ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 13, Issue 10, 2020

Online - 2455-3891
Print - 0974-2441

Research Article

STABILITY INDICATING VALIDATED HPLC METHOD FOR THE DETERMINATION OF ZANUBRUTINIB IN BULK AND PHARMACEUTICAL DOSAGE FORM

M. VIJAYA KUMARI[1,2]*, CH. BALASEKHAR REDDY[3]

[1]Department of Pharmaceutical Analysis, Brilliant Group of Technical Institutions, Abdullapurmet, Rangareddy (Dt.), Hyderabad, Telangana, India. [2]Research Scholar, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India. [3]Department of Pharmaceutical Analysis, Vagdevi College of Pharmacy, Gurazala, Andhra Pradesh, India. Email: vijjjmessa22@gmail.com

Received: 04 June 2020, Revised and Accepted: 15 August 2020

ABSTRACT

Objective: An accurate, rapid economical and straight forward, reliable assay technique was evolved and showed for the evaluation of zanubrutinib using reversed-phase high-performance liquid chromatography.

Methods: In the proposed method, efficient chromatographic separation was achieved applying acetonitrile and 0.1% orthophosphoric acid (50:50 v/v) as a mobile phase with a flow of 1 ml/min and the wavelength was observed at 220 nm. Chromatography was administered isocratically at ambient temperature and run time was approximately 6 min and the retention time (Rt) was observed as 4.358 min.

Results: The method was justified as per ICH guidelines. System suitability parameters were studied by injecting the quality six fold and results were well under acceptance criteria. Linearity study was administered between 10% and 150% levels, regression coefficient value was observed as 0.999. Limit of detection and limit of quantification were observed as 0.02 µg/ml and 0.2 µg/ml, respectively. Precision was found to be 0.74 for repeatability and 0.68 for intermediate precision. Recovery of the drug was found to be 98–102%, indicates that the recovery is in the acceptable limit. Validation results were found to be satisfactory and the method applicable for bulk and formulation analysis. Hence, it was evident that the proposed method was said to be suitable for regular analysis and quality control of pharmaceutical preparations.

Conclusion: The validation results were in good agreement with the acceptable limit. Relative standard deviation values which are <2.0% indicating the accuracy and precision of this method. Assay of retail formulation was administered and found to be 100.24% was present using the above method. Stress conditions of degradation in acidic, alkaline, peroxide, and thermal were studied. This developed method showed reliable, precise, accurate results under optimized conditions.

Keywords: Zanubrutinib, Validation, High-performance liquid chromatography, Stress studies.

 DOI: http://dx.doi.org/10.22159/ajpcr.2020.v13i10.38621

INTRODUCTION

The brand name of zanubrutinib was Brukinsa is used to cure adult patients who were suffering from the disease of mantle cell lymphoma (MCL) [1-3] who have received a minimum of one prior therapy. Zanubrutinib is judged as a Bruton's tyrosine kinase (BTK) inhibitor [4-6]. It is taken orally. Efficacy [7,8] was evaluated in BGB-3111-206 (NCT03206970), a phase II clinical trial open-label, multi-center, single-arm trial of 86 patients with MCL who received a minimum of one prior therapy. Zanubrutinib was given orally at 160 mg 2 times daily until disease [9-11] passage or intolerable toxicity [12]. Effectiveness was also estimated in BGB-3111-AU-003 (NCT 02343120), a phase I/II open-label, growth, global, multi-center, single-arm trial of B cell malignancies [13,14], including 32 previously treated MCL patients treated with zanubrutinib administered orally at 160 mg twice daily or 320 mg once daily.

METHODS

Chemicals

Acetonitrile, orthophosphoric acid (OPA), and water (high-performance liquid chromatography [HPLC] grade) were purchased from Merck (India) Ltd., Mumbai, India. API of zanubrutinib (purity-99.9%) as reference standard was procured from Glenmark Pharmaceutical Private Ltd., Andheri (E), Mumbai, India.

Equipment

Chromatographic system of e-2695 with a quaternary pump and a PDA detector of 2996 was used. The chromatographic data were analyzed with Empower Software of version 2.0.

Chromatographic conditions

Using chromatographic conditions, separation was administered in isocratic mode at temperature employing a Luna C_{18} (250 × 4.6 mm, 5 µ) column. The combination of 0.1% OPA and acetonitrile 50:50 v/v with a flow 1 ml/min was used as a mobile phase. The volume of injection was 10 µl and eluent was observed at 220 nm, so this was selected. The spectrum was shown in Fig. 1.

Preparation of standard solution

Weigh 20 mg of zanubrutinib working standard and transferred into a flask volume of 100 ml and diluted to volume with diluents. Further, dilute 5 ml of the prepared solution to 50 ml with diluents.

Preparation of sample solution

Transfer 38.5 mg of zanubrutinib sample into a flask of 100 ml and add 70 ml of diluents, sonicate to dissolve it, and makeup to the mark. Further, dilute 5 ml of the prepared sample solution to 50 ml with diluents.

RESULTS AND DISCUSSION

Method validation

In this method validation parameter [15] (system precision, linearity, precision, accuracy, limit of detection (LOD), limit of quantification (LOQ), robustness, forced degradation, and stability), studies were validated for the chosen drug of Zanubrutinib. Sample and standard chromatograms of the proposed technique were shown in Figs. 2 and 3.

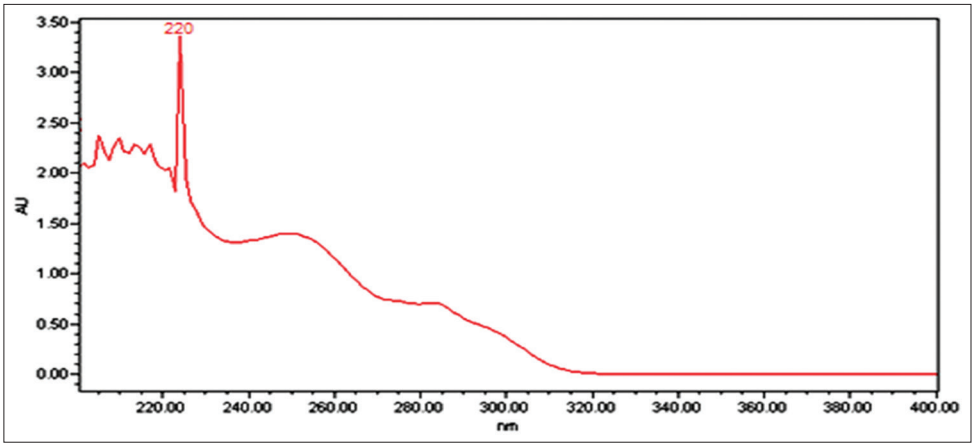

Fig. 1: PDA spectrum of zanubrutinib

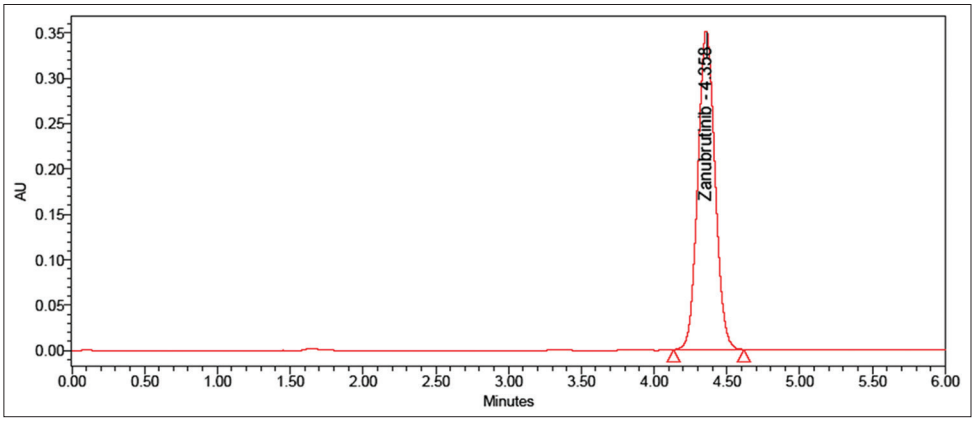

Fig. 2: Chromatogram of standard

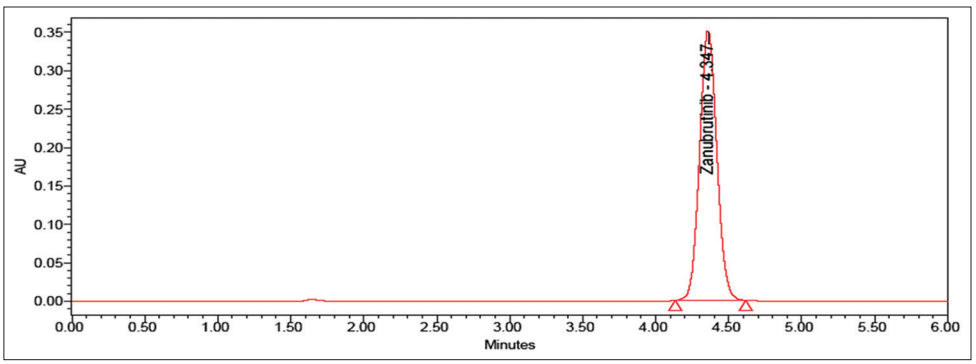

Fig. 3: Chromatogram of sample

System suitability

The HPLC system was stabilized for 60 min to urge a stable bottom line. Six replicate injections of the quality solution containing 20 µg/ml of zanubrutinib were assessed to see the system suitability. The amount of the theoretical plate counts was 6527 and tailing factor was 1.04, respectively. These parameters were found to be within the acceptable limit.

Linearity

Linearity of the tactic was evaluated by preparing a typical solution containing 20 µg/ml of zanubrutinib (100% of the targeted level of the assay concentration). Sequential dilutions were performed to the given solutions at 10%, 25%, 50%, 100%, 125%, and 150% of the target concentrations. These were injected, and therefore, the peak areas are to plot calibration curves against the concentration. The correlation coefficient value of those analytes was 0.999. The results were shown in Table 1 and Fig. 4.

LOD and LOQ

LOD and LOQ minimum concentration level at which the analyte is often reliably detected, quantified using the quality formulas (3.3 σ/s and 10 σ/s for LOD and LOQ, respectively). LOD value of zanubrutinib was 0.02 µg/ml and s/n value is 5. LOQ value of zanubrutinib was 0.2 µg/ml and its s/n value is 26.

Precision

Method precision was investigated by the analysis of six separately prepared samples of an equivalent batch. From these six separate samples, solution was injected, and therefore, the peak responses obtained wont to calculate mean and percentage relative standard

Table 1: Linearity data of zanubrutinib

Analyte	Linearity range	Equation of calibration curve	Correlation coefficient
Zanubrutinib	2–30 µg/ml	Y=142140x+2065	0.9999

Table 2: Intraday precision data of zanubrutinib

Analyte	Amount present	% assay (mean)	% RSD of assay
Zanubrutinib	19.98±0.38	99.7	0.38

Table 3: Results of accuracy study of zanubrutinib

% of target concentration	Zanubrutinib (% recovery)	Zanubrutinib (%RSD)
50	99.9	1.19
100	100.6	0.6
150	99.3	0.33

Table 4: Assay results of zanubrutinib

S. No	Formulation	Label claim	Amount taken	Amount found	% Assay
1	Brukinsa (capsule)	80 mg	20 mg	20.06±0.38	100.24

Table 5: Robustness study of zanubrutinib

Drug name	Flow plus (1.2 ml/min)	Flow minus (0.8 ml/min)	Org plus (55:45)	Org minus (45:55)
	% RSD			
Zanubrutinib	0.38	0.11	0.75	1.56

deviation (RSD) values. This technique was observed to be precise and RSD was 2.0% and also the share assay values were on the brink of 100%. The results were represented in Table 2.

Accuracy

Accuracy decided by recovery studies which were administered in three individual concentration levels (50%, 100%, and 150%). APIs with concentrations 10, 20, and 30 µg/ml were prepared. As per the test method, the test solution was injected to three preparations at each spike level and therefore the assay was performed. The percentage recovery values were observed in between 99.3 and 100.65 of zanubrutinib. The share recovery values were observed as 2%. The results were given in Table 3.

Assay results

Table 4 shows the assay results of zanubrutinib.

Ruggedness

Ruggedness of the tactic was studied and showed that the chromatographic patterns did not significantly change when different HPLC system, analyst, and column. The worth of the percentage of RSD was below 2% exhibits the ruggedness of the developed method.

Robustness

Robustness of the tactic was found to attract RSD should be but 2%. Slightly variations were wiped out the optimized method parameters like flow (±0.2 ml/min), organic content in the mobile phase (±10%). The results were shown in Table 5.

Stability

By observing the stability techniques of Shyamal *et al*. [16], Kalpana *et al*. [17], stability of ordinary and sample solutions is studied from initial to 24 h stored at RT. They were injected at different time intervals and difference between initial to 24 h percentage of the assay was not quite 2%. There is no effect in storage conditions for zanubrutinib drug. The results are shown in Table 6.

Table 6: Stability data of zanubrutinib

Time intervals	Zanubrutinib (% assay)	% Difference
Initial	100.2	0.00
6 h	99.3	-0.9
12 h	99.1	-1.1
18 h	98.5	-1.7
24 h	97.3	-2.89

Table 7: Forced degradation data of zanubrutinib

Degradation	Zanubrutinib (% label claim)	% of degradation
Control	100.1	−0.1
Acid degradation	67	33.1
Alkali degradation	68.6	31.4
Peroxide degradation	66.6	33.4
Reduction degradation	67.1	32.9
Thermal degradation	66.7	33.3
Hydrolysis degradation	66.5	33.5

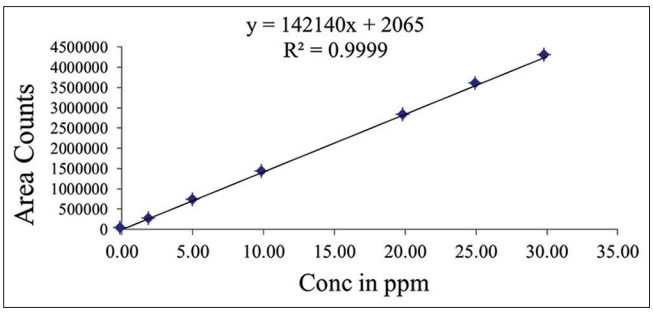

Fig. 4: Calibration curve of zanubrutinib

Forced degradation

Forced degradation conditions containing acidic, basic, peroxide, hydrolysis, reduction, and thermal stress were studied in 0.1 N and 1 N concentration levels (Table 7).

CONCLUSION

This method describes the quantification of zanubrutinib in bulk and pharmaceutical formulation according to ICH guidelines. The evolved technique was observed to be accurate, precise, linear, and reliable. The advantage lies within the simplicity of sample preparation and therefore, the less costly reagents were used. The proposed HPLC conditions ensure sufficient resolution and, therefore, the precise quantification of the compounds. Analysis of the testing results indicates that the precision and reproducibility data are satisfactory. The developed chromatographic technique was often successfully applied for routine analysis in drug research.

ACKNOWLEDGMENT

The authors are thankful to Professor Dr. Ch. Balasekhar Reddy for giving his valuable guidance and suggestions. I heartily thank full to Brilliant Group of Technical Institutions, Abdullapurmet, Rangareddy (Dt.) Hyderabad and Shree Icon Pharmaceutical Laboratories, Vijayawada, India, for providing research facilities for this work.

AUTHORS' CONTRIBUTIONS

Dr. Ch. Balasekhar Reddy has provided the design intellectual content, innovations, protocol for conducting the experiment along with

mentorship, and review the data. Vijaya Kumari has majorly performed the analysis in laboratory, literature collection, and sincerely authored the article.

CONFLICTS OF INTEREST

The authors declare that there were no conflicts of interest.

AUTHORS' FUNDING

The author declares that any funding or research grants are not received in this research work.

REFERENCES

1. Skarbnik AP, Goy AH. Mantle cell lymphoma. State of the art. Clin Adv Hematol Oncol 2015;13:44-55.
2. Ye H, Desai A, Zeng D. Front line treatment for older patients with mantle cell lymphoma. Oncologist 2018;23:1337-48.
3. Ye H, Desai A, Huang S. Paramount therapy for young and fit patients with mantle cell lymphoma. Strategies for front line therapy. J Exp Clin Cancer Res 2018;37:150.
4. Palsingh S, Dammeijer F, Hendriks RW. Role of Bruton's tyrosine kinase in B cell and malignancies. Mol Cancer 2018;17:57.
5. Montalban X, Arnold DL, Weber MS. Placebo controlled trial of an oral BTK inhibitor in multiple sclerosis. N Engl J Med 2019;380:2406-17.
6. Matsushita M, Yamadori T, Kato S, Takemoto Y, Inazawa J, Baba Y, *et al*. Identification and characterization of a novel SH3-domain binding protein, sab, which preferentially associates with Bruton's tyrosine kinase (BtK). Biochem Biophys Res Commun 1998;245:337-43.
7. Burzomato V, Beato M, Groot-Kormelink P, Colquhoun D, Sivilotti LG. Single channel behavior of heteromeric alpha 1 beta glycine receptors. An attempt to detect a conformational change before the channel opens. J Neurosci 2004;24:10924-40.
8. Lape R, Colquhoun D, Sivilotti L. On the nature of partial agonism in the nicotinic receptor super family. Nature 2008;454:722-8.
9. Hanne M, Hawken SJ. Metaphors for illness in contemporary media. Med Humanit 2007;33:93-9.
10. Mcwhinney IR. Health and disease problems of definition. CMAJ 1987;136:815.
11. Jhonson R. The concept of sickness behavior a brief chronological account of four key discoveries. Vet Immunol Immunopathol 2002;87:443-50.
12. Vaibhav D. A simple model to solve complex drug toxicity problem. Toxicol Res 2019;8:157-71.
13. Hong M, Lee T, Kang SY, Kim SJ, Kim W, Ko YH. Nasal type NK-T cell lymphomas are more frequently T rather than NK lineage based on T-cell receptor gene, RNA and protein studies, lineage does not predict clinical behavior. Mod Pathol 2016;29:430-43.
14. Bachanova V, Miller JS. NK cells in therapy of cancer. Crit Rev Oncog 2014;19:133-41.
15. Rao KP, Bhavani P, Mohan S. Stability indicating RP-HPLC method for the estimation of diethyl carbamazine citrate, guaiphenesin and chlorpheniramine maleate. Int J Appl Pharm 2018;10:124-35.
16. Shyamal, Swarupa A, Anitha P. Validated stability indicating RP-HPLC method for determination of asenapine. Indo Am J Pharm Sci 2018;5:4107-13.
17. Kalpana GL, Devalarao G, Raju MB, Kumar TP. Validated stability indicating high performance liquid chromatographic method for the quantification of asenapine. Int J Pham Pham Sci 2015;7:61-5.