International Journal of Pharmaceutical Archive-3(1), 2014, 269-272
Available online through www.ijpaonline.info ISSN 2319-7226

RESEARCH ARTICLE

THE ESTIMATION OF BORTEZOMIB IN POWDER FOR INJECTION DOSAGE FORMS BY RP-HPLC

Dr. VVSS. Appala Raju[1], Dr. Ahmed Bin Mohamud[2], Dr. P. Janaki Pathi*[3] and Dr. N. Appala Raju[4]

1. Department of Chemistry, MAHSA University, Koula lumpur, Malaysia.
2. Dean, Faculty of Pharmacy, MAHSA University, Koula lumpur, Malaysia.
3. Analytical Department, Vishnu Chemicals Limited, Hyderabad, India.
4. Department of Pharmaceutical Chemistry, Sultan-Ul-Uloom College of Pharmacy, Hyderabad-500 034, India.

(Received on: 17-11-13; Revised & Accepted on: 13-01-14)

ABSTRACT

A simple, precise, rapid and accurate reverse phase HPLC method was developed for the estimation of Bortezomib in powder for injection dosage form. An Inertsil ODS-3V analytical column (250 x 4.6 mm, 5 µm partical size) with mobile phase consisting of mixture of buffer 0.1% ortho-phosphoric acid in water and pH adjusted to 6.0 with Triethylamine and acetonitrile in the gradient program was used. The flow rate was 1.0 mL/min and the effluents were monitored at 270 nm. The retention time was 9.7 min. The detector response was linear in the concentration of 2-12 mcg/mL. The respective linear regression equation being y=1389.7x-1384.1. The limit of detection and limit of quantification was 0.005mcg/mL and 0.015mcg/mL respectively. The percentage assay of Bortezomib was 99.4 %. The method was validated by determining its accuracy, precision and linearity.

The results of the study showed that the proposed RP-HPLC method is simple, rapid, precise and accurate, which is useful for the routine determination of Bortezomib in bulk drug and in its pharmaceutical injections dosage form.

Key words: *Bortezomib, RP-HPLC and Injections.*

INTRODUCTION

Bortezomib is the first therapeutic proteasome inhibitor to be tested in humans. It is approved in the U.S. for treating relapsed multiple myeloma[1] and mantle cell lymphoma. Bortezomib for Injection is an antineoplastic agent available for intravenous injection or subcutaneous use. Bortezomib is a modified dipeptidyl boronic acid. The product is provided as a mannitol boronic ester which, in reconstituted form, consists of the mannitol ester in equilibrium with its hydrolysis product, the monomeric boronic acid. The drug substance exists in its cyclic anhydride form as a trimeric boroxine. Chemically[2], Bortezomib is: [(1*R*)-3-methyl-1-({(2*S*)-3-phenyl-2-[(pyrazin-2-ylcarbonyl) amino] propanoyl} amino)butyl]boronic acid. The empirical formula is $C_{19}H_{25}BN_4O_4$, with a molecular weight of 384.22 g/mol. The solubility of bortezomib, as the monomeric boronic acid, in water is 3.3 to 3.8 mg/mL in a pH range of 2 to 6.5 Literature survey reveals a few chromatographic methods have appeared in the literature for the quantification of bortezomib in using SPE–LC-MS/MS for bortezomib and its hydrolyzed metabolite in human urine and development and validation of a liquid chromatography–tandem mass spectrometric assay for Bortezomib and its hydrolyzed metabolite in human plasma[3-6] chromatographic methods for the estimation of Bortezomib from pharmaceutical dosage forms. The availability of an HPLC method with high sensitivity and selectivity will be very useful for the determination of Bortezomib in pharmaceutical formulations. The method was validated by determining its accuracy, precision and linearity as per ICH guidelines[7].

Corresponding author: P. Janaki Pathi[2]*
[2]Analytical Department, Vishnu Chemicals Limited, Hyderabad, India
E-mail: pjp02002@yahoo.com, Mobile No: +919000955407

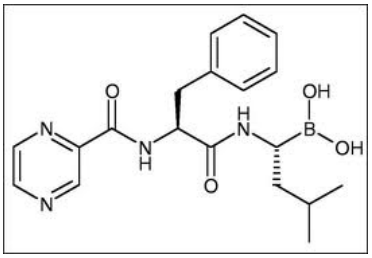


Fig 1: Structure of Bortezomib

EXPERIMENTAL

Materials and Methods

Bortezomib was obtained as a gift sample from M/s. Vishnu Chemicals Ltd, Hyderabad. Acetonitrile, *ortho*-phosphoric acid, Triethylamine and water used were of HPLC grade (Qualigens). Commercially available Bortezomib Injections 2.0mg (Bortecad® Cadila Pharma, India) were procured from local market.

Instrument

Quantitative HPLC was performed on liquid chromatograph, Agilent 1200 series system with equipped with Diode Array Detector and automatic injector with injection volume 20 µl. The HPLC data was analyzed with Chemstation Software.

HPLC Conditions

The contents of the mobile phase were mixture of buffer 0.1% *ortho*-phosphoric acid in water and pH adjusted to 6.0 with Triethylamine and acetonitrile in the gradient program was used (shown in table-IV). They were filtered before use through a 0.45 µm membrane filter, and pumped from the respective solvent reservoirs to the column at a flow rate of 1.0 mL/min. The run time was set at 20.0 min and the column temperature was ambient. Prior to the injection of the drug solution, the column was equilibrated for at least 20 min with the mobile phase flowing through the system. The eluents were monitored at 270 nm.

Preparation of Standard Stock solution

A standard stock solution of the drug was prepared by dissolving 10 mg of Bortezomib in 100 mL volumetric flask and dissolved in diluent (Acetonitrile and Water:50:50), sonicated for about 15 min and then made up to 100 mL with diluent get 100 mcg/mL standard stock solution.

Working Standard solution

1.0 mL of the above stock solution was taken with micropipette in 10 mL volumetric flask and thereafter made up to 10 mL with diluent (Acetonitrile and Water: 50:50) to get a concentration of 10mcg/mL.

Preparation of Sample solution

Five vials of powder for injection contains Bortezomib 2.0mg (Bortecad ® Cadila Pharma, India) were taken and weighed. A sample of the powdered injections, equivalent to 10mg of the active ingredient, was mixed with 30 mL of diluent in 100 mL volumetric flask. The mixture was allowed to stand for 15 min with intermittent sonication to ensure complete solubility of the drug, and then filtered through a 0.45 µm membrane filter, followed by adding diluent up to 100 mL to obtain a stock solution of 100mcg/mL. 1 mL of the above solution was taken and further diluted with diluent up to 10 mL to get working sample solution of 10 mcg/mL.

Linearity

Aliquots of standard Bortezomib stock solution were taken in different 10 mL volumetric flasks and diluted up to the mark with the diluent such that the final concentrations of Bortezomib are in the range of 2-12 µg/mL. Each of these drug solutions (20 µL) was injected three times into the column, and the peak areas and retention times were recorded. Evaluation was performed with Diode Array detector at 270 nm and a Calibration graph was obtained by plotting peak area versus concentration of Bortezomib (Fig 3).

The plot of peak area of each sample against respective concentration of Bortezomib was found to be linear in the range of 2–12 mcg/mL with correlation coefficient of 0.9999. Linear regression least square fit data obtained from the measurements are given in table I. The respective linear regression equation being y=1389.7x-1384.1. The regression characteristics, such as slope, intercept, and %RSD were calculated for this method and given in table I.

Assay

20 µL of sample solution was injected into the injector of liquid chromatograph. The retention time was found to be 9.7 minutes. The amount of drug present per vial containing powder for injection was calculated by comparing the peak area of the sample solution with that of the standard solution. The data are presented in table II.

Recovery Studies

Accuracy was determined by recovery studies of Bortezomib, known amount of standard was added to the preanalysed sample and subjected to the proposed HPLC analysis. Results of recovery study are shown in table II. The study was done at three different concentration levels.

Results and Discussion

The system suitability tests were carried out on freshly prepared standard stock solution of Bortezomib. The parameters studied to evaluate the suitability of the system are given in table III**.**

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) for Bortezomib were found to be 0.005 mcg/mL and 0.015 mcg/mL respectively. The signal to noise ratio is 3 for LOD and 10 for LOQ. From the typical chromatogram of Bortezomib as shown in fig 2, it was found that the retention time was 9.7 min. A mixture of buffer 0.1% *ortho*-phosphoric acid in water and pH adjusted to 6.0 with Triethylamine and acetonitrile in the gradient program was used (shown in table-IV) was found to be most suitable to obtain a peak well defined and free from tailing. In the present developed HPLC method, the standard and sample preparation required less time and no tedious extraction were involved. A good linear relationship (r^2=0.9999) was observed between the concentration range of 2-12 mcg/mL. Low values of standard deviation are indicative of the high precision of the method. The assay of Bortezomib powder for injections was found to be 99.4%. From the recovery studies it was found that about 99.2% of Bortezomib was recovered which indicates high accuracy of the method. The absence of additional peaks in the chromatogram indicates non-interference of the common excipients used in the injections. This demonstrates that the developed HPLC method is simple, linear, accurate, sensitive and reproducible. Thus, the developed method can be easily used for the routine quality control of parental dosage forms of Bortezomib within a short analysis time.

ACKNOWLEDGEMENTS

The authors are grateful to M/s Vishnu chemicals Limited, Hyderabad for the supply of as a gift sample Bortezomib and to the Management, Vishnu Chemicals Limited, Hyderabad, for providing the necessary facilities to carry out the research work.

Table I: Linear Regression Data for Calibration curves

Drug	Bortezomib
Concentration range (mcg/mL)	2-12
Slope (m)	1389.7
Intercept (b)	-1384.1
Correlation coefficient	0.9999
% RSD	0.32

Table II: Results of HPLC Assay and Recovery studies

Sample	Amount claim (mg/Injections)	% found by the proposed method	% Recovery*
1.	2	99.53	99.17
2.	2	99.45	99.15
3.	2	99.22	99.28

*Average of three different concentration levels.

Table III: Validation Summary

Validation Parameter	Results
System Suitability	
Theoretical Plates (N)	17542
Tailing factor	1.14
Retention time in minutes	9.7
% Area	99.89
LOD (mcg/mL)	0.005
LOQ (mcg/mL)	0.015

Table IV: Gradient Program in HPLC method

Time in mins	Buffer	Acetonotrile
0.01	80	20
3	80	20
5	20	80
15	20	80
18	80	20
20	80	20

Fig 2: Typical Chromatogram of Bortezomib by HPLC

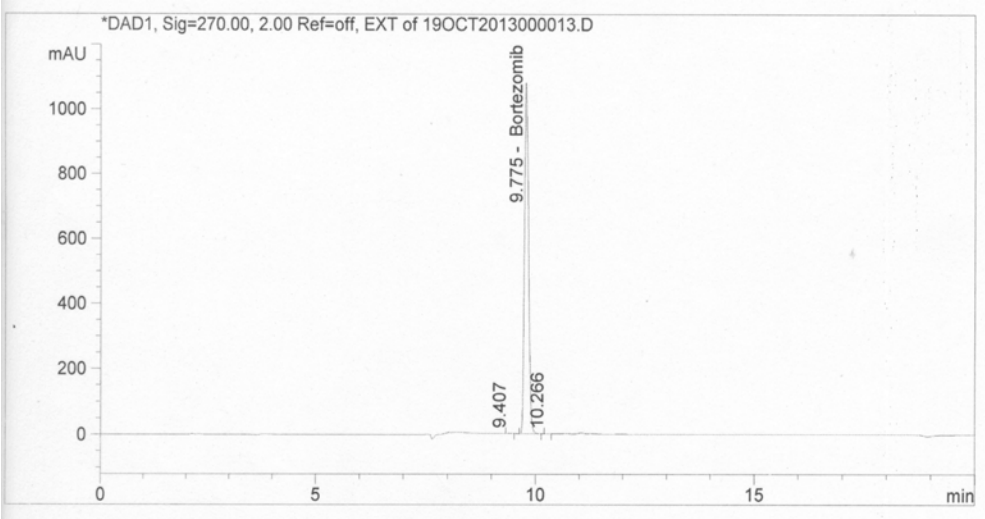
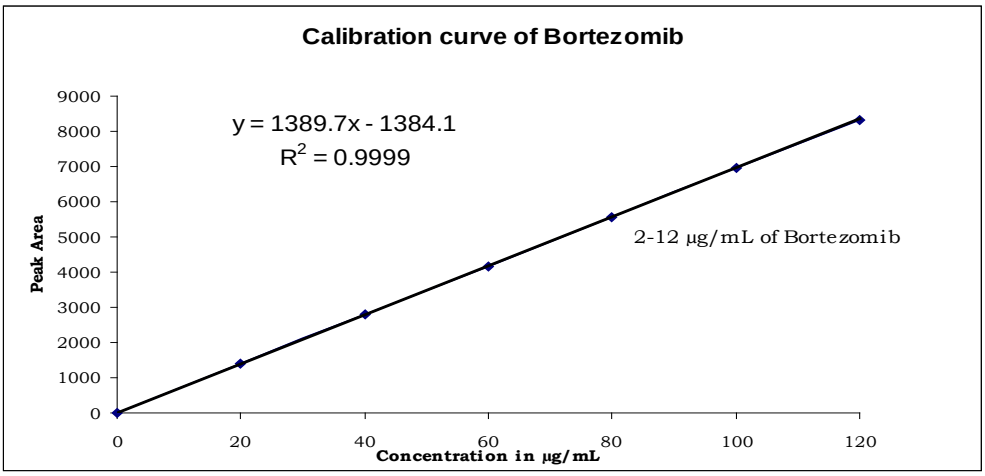


Fig 3: Calibration curve of the Bortezomib by RP-HPLC.



REFERENCES

1. Takimoto CH, Calvo E. "Principles of Oncologic Pharmacotherapy" in Pazdur R, Wagman LD, Camphausen KA, Hoskins WJ (Eds) Cancer Management: A Multidisciplinary Approach. 11 ed. 2008.
2. Adams J, Kauffman M: Development of the proteasome inhibitor Velcade (Bortezomib). Cancer Invest. 2004; 22(2):304-11.
3. Ji Y Zhang, Douglas M Fast, Alan P Breau (2003) Journal of Chromatography B 787: 333-344.
4. Rane VP, Patil kr, Sangshetti NJ, Yeole RD, Shinde DB (2009) Stability-indicating RP-HPLC method for analysis of bortezomib in the bulk drug and in a pharmaceutical dosage form 21: 619-629.
5. Venkataramanna M, Sudhakar Babu K, Anwar SKC (2012) A Validated Stability-Indicating UF LC Method for Bortezomib in the Presence of Degradation Products and its Process-Related Impurities. J Chromatograph Separat Techniq 3:117. doi:10.4172/2157-7064.100011.
6. Bakshi Singh MS (2002) Development of validated stability indicating assay methods-critical Review. J Pharm Biomed Anal 28: 1011-1040.
7. ICH Guidelines on validation of analytical Procedures definitions and terminology.

Source of support: Nil, Conflict of interest: None Declared

