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RESEARCH ARTICLE

Stability Indicating HPLC Method for Estimation of Bortezomib for Injection 3.5 mg/Vial

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ABSTRACT

An accurate, precise, simple and economical High Performance Liquid Chromatographic method for the Estimation of Bortezomib in its lyophilized dosage form has been developed. The method developed is Reverse Phase High Performance Liquid Chromatographic method using Hypersil BDS C_{18} column (Length: 150 mm, Diameter: 4.6mm, Particle size: 5µ) with a simple 0.1 % TFA buffer and Acetonitrile mixed in the proportion of 20:80v/v as a mobile phase, and Methanol: Water (90:10) as a diluent. 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 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 $C_{19}H_{25}BN_4O_4$.

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

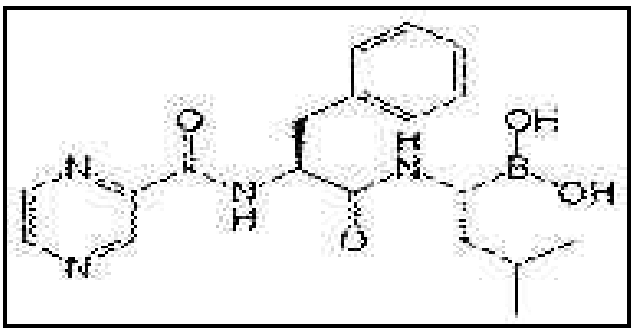

Figure 1: Structure Bortezomib

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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. Tri-fluro acetic acid, Acetonitrile and Methanol HPLC grade purchased from Merck chemicals.

Instrumentation

A 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 C_{18} column (150x4.6mm, 5µ) at 35°C temperature and autosampler Temperature at 15°C. The mobile phase was used is in the isocratic programme. The flow rate was 1.0ml/min and the retention time was about 3.5 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 was prepared by mixing 0.1 % TFA buffer and Acetonitrile in the ratio of 20:80. The mobile phase is then sonicated by using a Ultra-sonicator fore to remove the dissolved gases. Diluent used is methanol and water in the ratio of 90:10.

Preparation of Standard Solution

Weighed and transferred 6.0 mg of Bortezomib standard into 10 mL volumetric flask, dissolved and dilute to volume with dluent. And further diluted 2.0mL of this solution to 10.0 mL with diluent and mixed well.

Preparation of Sample Solution

Reconstitute 7 sample vials with 5.0 mL of diluent to each vial, Pooled together into 100 mL volumetric flask and rinsed the vials with diluent and mixed well. Further diluted 5.0 mL of this solution to 10.0mL with diluent and mixed well.

Experimental Procedure for Method Validation

The method was validated according to International Conference on Harmonization (ICH) guidelines for validation of analytical procedures[2-3].

Linearity

A series of standard curves were prepared over a concentration range of 50 to 150 % by diluting the standard solution of Bortezomib in the diluent. The data from peak area verses drug concentration plots were treated by linear Curve regression analysis. 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.

Accuracy

Spiked known quantity of Bortezomib standard at 50%, 80%, 100%, 120% and 150% of Assay specification limits into the placebo.

Performed precision at the lowest and the highest levels and for the other levels prepared in triplicate and injected in duplicate Calculated the % recovery from the results of Accuracy.

Precision of the Method

Precision was measured in accordance with ICH recommendations. The precision study was carried out by injecting sample preparation of 120µg/ml concentration six times separately. The % RSD for Retention time and area response was calculated and is well within 1.0 % and 2.0 % respectively.

System Suitability

System suitability was assessed by replicate analysis of 5 injections of the Bortezomib standard solution at a concentration of 120µg/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 five 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 organic phase composition ±2.0%, and column temperature by ±5°C from developed HPLC conditions. The concentration of the solution analyzed was 120.0µg/ml for standard and 120.0µg/mL for sample. It is found that the method is robust. The % RSD is well within 2.0 %.

Ruggedness

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

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 C_{18} 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 ratios for the analysis was found to be good with a flow rate of 1.0ml/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 50 to 150%. The area for each of the concentration obtained was plotted against the concentration of the analyte. The correlation coefficient (R2) was more than 0.995.

Linearity Stock Preparation

Weighed 37.210 mg of Bortezomib Standard into 50 mL volumetric flask, dissolved and diluted to volume with diluent and mixed well. Form that further dilutions are made as shown in table 1.

Table 1: Linearity Concentration

Level	Linearity Stock solution added (in mL)	Total volume made up with diluent(in mL)
1	1.0	10
2	1.2	10
3	1.4	10
4	1.6	10
5	1.8	10
6	2.0	10
7	2.2	10
8	2.4	10
9	2.8	10
10	3.0	10

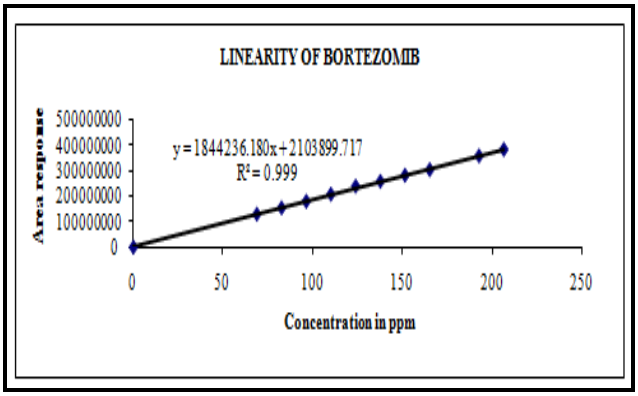

Figure 2: Graph for linearity

Accuracy

Accuracy of the method was expressed in terms of recovery of added compound. Percentage recovery was calculated by multiplying the ratiothe measured concentration with 100. Mean % recovery and %RSD were calculated and were found to be within 98 to 102% respectively. It can be obtained from table-2 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 five Bortezomib standard. The results are given in table-3.

System Suitability

The %RSD of the peak area and retention time of Bortezomib were within 1.0% and 2.0%. The efficiency of column is expressed by the number of theoretical plates for five replicate injections were found to be more than 5400 and the tailing factor was 1.0. Results are given in table-3

Specificity

Accelerated degradation studies under different conditions viz., acid treatment; base treatment, peroxide, thermal were conducted to demonstrate the specificity. The Sample is found to be degrading in acid, alkali, peroxide and UV light stressed conditions but slightly degraded in neutral, sun light and thermal stressed condition. However, unknown impurities are well separated from Bortezomib peak and impurities. The Bortezomib peaks are pure. Hence, the Assay method is considered specific & stability indicating.

Table 2: Results of recovery experiments

% Level (about)	Sample	Mean Area Response	*mg Added	*mg Recovered	%Recovery	Mean % Recovery	%RSD
50	1	125333623	0.0688	0.0695	101.0	**100.8**	**1.2**
	2	125478868	0.0688	0.0695	101.0		
	3	126048215	0.0688	0.0699	101.6		
	4	125717432	0.0688	0.0697	101.3		
	5	122168163	0.0688	0.0677	98.4		
	6	126265310	0.0688	0.0700	101.7		
80	7	196107502	0.1101	0.1087	98.7	**99.6**	**0.9**
	8	199301041	0.1101	0.1105	100.4		
	9	198178430	0.1101	0.1098	99.7		
100	10	248092823	0.1376	0.1375	99.9	**99.4**	**0.5**
	11	245839332	0.1376	0.1362	99.0		
	12	246498872	0.1376	0.1366	99.3		
120	13	299117066	0.1652	0.1658	100.4	**100.3**	**1.3**
	14	302921065	0.1652	0.1679	101.6		
	15	295256084	0.1652	0.1636	99.0		
150	16	365164355	0.2064	0.2024	98.1	**100.2**	**1.5**
	17	376822409	0.2064	0.2088	101.2		
	18	377104359	0.2064	0.2090	101.3		
	19	374402257	0.2064	0.2075	100.5		
	20	377422166	0.2064	0.2092	101.4		
	21	366483434	0.2064	0.2031	98.4		
%Mean					**100.0**		
% RSD for 21 levels					**1.2**		

Table 3: Precision results system precision

Injection No.	Bortezomib	
	Retention Time in	Area
1	3.530	2036054
2	3.531	2035470
3	3.531	2036022
4	3.531	2035322
5	3.532	2035651
6	3.530	2034872
Mean	**3.531**	**2035565**
% RSD	**0.0**	**0.0**

Table 4: Precision results Method precision

% Assay of Bortezomib	
Sample	**% Assay**
1	99.5
2	100.2
3	100.0
4	99.1
5	99.3
6	99.7
Mean	99.6
% RSD	**0.4**

Table 6: Results of Robustness

System suitability Parameter		% RSD for Area	Theoretical plates
Limit		**NMT 2.0**	**NLT 1500**
Original conditions		0.0	5479
Flow rate	1.2 mL/min	0.1	4798
	0.8 mL/min	0.0	5967
Column temperature	40°C	0.1	5532
	30°C	0.0	5210
Organic Phase	+2%	0.1	5244
	-2%	0.1	5323

Table 5: Results of Specificity by forced degradation

Stressed Condition	Bortezomib (in Assay %)	Bortezomib (% of Degradation)	*Peak purity	Peak purity factor of Bortezomib
Sample As such	99.1	-	P	1.000
Alkali	78.5	20.8	P	1.000
Acid	88.9	10.3	P	1.000
Peroxide	80.5	18.2	P	1.000
Neutral	94.9	3.6	P	1.000
Sun light	97.8	1.3	P	1.000
UV-light	91.7	7.5	P	1.000
Thermal	98.1	1.0	P	1.000

Ruggedness

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

Table 7: Ruggedness results

% Assay of Bortezomib	
Sample	**% Assay**
1	100.4
2	100.3
3	100.1
4	100.2
5	99.7
6	100.0
Mean	100.1
% RSD	0.2

CONCLUSION

A rapid and accurate RP-HPLC method was developed for the Estimation 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.

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