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RESEARCH ARTICLE

Analytical Method Development and Validation of Related Substance Method for Bortezomib for Injection 3.5 mg/Vial by RP-HPLC Method

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ABSTRACT

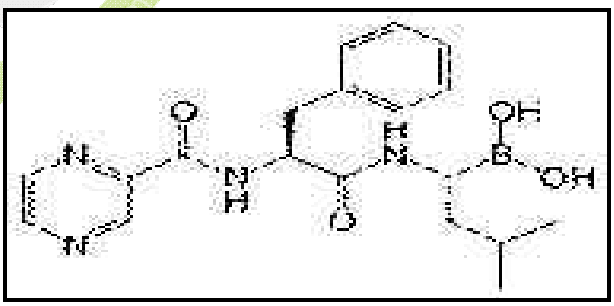
An accurate, precise, simple and economical High Performance Liquid Chromatographic method for the related substance determination of Bortezomib in its lyophilized dosage form has been developed. The method developed is Reverse Phase High Performance Liquid Chromatographic method using Hypersil BDS C18 column (Length: 150mm, Diameter: 4.6mm, Particle size: 5µ) with Gradient programmed and a simple Acetonitrile, Water and Formic acid in the ratio of 30:70:0.1 (v/v/v) respectively as mobile phase A and Acetonitrile, Water and Formic acid in the ratio of 80:20:0.1 (v/v/v) respectively. The method so developed was validated in compliance with the regulatory guidelines by using well developed analytical method validation tool which comprises with the analytical method validation parameters like Linearity, Accuracy, Method precision, Specificity with forced degradation, System suitability, Robustness, LOD, LOQ and Ruggedness. The results obtained were well within the acceptance criteria.

KEYWORDS

Bortezomib, HPLC, Hypersil BDS.

INTRODUCTION

Bortezomib, an anti-neoplastic agent is used for the treatment of multiple myeloma and mantle cell lymphoma. The chemical name of Bortezomib is [(1R)-3-methyl-1-[[(2S)-1oxo-3-phenyl-2-[(pyrazinylcarbonyl) amino] propyl]amino]butyl] boronic acid and its structure is shown in figure -1. It is a monomeric boronic acid analogue having a molecular weight of 384.237 g/mol and molecular formula of C19H25BN4O4. A Publication is available for Bortezomib and is on estimation of Bortezomib in bulk and its pharmaceutical dosage form by using a novel validated accurate reverse phase high performance liquid chromatography is reported but none have employed a method to determine Bortezomib in Injection form by RP-HPLC. In the present work, attempts were made to determine Impurities in Bortezomib Injection form by using RP-HPLC. Bortezomib was approved for the treatment of patients with relapsed or refractory multiple myeloma in May 2003 by the US food and drugs Administration



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and in April 2004 by the Committee for the treatment of mantle cell lymphoma.6-8.

MATERIALS AND METHODS

Chemicals and Reagents

Bortezomib reference material was procured from a reputed reference material supplier in India. A commercial local vial formulation was used in this study. Formic acid, Acetonitrile HPLC grade purchased from Merck chemicals.

Instrumentation

An Agilent HPLC separation module 1200series equipped with DAD UV-Vis detector was used for all the experiments. Data acquisition was performed by EZ-Chrome software. Analysis was carried out at 270nm with a Hypersil BDS C18 column (150x4.6mm, 5µ) at 30°C temperature. The mobile phase was used is in the gradient programme. The flow rate was 1.2ml/min and the retention time was about 10.5 min. The mobile phase was degassed and filtered through 0.45µm membrane filter before pumping into the HPLC system.

Preparation of Solutions

Preparation of Mobile Phase

The Mobile phase A- Mixture of Acetonitrile, Water and Formic acid in the ratio of 30:70:0.1 (v/v/v) respectively. Mobile phase B- Mixture of Acetonitrile, Water and Formic acid in the ratio of 80:20:0.1 (v/v/v) respectively. Diluent used is mobile phase-A. Gradient programme used is 0-15[100/0], 15-45[0/100],47-50[100/0].

Preparation of Standard Solution

Standard solution of Bortezomib was prepared for to obtain a concentration of 1µg/mL by dissolving in mobile phase A. The solution was sonicated for about 2 min for complete dissolution of the standard.

Preparation of Sample Solution

The bortezomib marked formulation of 3.5 mg/vila is used and prepared a solution of 1000 µg/mL.

Experimental Procedure for Method Validation

The method was validated according to International Conference on Harmonization (ICH) guidelines for validation of analytical procedures2-3.

Linearity

A series of standard curves were prepared over a concentration range of LOQ to 300 % by diluting the standard solution of Bortezomib and its impurities in mobile phase. The data from peak area verses drug concentration plots were treated by linear Curve regression analysis. Bortezomib and impurities are linear between LOQ to 300% level. The correlation & regression coefficients are more than 0.995.In addition, the analysis of residual shown that the values are randomly scattered around zero, the P-value was determined.

LOD and LOQ

Theoretical LOD and LOQ Concentrations of bortezomib and its impurities are calculated from the linearity curve and freshly prepared solution are injected. The precision at LOQ for bortezomib and impurities are calculated are well within the 5 %. The LOQ is found to be NMT 0.05%

Accuracy

Accuracy was performed by injecting the sample in mg added verses mg recovered, from LOQ to 300% to the sample concentration. The experiment was performed in triplicate % recovery, mean % recovery, RSD (%) were calculated for each concentration.

Precision of the Method

Precision was measured in accordance with ICH recommendations. The precision study was carried out by injecting sample preparation of 1000µg/ml concentration six times. The % RSD for Retention time and area response are calculated and are well within 1.0 % and 5.0 % respectively.

System Suitability

System suitability was assessed by replicate analysis of 6 injections of the Bortezomib standard solution at a concentration of 1.0µg/ml and the chromatogram was obtained. The system suitability parameters such as tailing factor, theoretical plate count and reproducibility (%RSD) of analyte retention time and area of the six replicates calculated from the chromatogram.

Specificity

The analyte was subjected to forced degradation studies using photolytic, peroxide, thermal, acid and alkali treatments for demonstration of specificity of the method. Bortezomib was analyzed under these conditions for purity, indicating that the developed HPLC method effectively separated the degradation products from the Bortezomib standard peak. It is found that the bortezomib and its impurities are well separated from each other. There is no any interference of any other peak with the peak of interest. The peak purities found to be 1.0

Robustness

The different variations are in flow rates by ±0.2ml/min, in mobile phase composition ±5ml, and column temperature from developed HPLC conditions. The concentration of the solution analyzed was 1µg/ml for standard and 1000µg/mL for sample. It is found that the method is robust. The % RSD is well within 5.0 %.

Ruggedness

The ruggedness of the method was demonstrated by analysis of the sample as for precision study by a second analyst.

RESULTS AND DISCUSSION

Method Development and Optimization

Bortezomib was analyzed by using different solvents and by changing the ratio of their composition. In all these cases Bortezomib was analyzed using column (Hypersil BDS C18 column (150x4.6mm, 5µ)). Various buffer strengths, flow rates, mobile phase compositions with gradient elution were examined. All the experiments were monitored using UV detector at a wavelength of 270nm. Optimum mobile phase gradient ratios for the analysis was found to be good with a flow rate of 1.2ml/min. Best separation, good peak shape was observed.

Method Validation

Linearity

The calibration curve constructed was evaluated by using correlation coefficient. The peak area of the drug was linear in the range of LOQ to 300%. The area for each of the concentration obtained was plotted against the concentration of the analyte. The correlation coefficient (R2) is 0.995.

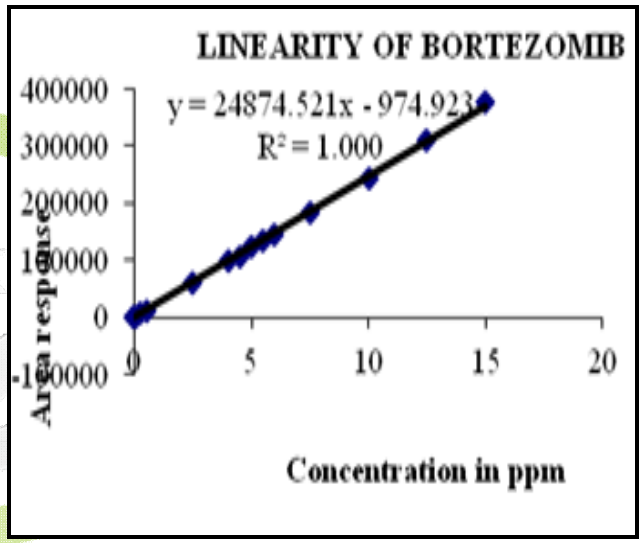

Figure 1: Graph for linearity

Accuracy

Accuracy of the method was expressed in terms of recovery of added compound. Percentage recovery was calculated by multiplying the ratio of the measured concentration with 100. Mean % recovery and %RSD were calculated and were found to be within 85 to 115% respectively. It can be obtained from table-1 that the developed HPLC method is accurate.

Precision

The precision of the method was calculated from the Retention time and area reproducibility of the area of standard solution and % RSD of six Bortezomib standard samples. The results are given in table-2.

Table 1: Results of Recovery Experiments

Individual and mean% recovery for Boretzomib & Imp.	Imp-A	LOQ%	50%	100%	200%	300%
	Imp-B	100	98	95	99	101
	Imp-C	100	99	98	100	98
	Imp-G	100	97	97	102	99
	Imp-H	100	95	96	98	96
	Bortezomib	100	98	97	96	97

Table 2: Precision Results

A	**B**	**C**	**G**	**H**	**UN**	**Total**
0.99	1.06	1.04	0.94	1.04	0.03	5.10
1.00	1.07	1.04	0.95	1.04	0.03	5.13
1.00	1.08	1.04	0.96	1.04	0.03	5.15
0.99	1.07	1.03	0.97	1.03	0.03	5.12
1.00	1.05	1.04	0.94	1.05	0.03	5.11
1.02	1.06	1.04	0.98	1.04	0.03	5.17
1.00	**1.07**	**1.04**	**0.96**	**1.04**	**0.03**	**5.13**
1.1	**1.0**	**0.4**	**1.7**	**0.6**	**0.0**	**0.5**

Table 3: Robustness Results

Sr.No	**Parameter**	**% RSD of Bortezomib**	**Therotical plates.**
1	Increase flow	1.1	2089
2	Decrease flow	1.5	2569
3	Increase Temp	1.3	3256
4	Decrease Temp	3.2	5628
5	Increase organic phase	2.2	2855
6	Decrease flow	1.9	3265

System Suitability

The %RSD of the peak area and retention time of Bortezomib were within 2%. The efficiency of column is expressed by the number of theoretical plates for six replicate injections were found to be 2538 and the tailing factor was 1.58. Results are given in

Specificity

Accelerated degradation studies under different conditions viz., acid treatment; base treatment, peroxide, thermal were conducted to demonstrate the specificity. The Sample was found to be degraded in acid, alkali, and peroxide stressed conditions. However, unknown impurities are well separated from Bortezomib peak and impurities. The Bortezomib peaks are pure. Hence, the Related substances method was considered specific & stability indicating.

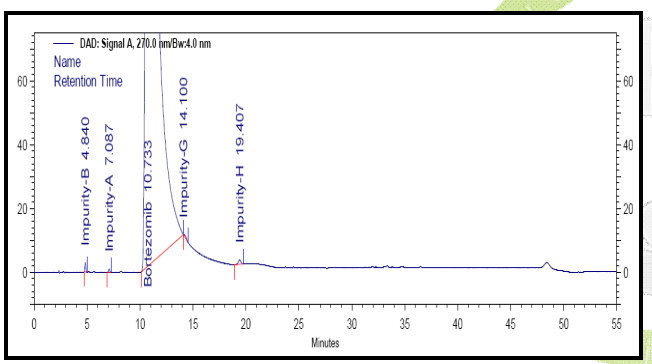

Figure 2**:** Specificity Chromatogram

Robustness

Robustness was performed by small variations in chromatographic conditions like volume of the mobile phase Composition, flow rate, and column temperature.

Ruggedness

The results were well within acceptable limits these results indicate that the developed HPLC method was rugged.

CONCLUSION

A rapid and accurate RP-HPLC method was developed for the determination Related substances of Bortezomib for injection in vial dosage form. The method was evaluated for specificity, linearity, accuracy, precision, ruggedness and robustness as per ICH guidelines and proved to be economical and effective for the quality control of the drug in the given application.

Table 4: Ruggedness results

A	B	C	G	H	UN	Total
1.02	1.23	1.02	1.08	1.31	0.03	5.69
0.99	1.18	0.99	1.03	1.29	0.03	5.51
0.99	1.18	1.00	1.03	1.31	0.03	5.54
1.02	1.23	1.03	1.06	1.32	0.03	5.69
0.99	1.18	1.00	1.01	1.31	0.03	5.52
1.02	1.23	1.03	1.05	1.33	0.03	5.69
1.00	**1.14**	**1.03**	**1.00**	**1.18**	**0.03**	**5.37**
1.4	**6.6**	**1.8**	**5.0**	**12.1**	**0.0**	**4.8**

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