Original Research Paper

Volume - 13 | Issue - 11 | November - 2023 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Pharmaceutical Science

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR ESTIMATION OF IBRUTINIB IN BULK AND TABLET DOSAGE FORM

Jayanti Mukherjee*	Professor, Department of Pharmaceutical Analysis, CMR College of Pharmacy, Hyderabad, Telangana. *Corresponding Author
S. Sriya	Associate Professor, Department of Pharmaceutical Analysis, CMR College of Pharmacy, Hyderabad, Telangana
A. Raja Reddy	Associate Professor, Department of Pharmaceutical Analysis, CMR College of Pharmacy, Hyderabad, Telangana
T. Rama Rao	Professor, Department of Pharmaceutical Analysis, CMR College of Pharmacy, Hyderabad, Telangana.

ABSTRACT **Background:** In the current study, a simple, improved, rapid, precise and accurate reverse phase liquid chromatographic method has been developed for the estimation of Ibrutinib in bulk form and as well as in marketed tablet dosage form. This drug is used to treat chronic lymphocytic leukemia and small lymphocytic lymphoma. Chromatography was carried out on a Phenomenex Luna C18 (4.6 x 150mm, 5m) column using isocratic mobile phase of methanol and acetonitrile in the ratio of 25:75 v/v using the at a flow rate of 1.0ml/min, the detection was carried out at 250 nm. **Results:** Ibrutinib was eluted at 2.379 ±0.02 min with a run time of 6 minutes. This method produced linear responses in the concentration range of 30-70 µg/ml of Ibrutinib with a correlation coefficient of 0.999. Limit of detection and limit of quantitation were found to be 2.4 µg/mL and 6.34 µg/mL, respectively. The system suitability parameters such as theoretical plate count, tailing and percentage RSD between five standard injections were within the limit. Ibrutinib was subjected for forced degradation stability study in conditions of thermal, acid, alkali, and oxidation and photo-degradation condition. The degradants were well resolved from the Ibrutinib main peak. Validation of the developed method is carried as per USFDA and ICH guidelines. **Conclusion:** The results of the analysis prove that the method is simple, improved, precise, accurate, and rapid for estimating the content of Ibrutinib in bulk drug and tablet dosage form and can be applied for routine analysis.

KEYWORDS : Ibrutinib, Stability-indicating RP-HPLC, Anticancer, Forced degradation.

INTRODUCTION

Ibrutinib is an anticancer drug that targets B-cell malignancies. The IUPAC name of Ibrutinib is 1-[(3R)-3-[4-amino-3-(4-phenoxyphenyl)-1H-pyrazol [3,4-d] pyrimidin-1-yl] piperidin-1-yl] prop-2-en-1-one[1]. Ibrutinib is a potent, irreversible inhibitor of Bruton's tyrosine kinase (BTK). It forms a covalent bond with a cysteine residue in the active site of BTK (Cys481), leading to its inhibition[2]. The inhibition of BTK plays a role in the B-cell receptor signaling and thus, the presence of Ibrutinib prevents the phosphorylation of downstream substrates such as PLC-γ[3,4]. Preclinical studies have shown that Ibrutinib effectively inhibits the proliferation and survival of malignant B cells in vivo, as well as cell migration and substrate adhesion in vitro[5-7]. The latter effect has been shown to produce regression in xenograft mouse models[8-10]. Ibrutinib was FDA-approved in 2013 for the management and treatment of chronic lymphocytic leukemia and mantle cell lymphoma[11]. Structurally, it consists of a fused aminopyrimidine-pyrazole core with a piperidine substitution on pyrazole[12]. More importantly, an acrylamide group is presently attached to piperidine nitrogen, responsible for its irreversible inhibitory action on Bruton's tyrosine kinase[13]. In the molecular modeling-based structural analysis of ibrutinib bound to BTK, the visible contacts include the H-bond interaction between the 4-amino group of ibrutinib and hinge residue Glu475[14]. Similarly, another H-bond interaction occurs between N3 of pyrazolo[3,4-d]pyrimidine nucleus and Met477. Hydrophobic interactions with Leu408, Met449, Ile472, and Leu542 along with vander Waals interaction with Asp539 are also observed[15]. More importantly, the acrylamide group lies closer to the thiol group of Cys481 supporting the covalent interaction. H-bond and hydrophobic contacts are essential for the proper orientation leading to the covalent modification between acrylamide and Cys48[16].

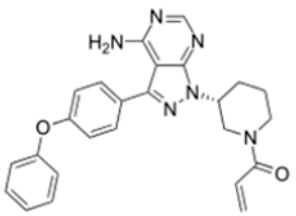

Fig. 1: Structure of Ibrutinib

As per literature survey, Ibrutinib was estimated individually by few methods like simple HPLC , Ultra HPLC, HPLC-MS 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.

Experimental Method

Chemical & Reagents: Ibrutinib was obtained from Sura Laboratories, Hyderabad, India and the tablet formulation Imbruvica (Brand name) is purchased from Janssen Cilag International Nv. All the reagents such as methanol, acetonitrile used were of analytical grade of Merck company.

Instrumentation and Chromatographic Conditions: A HPLC system (Waters, Model 2996) with software, Empower 2, fitted with Phenomenex Luna C18 (4.6 x 150mm, 5m) and PDA detector (at 250nm) was used for the complete analysis. Isocratic run with a flow rate 1ml/min was preferred for resolving the drug.

Preparation of Mobile Phase: Accurately measured 250 ml (25%) of HPLC Methanol and 750 ml of HPLC Water (75%) were mixed and degassed in a digital ultra-sonicater for 15 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Preparation of Standard Solution: Accurately weighed 10 mg of Ibrutinib working standard was transferred into a 10ml of clean dry volumetric flasks, added about 7ml of methanol and sonicated to dissolve and removal of air completely. Finally, the volume was made up to the mark with the same solvent methanol. Further 0.5ml of the above Ibrutinib stock solution was pipetted into a 10ml volumetric flask and diluted up to the mark with methanol. The average retention time was found to be 2.380 min.

Preparation of Sample Solution and Assay: An equivalent weight of 10 mg of Ibrutinib was transferred into a 10mL clean dry volumetric flask and added about 7mL of diluent and sonicated to dissolve it completely to make up the volume up to the mark with the same solvent. Further 0.5ml of Ibrutinib above stock solution was pipetted into a 10ml volumetric flask and diluted up to the mark with the diluent. The three replicate injections of standard and sample solutions were

injected and calculated the assay value.

Forced Degradation Studies

The specificity of the method can be demonstrated by applying stress conditions using acid, alkaline, peroxide, photolytic and thermal degradations. The sample was exposed to these conditions and the main peak of the drug was studied for peak purity which indicated the method effectively separating the degradation products from the pure active ingredient.

Acid Degradation Studies: To 1 ml of Ibrutinib stock, 1 ml of 5N HCl was added and refluxed for 1 hr at 60 ºC. The resultant solution was neutralized with 1 ml 5N NaOH and made up the final volume with diluent. The solution was cooled to the room temperature and filtered through 0.45µm membrane filter. A sample of 10µl was injected into the HPLC system, and the chromatograms were recorded to assess the stability of the sample.

Alkali Degradation Studies: To 1 ml of stock solution of Ibrutinib, 1 ml of 5N sodium hydroxide was added and refluxed for 30 min at 60 ºC. The resultant solution was neutralized with 1 ml of 5N HCl and made up the final volume with diluent. The solution was cooled to the room temperature and filtered through 0.45µm membrane filter. A sample of 10µl was injected into the HPLC system, and the chromatograms were recorded to assess the stability of the sample.

Oxidation Degradation Studies: To 1 ml of stock solution of Ibrutinib, 1 ml of 30% hydrogen peroxide (H_2O_2) was added separately. The solution was kept for 2hr 30 min at 60ºC. For HPLC study, the resultant solution was diluted up to the mark with diluent. The solution was cooled to the room temperature and filtered through 0.45µm membrane filter. A sample of 10µl was injected into the HPLC system, and the chromatograms were recorded to assess the stability of the sample.

Dry Heat Degradation Studies: A volume of 1 ml of standard drug solution was placed in the oven at 60ºC for 4h to study dry heat degradation. For HPLC study, the resultant solution was made up to final volume with diluent. The solution was cooled to the room temperature and filtered with 0.45µm membrane filter. A sample of 10µl was injected into the HPLC system, and the chromatograms were recorded to assess the stability of the sample.

Photo Degradation Studies: The photo stability of the drug was studied by exposing the stock solution to UV light for 2 days or 200 Watt-hours/m^2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain (40µg/mL and 20µg/mL) solution and filtered through 0.45µm membrane filter. A sample of 10µl solution was injected into the system, and the chromatograms were recorded for the assessment of sample stability.

Method Validation: The developed method was validated as per ICH Q2 (R1) and USFDA guidelines. The method was validated for the parameters such as linearity and range, specificity, accuracy, precision, robustness, ruggedness, limit of detection (LOD), and limit of quantitation (LOQ). The specificity was determined by injecting samples of blank, placebo (except Ibrutinib), standard solution, and sample solution from the formulation.

Stability-indicating Tests: After performing the chromatographic run with several solvent mixtures, the mobile phase consisting of methanol: acetonitrile (25:75 v/v) was found to furnish sharp, well-defined peaks with good symmetry. The percentage degradation and percentage recovery data for stress degradation studies are summarized in Table-1. From the forced degradation conditions, it was observed that the degradation of Ibrutinib is higher in alkaline and lesser in thermal conditions. As per ICH guidelines, the limit of acceptable forced degradation is less than 20%. In the proposed method, the degradation of Ibrutinib was less than 20%, which represents the stability-indicating method.

RESULTS AND DISCUSSION

Method development and optimization of chromatographic conditions: To achieve satisfactory separation of Ibrutinib, different solutions at various pH conditions and solvents of different proportions were tested as binary and tertiary eluents. However, the mobile phase was adjusted with Methanol and Acetonitrile at a ratio of 25:75v/v with a run time of 6 min to achieve good satisfactory results at a flow rate of 1.0 mL/min measured at a detection of 250 nm. The optimized chromatogram and optimized conditions are mentioned in Fig. 2.

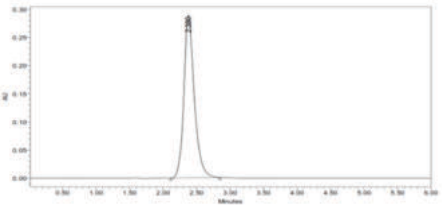

Fig. 2: Typical Optimized Chromatogram Of Ibrutinib

System Suitability

The system suitability test was performed to check whether the complete testing system was suitable for the required application. The standard solution was injected for five times and the area for all five injections in HPLC were measured. Peak area, retention time, theoretical plates and tailing factor were measured. The experimental result shows that the parameters tested were within acceptable limit for % RSD of peak area, theoretical plates and tailing factor.

Method Validation

Linearity: The plot of Concentration (x) versus the Average Peak Area (y) data of Ibrutinib is a straight line.

y = mx + c Slope (m) = 605

Intercept (c) = 19359

Correlation Coefficient (r) = 0.999

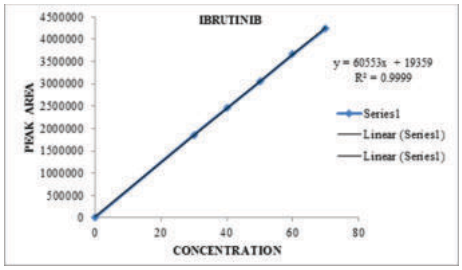

Fig 3: Linearity Graph Of Ibrutinib

Validation Criteria: The response linearity is verified if the Correlation Coefficient is 0.999 or greater as shown in Fig 3. Correlation Coefficient (r) is 0.99, and the intercept is 19359. These values meet the validation criteria.

Precision: The repeatability was performed by taking standard solution which is injected for five times and measured the area for all five injections in HPLC. The % RSD for the area of five replicate injections was found to be within the specified limits. The same study was performed by two different analysts to determine the inter-day precision in which standard solution was injected for six times and measured the area for all six injections in HPLC. The % RSD for the area of six replicate injections was found to be within the specified limits. The % RSD values for system and inter- day precision study was 0.176 % and 0.204 % respectively, which confirms that the method was sufficiently precise.

Accuracy: The three replicate injections of individual concentrations (50%, 100%, 150%) were injected under the optimized conditions. Later the chromatograms were recorded and measured the peak responses. Difference between the peak areas obtained for fortified and unfortified solutions were used to calculate percentage recovery of drugs. The recovery data indicates that excellent recoveries observed despite the presence of the degradation product of the drugs.

Specificity: The specificity was established by injecting the sample solutions of the drugs in presence of their degradation products and determining the peak purity. The complete separation of the drugs from their degradation product was noticed. The average retention times for the degraded products were furnished.

Limit Of Detection And Limit Of Quantification: The LOD and LOQ of the drug were determined by using a signal to noise ratio of 3 and 10 respectively. The LOQ was verified by injecting 6 replicates at its concentration. The limit of detection (LOD) and limit of quantification (LOQ) were 2.4 µg/mL and 6.34 µg/ml respectively.

Low value of LOD and LOQ indicate the method is sensitive.

Robustness: The separation of drug and degradation products is achieved after changing the flow rate from 0.9-1.1 mL/min, variation of mobile phase i.e., Methanol: Acetonitrile was taken in the ratio and 30:70, 20:80 instead of 25:75, column from different manufactures and solvents of different lots.

Analysis Of Marketed Sample: The amount of Ibrutinib tablets expressed as percentage of label claim and they were in good agreement i.e; suggesting no interference from the excipients of the tablet. The percentage assay results were found to be 99.8%.

Table 1 : Summary Of Forced Degradation Studies Of Ibrutinib

Degradation Conditions	% Degradation of Ibrutinib
Acid	2.15
Base/Alkaline	3.24
Oxidative	0.592
Photolytic	0. 729
Thermal	0.256

Table 2: Summary Of Validation And System Suitability Parameters

Parameters	Ibrutinib
Linearity	30-70µg/ml
Correlation Coefficient	0.999
LOD (µg/mL) LOQ (µg/mL)	2.4µg/ml 6.34µg/ml
Accuracy (%)	100.54%
System Precision	0.17653%
Intermediate Precision	0.204563%
Robustness	Robust
Assay (% mean assay)	99.80%

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Ibrutinib in bulk drug and pharmaceutical dosage forms. This technology made use of a reasonably priced solvent system. The % RSD readings were within limits, and the method was found to be precise. The RP-HPLC method produced good results as per the ICH guidelines. 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 30-70g/ml levels, R^2 value was found to be as 0.999. The method was successfully validated according to USFDA guidelines for all the parameters which were found within acceptance criteria. The developed method may be useful for routine analysis of Ibrutinib tablets or for assay of Ibrutinib tablets from stability batches.

REFERENCES

1. Dr. Kealey and P.J Haines, Analytical Chemistry, 1st edition, Bios Publisher, (2002), PP 1-7.
2. A.BraithWait and F.J.Smith, Chromatographic Methods, 5th edition, Kluwer,Academic Publisher, (1996), PP 1-2.
3. Andrea Weston and Phyllisr. Brown, HPLC Principle and Practice, 1st edition, Academic press, (1997), PP 24-37.
4. Yuri Kazakevich and Rosario Lobrutto, HPLC for Pharmaceutical Scientists, 1st edition, Wiley Interscience A JohnWiley & Sons, Inc., Publication, (2007), PP 15-23.
5. Sahajwalla CG a new drug development, vol 141, Marcel Dekker Inc., New York, (2004), PP 421–426.
6. Kim ES, Dhillon S: Ibrutinib: a review of its use in patients with mantle cell lymphoma or chronic lymphocytic leukaemia. Drugs. 2015 May;75(7):769-76.
7. Wilson and Gisvold's Textbook of Organic Medicinal and Pharmaceutical Chemistry, 11th edition, Lippincott Williams & Wilkins, New york, 2004.
8. Burger's Medicinal Chemistry and drug discovery, 6 th edition, Wiley Interscience, New Jersey, 2007.
9. Code Q2B, Validation of Analytical Procedures; Methodology. ICH Harmonized Snyder LR practical HPLC method development, 2nd edition. John Wiley and sons, New York, (1997), PP 180-182.
10. Skoog D A, West D M, Holler FJ: Introduction of analytical chemistry. Sounder college of publishing, Harcourt Brace college publishers. (1994), PP 1-5.
11. Sharma B K, Instrumental method of chemical analysis Meerut. (1999), PP 175-203.
12. Breaux J and Jones K: Understanding and implementing efficient analytical method development and validation. Journal of Pharmaceutical Technology (2003), 5, PP 110-114.
13. Willard, H. y. Merritt L.L, Dean J.A and Settle F.A "Instrumental methods of analysis" 7th edition CBS publisher and distributors, New Delhi, (1991), PP 436-439.
14. ICH Q2A, "validation of analytical methods, definitions and terminology", ICH Harmonized tripartite guideline, (1999). Tripartite Guidelines, Geneva, Switzerland, (1996), PP 1- 8.
15. Foye's "Principles of Medicinal Chemistry", 6th edition, Lippincott Williams & Wilkins, New york, 2008.
16. Drugs & Cosmetics Act, 1940 & Rules, 1945, 2nd edition, Susmit publishers, Mumbai, India, 2000. 11. Indian Pharmacopoeia, Ministry of Health & Family Welfare, Government of India, New Delhi, 1996.