CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES
SJIF Impact Factor: 7.187
https://doi.org/10.5281/zenodo.22031799

Available online at: http://www.iajps.com Research Article

DEVELOPMENT AND VALIDATION OF NEW ANALYTICAL METHOD FOR THE DETERMINATION OF IBRUTINIB IN BULK AND TABLET DOSAGE FORM

Sneha N U[1*], Suresha D N[2], Naveen Kumar G S[3]

[1]2nd year M pharma, Student of department of pharmaceutical analysis, Bharathi college of pharmacy, Bharathinagara, Mandya, Karnataka, India -571422

[2]Associate professor of Department of pharmaceutical analysis, Bharathi college of pharmacy, Bharathinagara, Mandya, Karnataka, India -571422

[3]Professor and HOD of Department of pharmaceutical analysis, Bharathi college of pharmacy, Bharathinagara, Mandya, Karnataka, India -571422

Abstract:

Simple, precise and accurate area under curve spectroscopic method has been developed and validated for the determination of Ibrutinib in bulk and tablet dosage form. The drug shows maximum absorption (λ max) at 258nm in Acetonitrile solution and Area under Curve [AUC] in absorption spectra were measured between the wavelength range 253 to 263nm which obeys Beer's law in the concentration range of 2-12 µg/ml. The linearity study was carried out and regression coefficient was found to be 0.9999 and it has showed good linearity, precision during this concentration range. The % recovery was found to be 99.61- 99.99. The LOD and LOQ were found to be 0.0396 and 0.12µg/ml. The % relative standard deviation was found to be less than 2. According to ICH guidelines the method has been validated for linearity, precision, accuracy, robustness, ruggedness, LOD and LOQ. The developed and validated method can be successfully applied for routine quantitation of Ibrutinib in bulk and tablet dosage form.

Keywords: *Ibrutinib, Area under curve spectroscopy, validation, pharmaceutical formulations.*

Corresponding author:

Sneha N U,

Department of Pharmaceutical Analysis, Bharathi College of Pharmacy, Bharathi Nagara, Maddur Taluk, Mandya District, Karnataka, India–571422

Mobile: 9611440993

Email Id: *snehagowdanu@gmail.com*

QR CODE

Please cite this article in press Sneha N U et al., Development And Validation Of New Analytical Method For The Determination Of Ibrutinib In Bulk And Tablet Dosage Form. Indo Am. J. P. Sci, 2026; 13(08).

INTRODUCTION:

Ibrutinib belongs to a family of drugs known as kinase inhibitors[1]. Ibrutinib, a United States Food and Drug Administration approved drug, chemically known as 1- [(3R)-3 - [4- Amino-3-(4-phenoxyphenyl)-1H-pyrazolo [3, 4-d] pyrimidin-1-yl] piperidin-1-yl] prop-2-en-1-one is a white to off-white solid powder soluble in polar solvents like acetonitrile, methanol and water. It is irreversible and elective small molecule which is used in the management of patients with chronic lymphocytic leukemia by binding perpetually to a protein, Bruton's tyrosine kinase (BTK), that inhibits B cell antigen receptor (BCR) signaling in human B cells via specific active-site occupancy2It works by stopping the function of the abnormal protein that sends a proliferation signal to cancer cells[1]. It is useful in the treatment of Waldenstrom's macro globulinemia, lymphocytic leukemia and second-line treatment for marginal zone lymphoma, chronic graft versus host disease and mantle cell lymphoma[3].

Fig.1: Chemical structure of Ibrutinib

It has a molecular formula of $C_{25}H_{24}N_6O_2$ and molecular weight of 440.5g/mol. It has the structural formula (Fig.1) [8].

The literature survey reveals that various analytical methods have been developed such as Stability indicating HPLC [13], UV spectroscopy methods [9-11] for the determination of ibrutinib present in combinational pharmaceutical formulations and Comparative purity study of UV& FTIR Techniques [12] for the determination of Ibrutinib tablets.

The aim of present work is to develop and validate a novel, rapid, simple, precise and specific Area under curve Spectrophotometric method for determination of Ibrutinib in bulk and tablet dosage form.

MATERIALS AND METHODS:

Instrument: UV-Visible double beam spectrophotometer, SHIMADZU (model UV-1800) with UV probe software. All weights were taken in analytical balance.

Chemicals: Ibrutinib was given as a gift sample by Pharma industry Bangalore. Tablets of Ibrutinib were procured from local market.

Solvent: Acetonitrile is used as a solvent.

Selection of analytical wavelength: Appropriate dilutions were prepared for drug from the standard stock solution and the solutions were scanned in the wavelength range of 200-400 nm. The absorption spectra obtained was showing the absorption maxima at 258 nm, as the wavelength for detection.

Preparation of standard stock solution: 100mg of Ibrutinib was weighed accurately transferred into 100 ml of volumetric flask and diluted in Acetonitrile up to the mark. From this, the solution was further diluted into 100µg/ml and pipetted out 0.2, 0.4, 0.6, 0.8, 1.0 and
1.2 ml into 10 ml individual volumetric flask and diluted in Acetonitrile up to the mark, this gives 2, 4, 6, 8, 10 and 12µg/ml concentration.

Preparation of sample solution: 20 tablets of Ibrutinib marketed formulations was weighed and powdered. A quantity of tablet powder equivalent to 100mg of Ibrutinib was transferred into 100ml volumetric flask then it was diluted with Acetonitrile and make up to the mark.

METHOD AND VALIDATION:

The method was validated according to the ICH guidelines[15-17].

RESULTS AND DISCUSSION:

Method: Area under curve spectroscopy.

Linearity: The linearity of an analytical method is its dimensions to show the test results that are directly proportional to the concentration of the analyte in the sample within the range. The linearity was established in the range of 2-12µg/ml and Area under Curve [AUC] in absorption spectra were measured between the wavelength of 253 to 263nm as absorbance values are shown in table-1 (Fig-4). The calibration curve was prepared by plotting graph against the concentration and absorbance and therefore the graph

shown in (Fig-4). Statistical variables like slope, intercept, regression equation, correlation coefficient and Sandell's sensitivity were determined. (table-2).

Precision: The precision of an analytical method expresses the closeness of a series of individual analyte measurements obtained from multiple sampling of the equivalent sample. Precision was determined by intra-day and inter-day study. Intra-day precision was determined by analysing the same concentration for six times in a same day. Inter-day precision was determined by analysing the same concentration daily for six days. (table-3).

Accuracy: The accuracy of an analytical method says that closeness of test results obtained by that method to the true value. To assess the accuracy of the developed method, recovery studies were carried out at three different levels as 50%, 100% and 150%. In which the formulation concentration kept constant and varied pure drug concentration. (table-4).

Ruggedness: The ruggedness is defined as the reproducibility of results when the method is performed under the variation in conditions. This includes different analyst, laboratories, instruments, temperature etc. Ruggedness was determined between different analyst; the value of %RSD was found to be less than 2. (table-5).

LOD and LOQ: The limit of detection is an individual analytical method is the smallest amount of analyte in a sample which can be reliably detected by the analytical method. The limit of quantitation is an individual analytical procedure is the smallest amount of analyte in a sample which can be quantitatively determined. LOD and LOQ were calculated by utilizing fallowing formula.
LOD = 3.3(SD)/S and LOQ = 3(LOD)

LOD and LOQ value of were found Ibrutinib be 0.0396 and 0.12µg/ml.

TABLES:

Table 1: Results of calibration curve at 253-263nm by Area under curve method

Sl no	ıtration in µg/ml	Absorbance ± Standard deviation*
1	0	0
2	2	0.113±0.00235
3	4	0.215±0.00211
4	6	0.325±0.00205
5	8	0.440±0.00170
6	10	0.545±0.00197
7	12	0.653± 0.00170

*Average of six determinations.

Table 2: Regression parameter for Ibrutinib at 253-263nm by Area under curve method.

Regression parameter	Results
Range(µg/ml)	2-12
Detection wavelengths(nm)	253-263
Regression Equation	Y= 0.0544x+0.0007
Slope(b)	0.0544
Intercept(a)	0.0007
Correlation coefficient(r^2)	0.9999
Sandell's equation	0.018
Limit of detection(µg/ml)	0.0396
Limit of quantitation(µg/ml)	0.12

Table 3: Determination of precision results for Ibrutinib at 253-263nm by Area under curve method.

centration (µg/ml)	Intra-day Absorbance ±Standard deviation*	%RSD**	Inter-day Absorbance ±Standard deviation*	%RSD**
2	0.113±0.0015	1.15	0.113±0.00095	0.84
4	0.218±0.00129	0.59	0.217±0.00138	0.63
6	0.326±0.00089	0.27	0.327±0.00106	0.32
8	0.439±0.000106	0.24	0.439±0.00068	0.15
10	0.545±0.00137	0.25	0.546±0.00089	0.16
12	0.652±0.00157	0.24	0.653±0.001354	0.20

***Average of six determinations, **percentage relative standard deviation.*

Table 4: Determination of Accuracy results for Ibrutinib at 253-263nm by Area under curve method.

Spiked Levels	mount of Sample (µg/ml)	Amount of Standard (µg/ml)	Amount Recovered	% Recovery ± Standard deviation*	%RSD**
50	6	3	9.001	99.99±0.7338	0.73
100	6	6	11.95	99.61±1.0667	1.07
150	6	9	14.98	99.85±0.4716	0.47

*****Average of six determinations, **percentage relative standard deviation.*

Table 5: Determination of Ruggedness results for Ibrutinib at 253-263nm by Area under curve method.

Analysts	Analyst 1	Analyst 2
Mean absorbance	0.337	0.327
±Standard deviation*	0.00125	0.00134
%RSD	0.366	0.397

*****Average of six determinations, **percentage relative standard deviation.*

Table 6: Determination of LOD and LOQ results for Ibrutinib at 253-263nm by Area under curve method.

Sl. no	Parameters	Values
1	**SD of Intercepts****	0.0007
2	**Average of Slopes****	0.0544
3	**LOD(3.3×SD of Intercepts/average of slopes)**	0.0396
4	**LOQ(10×SD of Intercepts/ average of slopes)**	0.12

**Mean value obtained from six calibration curves.

FIGURES:

Fig.2: Area under curve spectrum of Ibrutinib at 253-263nm.

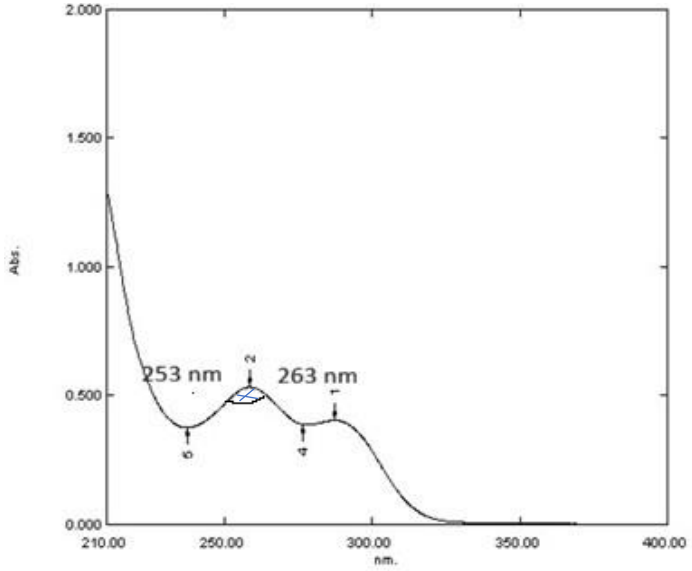


Fig.3: Area under curve overlain spectra of Ibrutinib showing absorbance at 253-263nm.

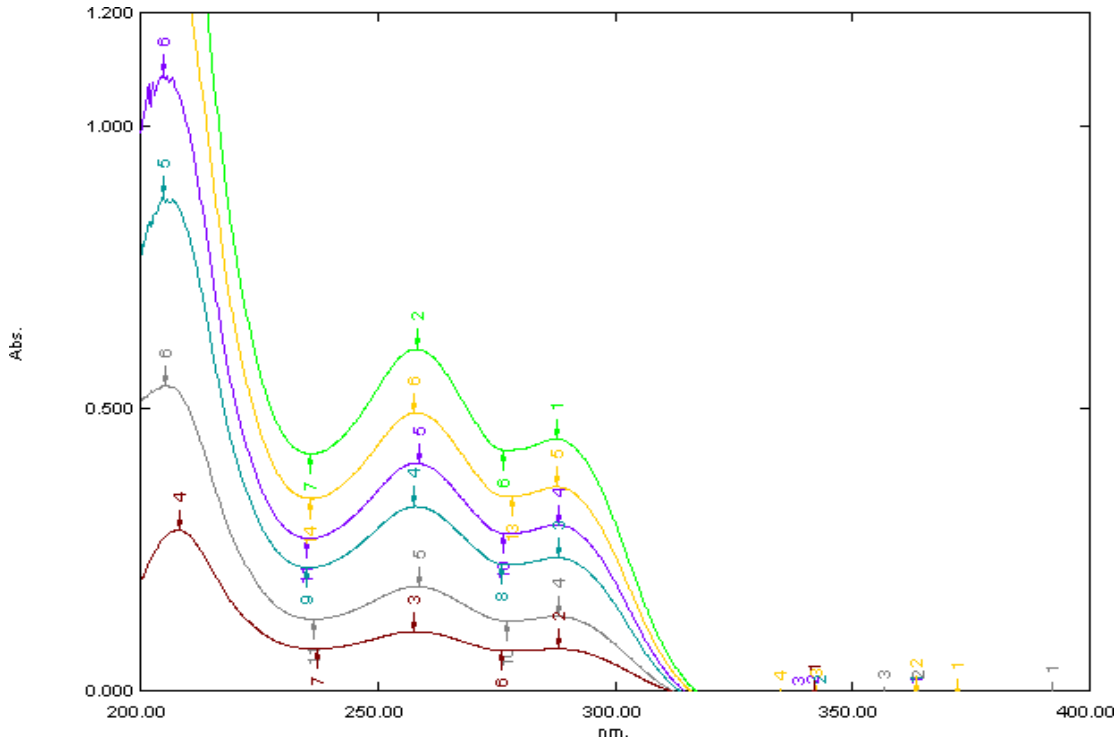
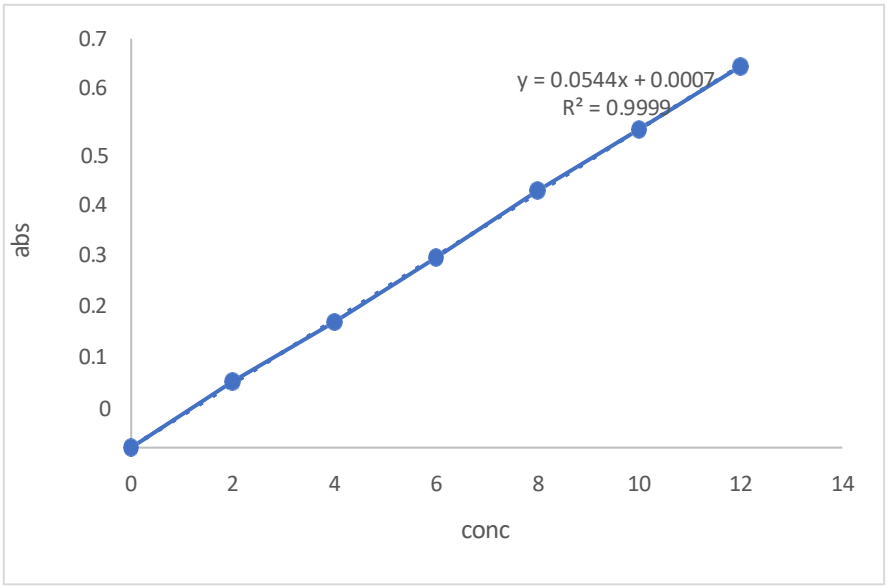


Fig.4: Calibration curve of Ibrutinib at 253-263nm by Area under curve



CONCLUSION:

As per ICH guidelines, the developed analytical method meets the acceptance criteria. It was concluded that the method is simple, specific, accurate, economical, sensitive and can be used for routine analysis of Ibrutinib in bulk drug and in tablet dosage forms.

ACKNOWLEDGEMENT: We authors wish to thank our management, principal of pharmacy college for providing all facilities in the college.

REFERENCES:

1. Akbel E, Güngör S, Bulduk İ. Alternative analytical methods for ibrutinib quantification in pharmaceutical formulation: A statistical comparison. Reviews in Analytical Chemistry. 2022 Jul 20;41(1):146-57.
2. Mehta L, Naved T, Grover P, Bhardwaj M, Mukherjee D. Development and validation of novel and highly sensitive stability-indicating reverse phase ultra performance liquid chromatography method for quantification of Ibrutinib and its ten degradation products. Indian Journal of Pharmaceutical Sciences. 2020 Dec 1;82(6):958-66.
3. Hepsebah NJ, Kumar AA. Bioanalytical method development and validation of ibrutinib in biological matrices by LC-MS/MS. Int J Pharm Pharm Sci. 2019;11:22-6.
4. Sultana S, Havannavar NT, Fathima H. Determination of ibrutinib in dosage form and in bulk drug by UV spectrophotometric and colorimetry methods.
5. Konduru N, Gundla R, Katari NK, Paidikondala K, Reddy AS, Jagadabi V. Development and validation of a stability-indicating method for ibrutinib: Identification and separation of degradation products, known and genotoxic impurities using RP-HPLC/PDA and QDa mass detectors. Analytical Chemistry Letters. 2020 Jan 2;10(1):113-36.
6. Bindu GH, Annapurna MM. 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):4587-91.
7. Prasad SS, Mohan GK, Babu AN. A quality by design approach for development of simple and robust reversed phase stability indicating HPLC method for determination of ibrutinib and its impurities. analytical methods. 2019 Jul;12(3):1434-45.
8. Uppala V, Divya N, Charishma E, Harshavardan K. PHARMACEUTICAL SCIENCES.
9. Validation of stability indicating RP-HPLC method for the assay of ibrutinib in pharmaceutical dosage form.
10. Gopireddy RR, Maruthapillai A, Mahapatra S. A stability indicating method development and validation for separation of process related impurities and characterization of unknown impurities of tyrosine kinase inhibitor ibrutinib using QbD approach by RP-HPLC, NMR spectroscopy and ESI-MS. Journal of chromatographic science. 2021 Oct;59(9):830-46.
11. Illendula S, Sharma S. UPLC method development and validation of acalabrutinib in bulk and pharmaceutical dosage form. Internatiolnal jouirnal of chemical and biochemical Sciences (IJCBS). 2024;25(14):397-411.
12. Mehta L, Naved T, Grover P, Bhardwaj M, Mukherjee D. Development and validation of novel and highly sensitive stability-indicating reverse phase ultra performance liquid chromatography method for quantification of Ibrutinib and its ten degradation products. Indian Journal of Pharmaceutical Sciences. 2020 Dec 1;82(6):958-66.
13. Sun S, Cheng D, Kong S, Li X, Li T, Yu Q, Wang L. A rapid and sensitive method for quantification of ibrutinib in rat plasma by UPLC–ESI–MS/MS: validation and application to pharmacokinetic studies of a novel ibrutinib nanocrystalline. Biomedical Chromatography. 2020 Jan;34(1):e4703.
14. Hepsebah NJ, Kumar AA. Bioanalytical method development and validation of ibrutinib in biological matrices by LC-MS/MS. Int J Pharm Pharm Sci. 2019;11:22-6.
15. ICH, Q2A Text on Validation of Analytical Procedures; 1994.
16. ICH, Q2B Validation of Analytical Methodology; 1996.
17. ICH, Q2 (R1) Validation of Analytical Procedures: text and methodology; 2005.