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RESEARCH ARTICLE

A Novel Gradient RP-HPLC Method Development for Bortezomib in Parenteral Dosage Form

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ABSTRACT

The objective of present study was the development of gradient RP-HPLC method for the estimation of bortezomib in parenteral dosage form. The analysis was performed on Hypersil C_{18} column (250 x 4.6mm) with mobile phase composed of acetonitrile: water: THF (65:35:5), maintaining flow rate at 1ml/min. with retention time of 3.5±0.5min. The detection was done at 280nm. A regression coefficient was 0.999 obeying the Beer's law at concentration range of 20 – 120 µg/ml. The proposed method was validated for specificity, linearity, accuracy, precision and robustness as per ICH guidelines and used successfully for bortezomib determination in routinely quality control.

KEY WORDS: Validation, RP-HPLC, Bortezomib.

INTRODUCTION:

Bortezomib, an [(1*R*)-3-methyl-1-{[(2*S*)-1-oxo-3-phenyl-2-[(pyrazinylcarbonyl) amino] propyl]-amino} butyl] boronic acid, is used for the treatment of multiple myeloma as an inhibitor of 26 proteosome. A typical chemical structure of bortezomib is given in fig.1.Bortezomib is available for i.v. injection as a sterile lyophilized powder in single dose vial containing 3.5mg bortezomib and 35mg of mannitol, USP.

A plethora of pharmacokinetic and pharmacodynamic studies have been described for bortezomib, some of are pharmacokinetic and pharmacodynamic studies of two doses in patients with relapsed multiple myeloma, (Reece DE, et al. 2011); clinical pharmacokinetics of bortezomib (Dominique Leveque 2007); while others include characterization of drug. However no analytical method for bortezomib estimation in dosage form has been reported in the literature. Hence this paves the path to the new method development for the estimation of bortezomib in pharmaceutical dosage form.

Parental dosage form with Intra Venous route of administration having 100% bioavailability.So there is need of a sensitive method like HPLC. So taking this as a vive we are developed a novel RP-HPLC separating technique which separate the analyte (bortezomib) with excipients (mannitol) with excellent sensitivity of detection by using SPD-20A prominence UV-Visible detector. In present research work Bortezomib has retention time 3.5±0.5min. This shows that developed method is more efficient using very less time to resolve the sample as well as also consuming less amount of solvent. Thus this shows the novelty of research. The validation studies were according to the ICH guidelines (ICH 2005; USP (2002).

EXPERIMENTAL:

MATERIAL:

Bortezomib standard and mannitol was provided by Venus Remedies Ltd., Baddi (India).Water (HPLC grade), THF (HPLC grade), Acetonitrile (HPLC grade) and Methanol (AR) were purchased from Merck chemicals (Mumbai).

CHROMATOGRAPHIC CONDITIONS:

Shimadzu HPLC system equipped with prominence LC-20 AD pump, used Hypersil C_{18} column (256 x 4.6mm) as stationary phase with SPD – 20 A prominence UV- Visible detector. The mobile phase consisted of Acetonitrile: Water: THF (60:35:5). The manual sampler was used with loop 20µl with detection wavelength at 280 nm. The flow rate of analysis was optimized to 1ml/min with run time 10mins. A typical Chromatogram of Bortezomib is given in figure - 2.

STANDARD AND SAMPLE PREPARATION:

Bortezomib standard and marketed formulation (equivalent to 35mg was accurately weighed and transferred in two separate 100 ml of volumetric flask containing 25ml of water (HPLC grade), sonicated for 10 min diluted with water (HPLC grade), up to the mark and filtered it through 0.45µm membrane filter to get this stock solution (1mg/ml).

DEVELOPMENT AND OPTIMIZATION OF HPLC METHOD:

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The selection of method completely depends upon the nature of analyte and its solubility. Bortezomib is soluble in polar solvent thus RP-HPLC was selected to estimate Bortezomib. To develop a rugged and suitable HPLC assay method for the determination of bortezomib different parameters were analyzed and then selected. Preliminary trial was the selection of mobile phase which was decided after testing /analyzing different compositions of mobile phase that included the solvents as Acetonitrile, chloroform, methanol, THF, to obtain good peak.

VALIDATION OF DEVELOPED METHOD:

Analytical method validation is a process of performing several tests designed to verify that an analytical test system is suitable for its intended purpose and is capable of providing useful and valid analytical data. A validation study involves testing multiple attributes of a method to determine that it can provide useful and valid data when used routinely.

SYSTEM SUITABILITY:

A system suitability testing is an integral part of analytical method. The tests are based on the concept of equipment, electronics and analytical operations. The peak area, Retention Time, theoretical plates and peak symmetry were calculated six times for the standard solution. The values obtained (table no.1) demonstrated the suitability of the system for the analysis.

LINEARITY:

Linearity study was carried out with sample solution containing Bortezomib in five different concentration 25-150% of the target concentration showing a typical linearity curve depicted in figure -3. Each concentration was injected in triplicate and linearity curve was plotted between concentration and area response. Statistical analysis of the calibration was done. Results obtained are arranged in table 2. The response of drug was found to be linear within the investigation concentration and linear regression equation was *y=199x – 31.10* with correlation coefficient 0.999. ***Precision:***

The precision of assay method was evaluated in terms of repeatability by carrying out six independent assay of standard preparation and %RSD of assay was calculated. Intermediate precision of the method was performed by different analyst under same experimental conditions. The results of intraday and interday precision study was found to be precise as %RSD value for repeatability and intermediate precision were 0.479% & 0.04% respectively, thus confirms the method is precise.

ACCURACY:

Accuracy was performed by adding known amounts of bortezomib to placebo preparation. The actual and measured concentrations were compared. %Recovery was evaluated at three different concentration level, three sets were prepared and injected in duplicate. The peak area responses for accuracy determination are depicted in table no.3.The result shows that best recovery i.e 100.556% of drug were obtained, indicating method was accurate.

ROBUSTNESS:

The robustness of study was carried out to evaluate the influence of small but deliberate variations in chromatographic conditions. The factors chosen for this study were Flow rate (±0.1ml/min).The summarized results shown in table 4, indicates that all variance conditions, assay value of the test preparation solution was not affected and it was in accordance with that of actual. System suitability parameters were also found satisfactory, hence analytical method would be concluded as robust.

SPECIFICITY:

The specificity of method determined by checking the interference of placebo with analyte which was eluted by checking the peak purity of Bortezomib. The HPLC chromatograms have shown no other peak within the retention time of 3.5±0.5min. Thus method is specific.

LOD & LOQ:

LOD and LOQ were determined as concentration with signal to noise ratio of 3 & 10 respectively. Each blank calculated concentration, standard meets the acceptance criteria i.e. LOQ is 2.43ppm while LOD is 1.662ppm.

RESULTS AND DISCUSSIONS:

A new RP-HPLC method has been developed to be routinely applied to determine bortezomib in parenteral dosage form. The method was validated in accordance with ICH guidelines. The method has been proved to be specific, linear, precise, accurate, robust and suitable. Hence method is recommended for routine analysis of bortezomib in parenteral dosage form. Summary of method validation results are depicted in table 4.

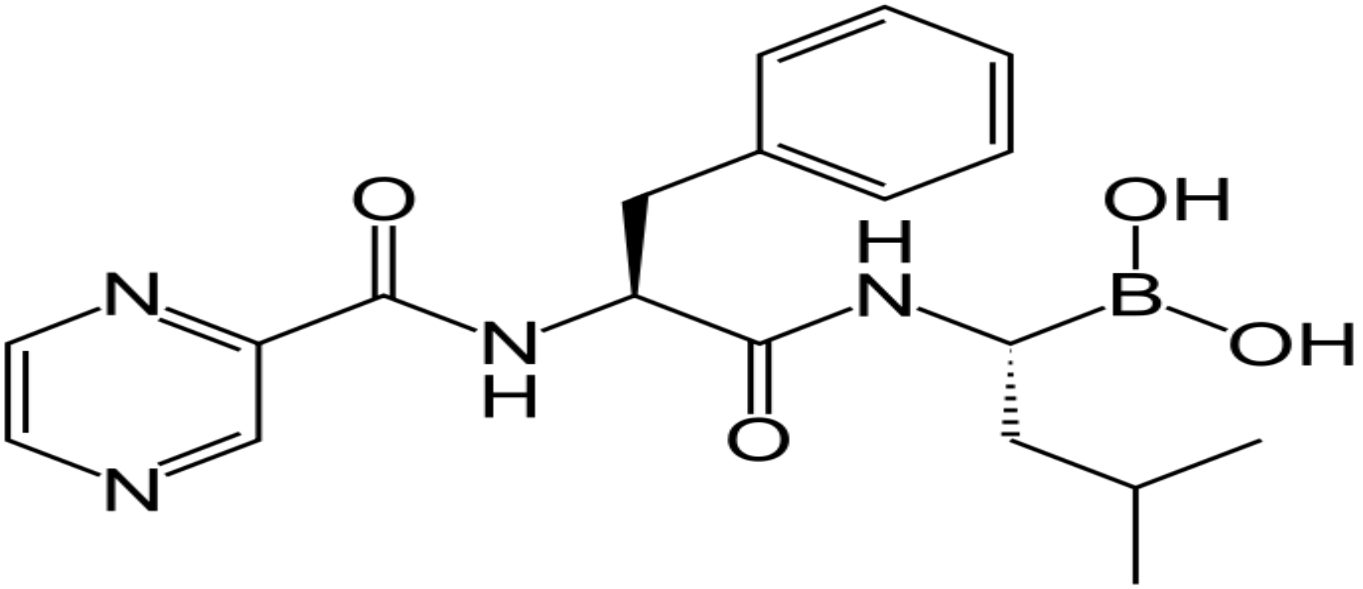

Figure 1: Chemical structure of Bortezomib

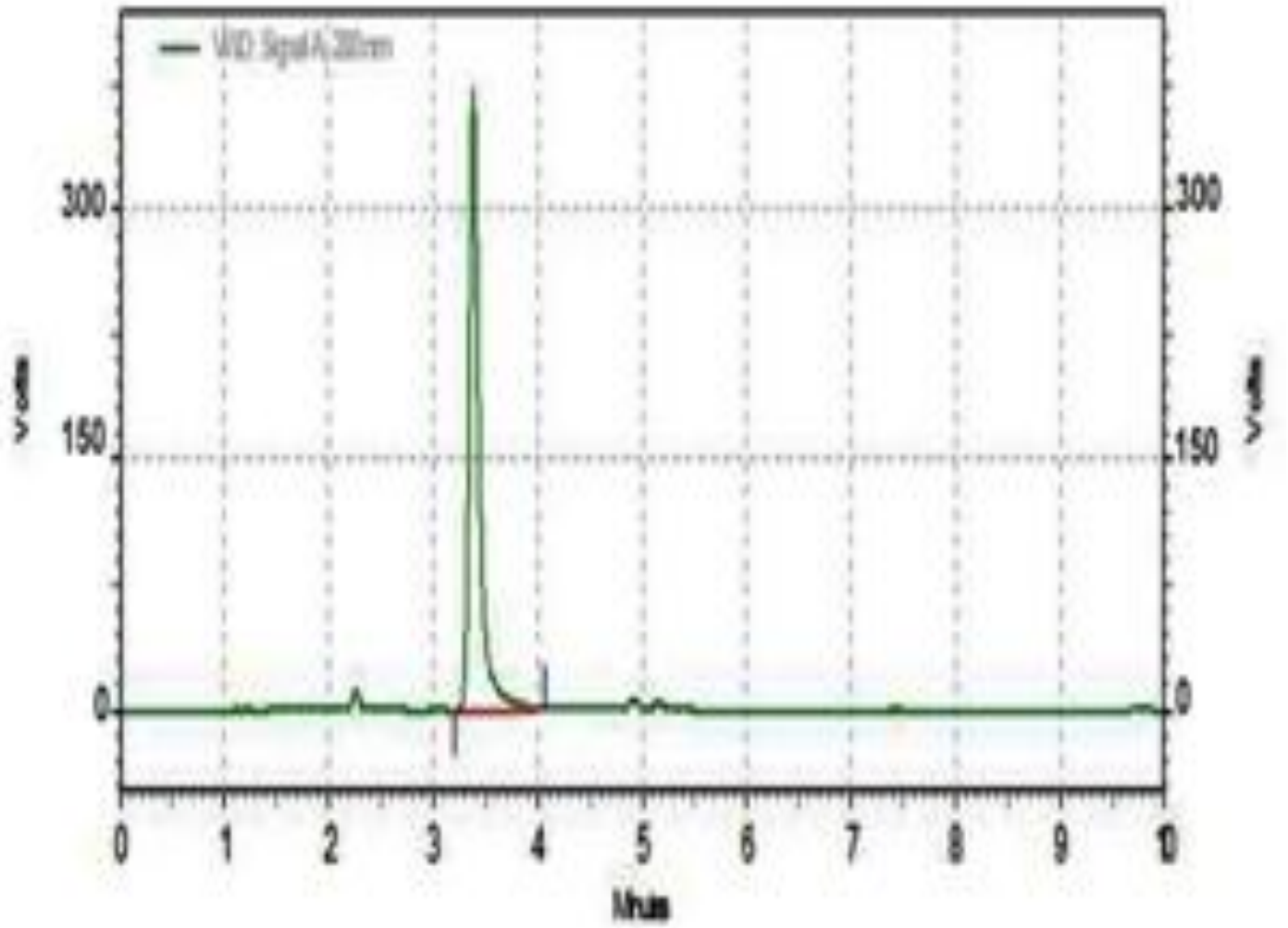

Figure 2: A typical Chromatogram of Bortezomib

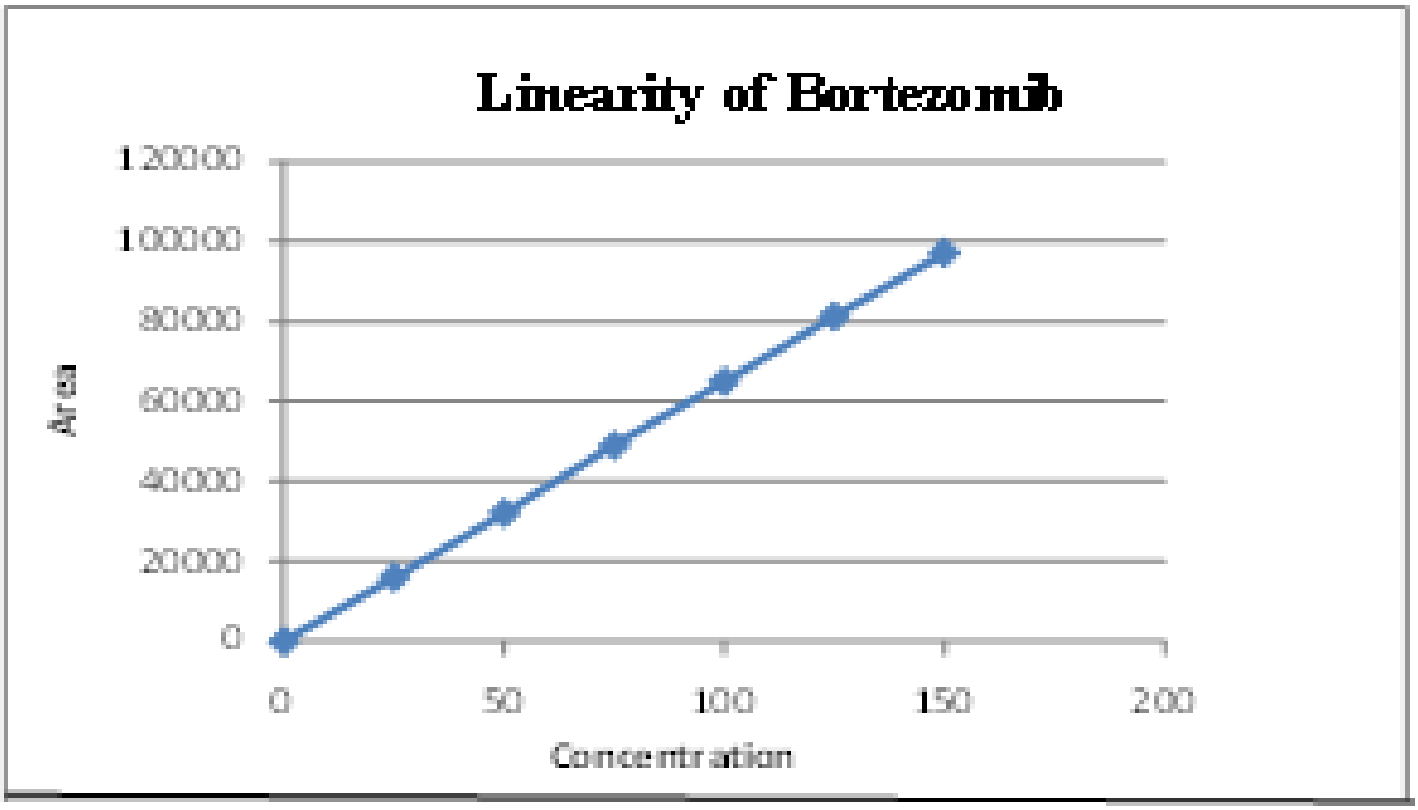

Figure 3: Linearity curve for Bortezomib

Table 1: Evaluation data of System suitability

Parameter	Bortezomib (n=6)
Theoretical Plate (Per column length)	$\bar{X}$ = 7401.5 ± 8.7 SD = 21.3892 RSD = 0.289%
Asymmetry of the peak	$\bar{X}$ = 1.202305 ± 4.08 SD = 0.0010 RSD = 0.083%
Retention time (min)	$\bar{X}$ = 3.380 ± 0.00163 SD = 0.004 RSD = 0.107%
Peak area	$\bar{X}$ = 56677304.67±110927.90 SD = 271706.7993 RSD = 0.479%

$\bar{X}$ is mean ± Standard error of the mean, SD is standard deviation, RSD is relative standard deviation and 'n' is average of 6 samples.

Table 2: Linearity table of Bortezomib

Concentration (%)	Peak area ratio	Statistical analysis
0	0	Slope : 0.0015382 Intercept : 0.1235826 Correlation coefficient : 0.999
25	16044	
50	32145	
75	49009	
100	65050	
125	81263	

Table 4: Summary of Validation parameters (Bortezomib)

Parameter	Bortezomib
Specificity	Specific
Linearity (r2)	0.999
Recovery	100.19%
LOD	1.662ppm
LOQ	2.435ppm
Precision Intraday	0.479%
Interday	0.045%
Robustness	
Change in flow (Increase)	0.61%
Change in flow (Decrease)	0.13%

RSD is relative standard deviation, r^2 is correlation coefficient, **LOD** is the limit of detection and **LOQ** is limit of quantification.

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