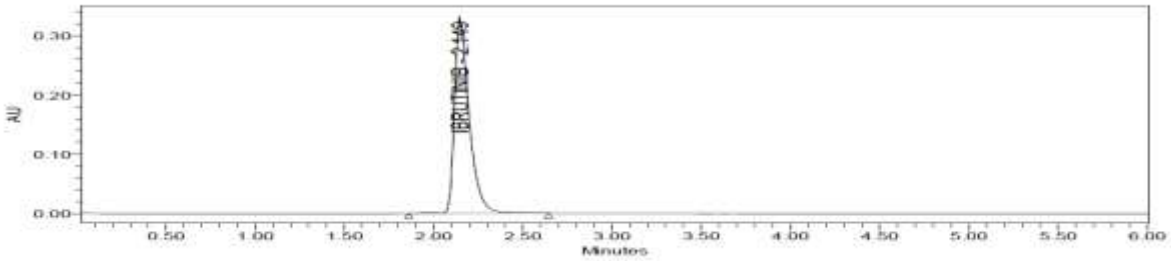

Fig-8: Linearity 125% Chromatogram

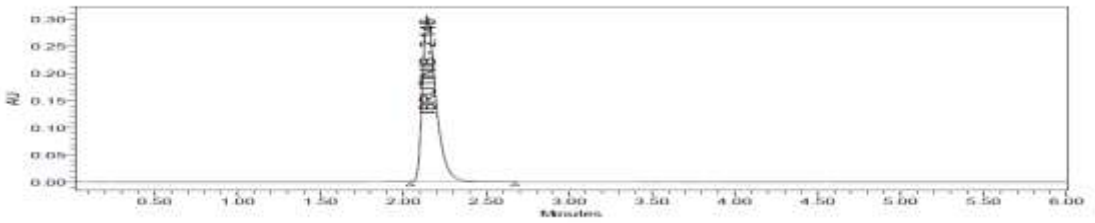

Fig-9: Linearity 150% Chromatogram

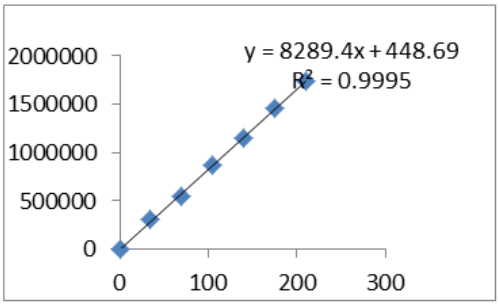

Fig 10: Linearity Plot

Accuracy

Three Concentrations of 50%, 100%, 150% are Injected in a triplicate manner and %Recovery was calculated as 99.80 and %RSD was found to be 1.32 and chromatograms were shown in fig 6.11-6.13.

Table-4: Accuracy data

% Level	Amount Spiked (µg/mL)	Amount recovered (µg/mL)	% Recovery	Mean %Recovery
50%	70	68.684	98.12	99.80%
	70	71.68	102.14	
	70	69.384	99.12	
100%	140	139.25	99.47	
	140	142.08	101.49	
	140	138.92	99.23	
150%	210	210.54	100.26	
	210	206.89	98.52	
	210	209.79	99.90	

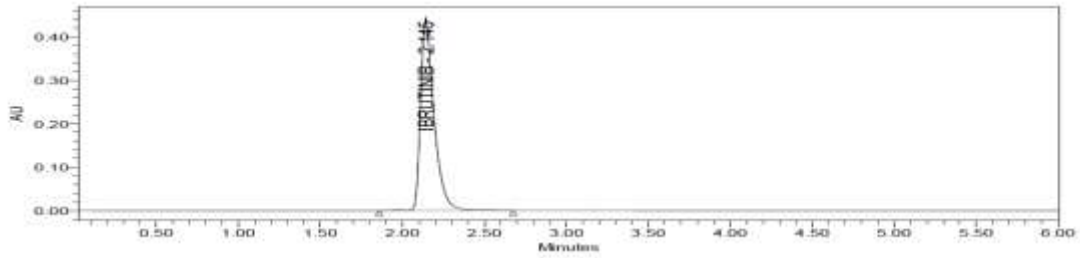

Fig-11: Accuracy 50% Chromatogram

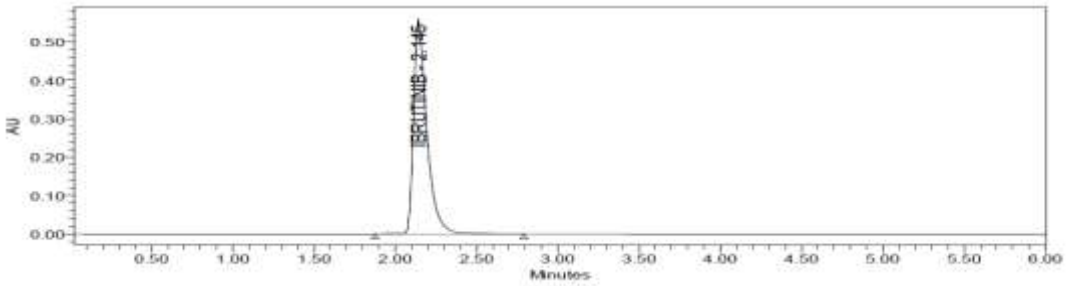

Fig 12: Accuracy 100% Chromatogram

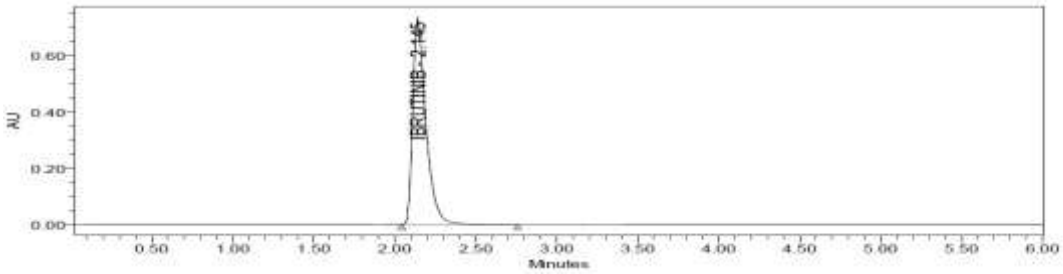

Fig 13: Accuracy 150% Chromatogram

LOD: Ditection limit of the Ibrutinib in this method was found to be 0.13µg/ml.

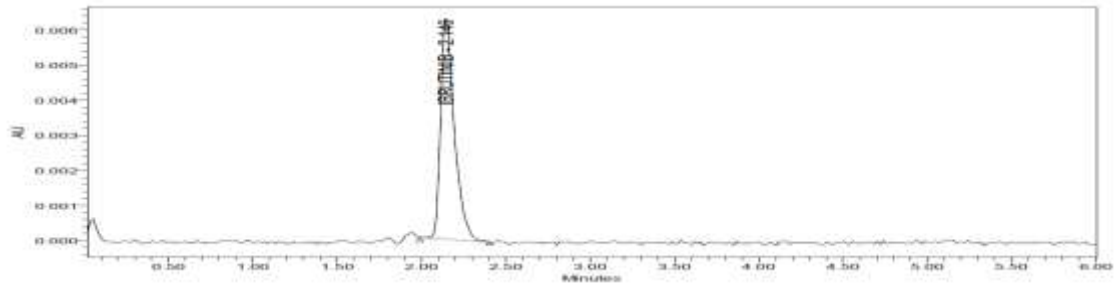

Fig-14: LOD Chromatogram of Ibrutinib

LOQ: Quantification limit of the Ibrutinib in this method was found to be 0.39µg/ml.

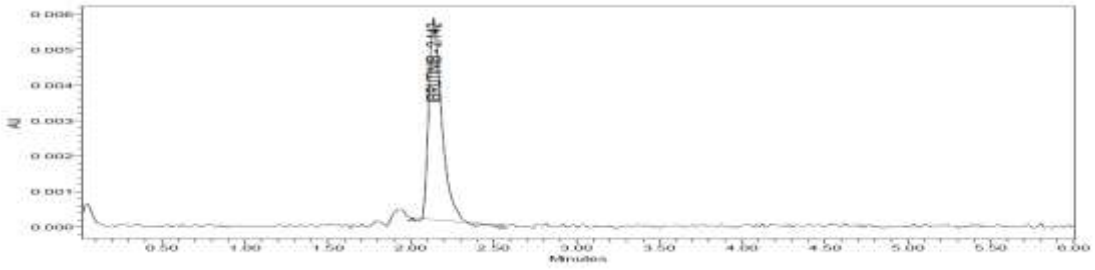

Fig-15: LOQ Chromatogram of Ibrutinib

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 is calculated.

Table-5: Robustness Data

Parameter	%RSD
Flow Minus	0.3
Flow Plus	0.1
Mobile phase Minus	0.1
Mobile phase Plus	0.8
Temperature minus	0.1
Temperature plus	0.7

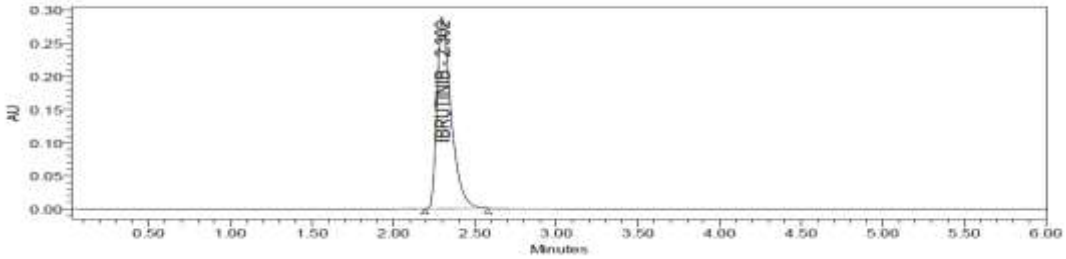

Fig-16: Flow minus Chromatogram of Ibrutini

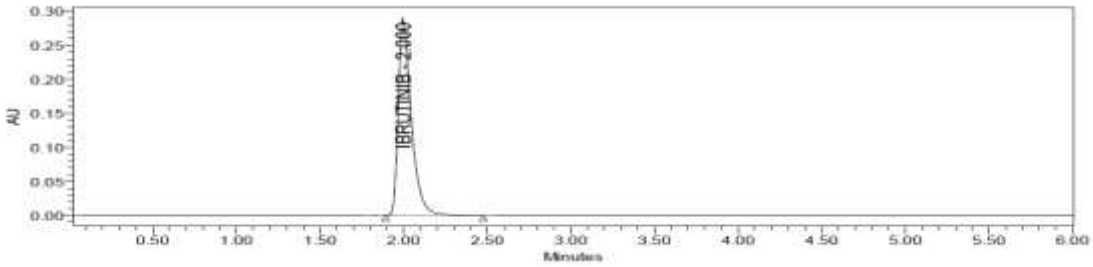

Fig 17: Flow plus Chromatogram of Ibrutinib

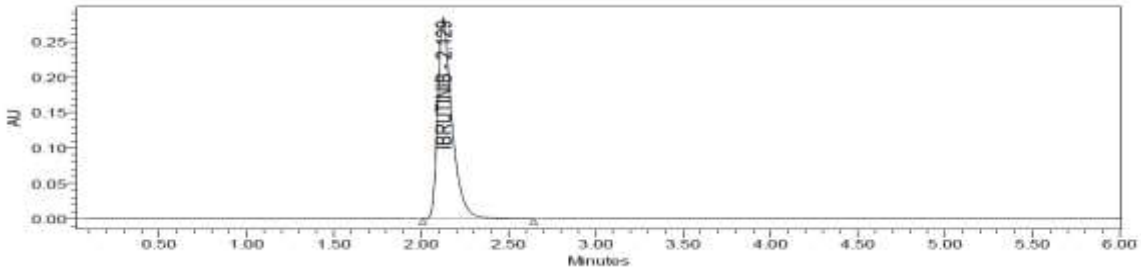

Fig 18: Mobile phase minus Chromatogram of Ibrutinib

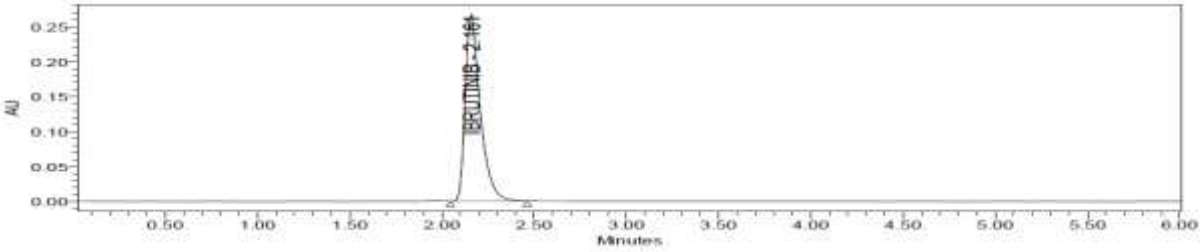

Fig 19: Mobile phase plus Chromatogram of Ibrutinib

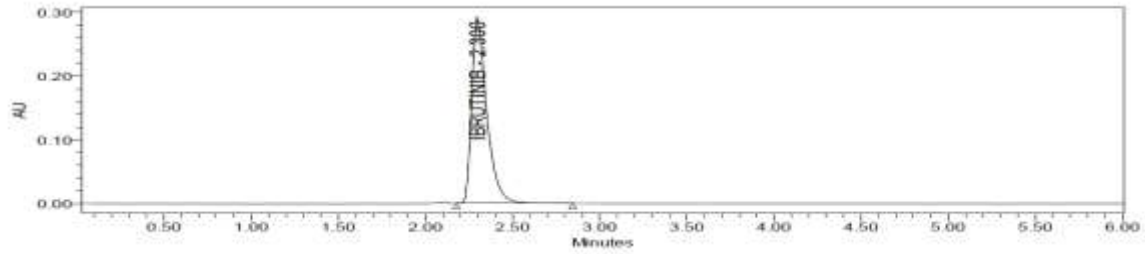

Fig 20: Temperature minus Chromatogram of Ibrutinib

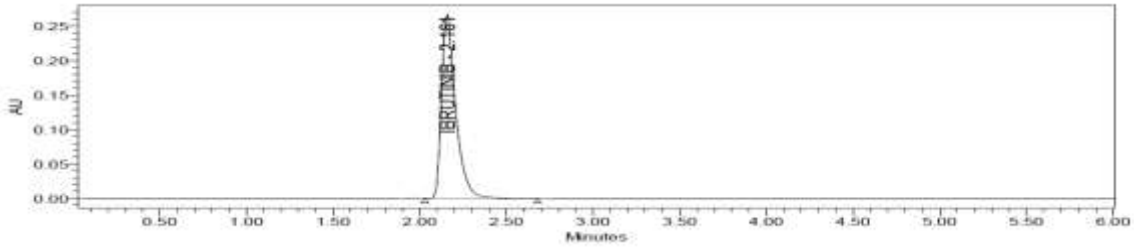

Fig-21: Temperature plus Chromatogram of Ibrutinib

ASSAY OF MARKETED FORMULATION

Standard solution and sample solution were injected separately into the system and chromatograms were recorded and drug present in sample was calculated using before mentioned formula.

Table-6: Assay of Formulation

Sample No	%Assay
1.	101.66
2.	101.52
3.	101.18
4.	99.41
5.	101.60
6.	101.86
AVG	101.21
TDEV	0.9055
%RSD	0.89

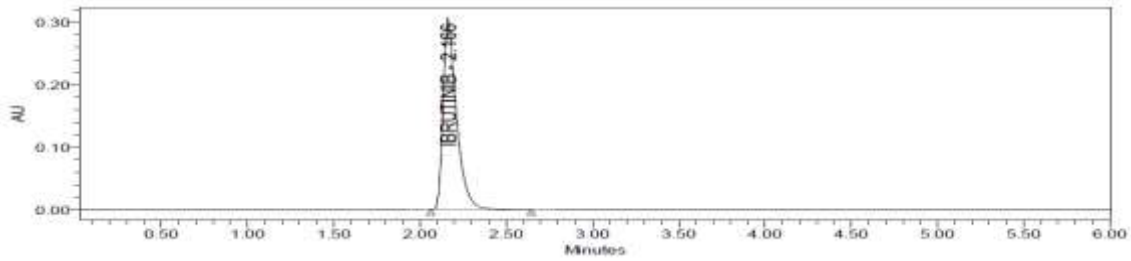

Fig-22: Assay Chromatogram

Degradation Studies

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

Table-7: Degradation Data of Ibrutinib

S.NO	Degradation Condition	% Drug Degraded	Purity Angle	Purity Threshold
1	Acid	8.629612	0.200	0.281
2	Alkali	6.548312	0.261	0.285
3	Oxidation	4.156025	0.256	0.286
4	Thermal	3.119288	0.269	0.286
5	UV	1.436557	0.236	0.275
6	Water	0.229449	0.243	0.275

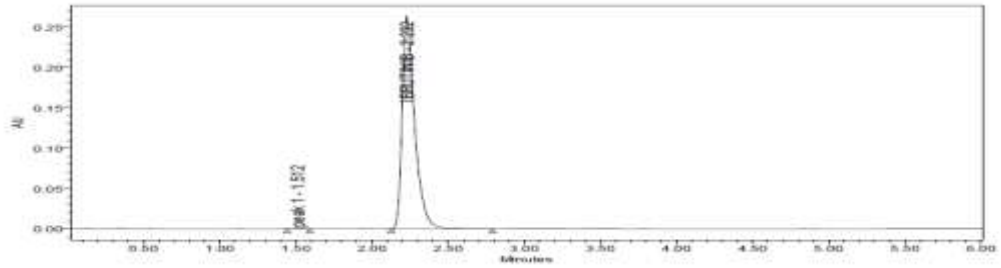

Fig-23: Acid degradation chromatogram

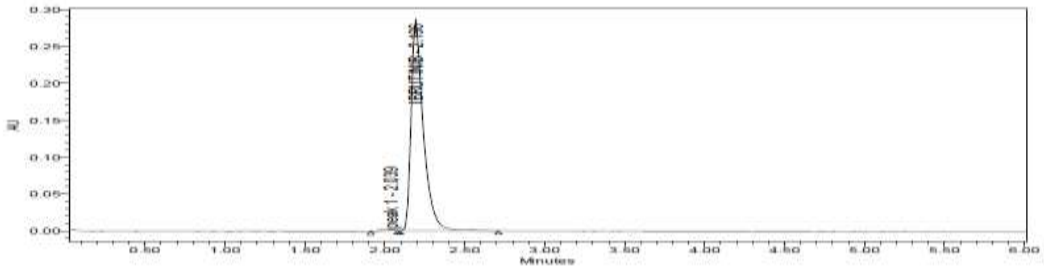

Fig 24: Base degradation chromatogram

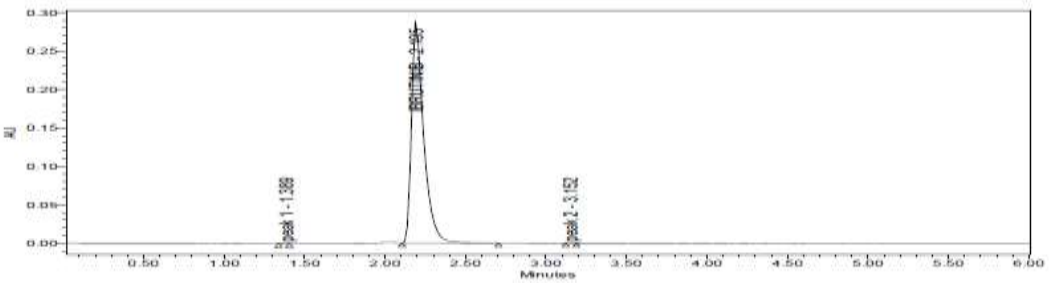

Fig-25: Peroxide degradation chromatogram

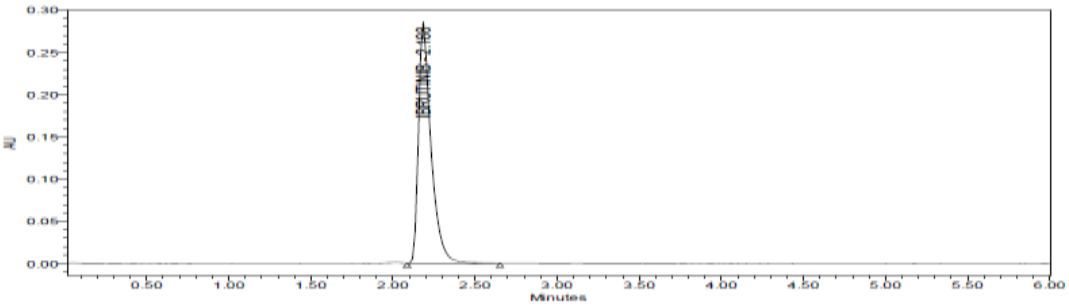

Fig-26: Thermal degradation chromatogram

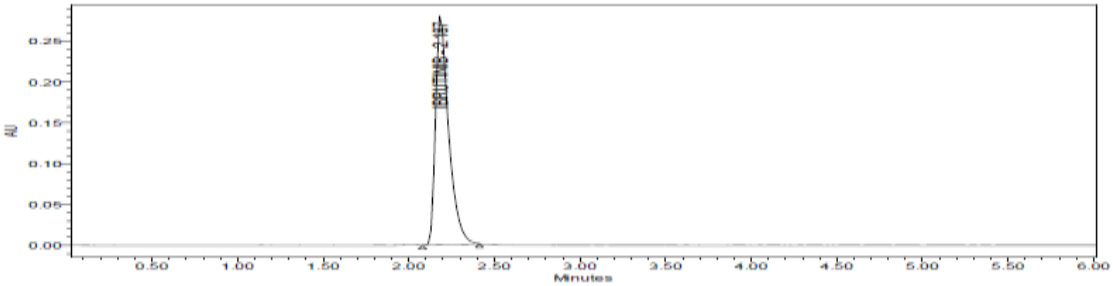

Fig-27: UV degradation chromatogram

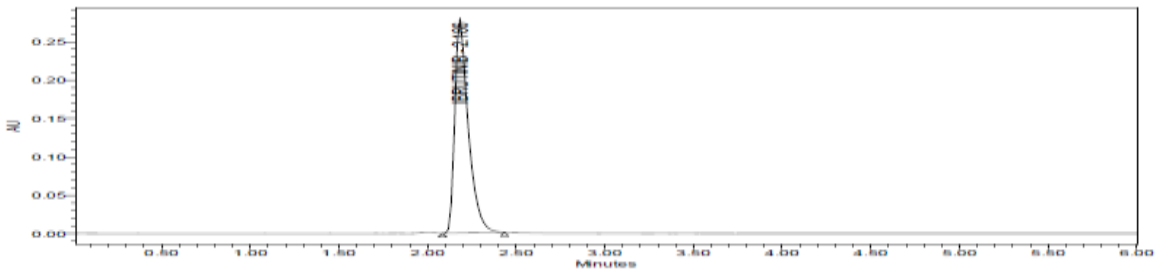

Fig-28: Water degradation chromatogram

SUMMARY AND CONCLUSION

Ibrutinib is a small-molecule inhibitor of BTK. Ibrutinib forms a covalent bond with a cysteine residue in the BTK active site, leading to inhibition of BTK enzymatic activity, helps in anticancer. Chromatographic conditions used are stationary phase Std Agilent (150mm*4.6mm), Mobile phase buffer: acetonitrile in the ratio of 70:30and flow rate was maintained at 1ml/min, detection wave length was 290nm, column temperature was set to 30^{o}C 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 0.89 for repeatability and 0.66 for intermediate precision. LOD and LOQ are 0.13µg/ml and 0.39µg/ml respectively

By using above method assay of marketed formulation was carried out 101.21% was present. Degradation studies of Ibrutinib were done, in all conditions purity threshold was more than purity angle and within the acceptable range.

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