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Research Article

Method Development and Validation for Quantitative Estimation of Bortezomib in its Bulk and Pharmaceutical Formulation

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Abstract

A new method was established for estimation of Bortezomib by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Bortezomib by using Phenomenex Luna C_{18} column 250x4.6mm 5µm, flow rate was 1.0 ml/min, mobile phase ratio was Acetonitrile : 0.1% formic acid (50: 50 v/v),detection wavelength was 280 nm. The retention time was found to be 5.3 mins. The % purity of Bortezomib was found to be 100.443% respectively. The system suitability parameters for Bortezomib such as theoretical plates and tailing factor were found to be more than 2000 and less than 2 respectively, the resolution was found to be less than 2. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1). The linearity study for Bortezomib was found in concentration range of 20µg-120µg/ml and correlation coefficient (r^2) was found to be 0.9994, % recovery was found to be 101.37%, % RSD for repeatability was 1.778, % RSD for intermediate precision was 1.537 respectively. The precision study was precise, robust, and repeatable. LOD value was 2.6402, and LOQ value was 7.281 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Bortezomib in API and pharmaceutical dosage form.

Keywords: Analytical Validation, Bortezomib, Phamarmaceutical, formulation.

INTRODUCTION

Chromatography is a powerful separation tool that is used in all branches of science, and is often the only means of separating components from complex mixtures. It is mostly implemented in science subjects such as chemistry and life sciences, especially in biochemistry. Chromatography comes from the Greek words, chroma meaning 'color' and graphein meaning 'to write'. Chromatography can be analytical or preparative, based on the main objective for which it has been followed. Analytical chromatography makes use of only a small amount of a mixture and determines the components of that particular mixture. Depending on the techniques used in chromatography, the process is broadly classified as, Adsorption chromatography, Partition chromatography [1]. New types of chromatography developed during the 1930s and 1940s made the technique useful for many separation processes. Chromatography technique was developed substantially as a result of the work of Archer John Porter Martin and Richard Laurence Millington Synge during the 1940s and 1950s. They established the principles and basic techniques of partition chromatography, and their work encouraged the rapid development of several chromatographic methods like, Paper Chromatography, Gas Chromatography, High Performance Liquid Chromatography. Advances are continually improving the technical performance of chromatography, allowing the separation of increasingly similar molecules. Chromatography is used as a technique to separate the additives, vitamins, preservatives, proteins and amino acids. Some other uses are in the detection of drugs or medications in the urine. It is used by pharmaceutical companies to prepare large amounts of pure materials that are further required in making medicines. Also, it is used to check the presence of any contamination in the manufactured compounds. It is also very popular in forensic science for investigative purposes [2].

MATERIALS AND METHODOLOGY:

Materials:

For the determination of Bortezomib in tablet dosage form by HPLC method the following Chemicals were used such as Methanol, Distilled water, Acetonitrile, Formic acid.

Method Development

Chromatographic trails for estimation of Bortezomib by RP-HPLC.

Table 1: Trial 1: Chromatographic trails for estimation of Bortezomib by RP-HPLC

Mobile phase	Methanol : water (50: 50 v/v)
Column	C18 Phenomenex Luna (250x4.6 mm;5µ)
Flow rate	1.0 ml/min
Temperature	Ambient
Wavelength	280 nm
Injection volume	20 µl
Run time	10 min
Retention time	7.6min
Inference	Broad peak and peak shape is not good

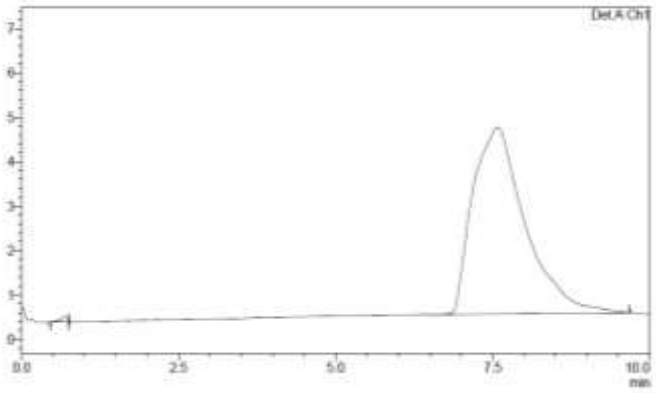

Figure 1: CMG showing trial-1 chromatogram

Table 2: Trail 2: CMG showing trial-1 chromatogram

Mobile phase	Acetonitrile : water (50: 50 v/v)
Column	C18 Phenomenex Luna (250x4.6 mm;5µ)
Flow rate	1.0 ml/min
Temperature	Ambient
Wavelength	280 nm
Injection volume	20 µl
Run time	10 min
Retention time	6.3min
Inference	Broad peak and peak shape is not good

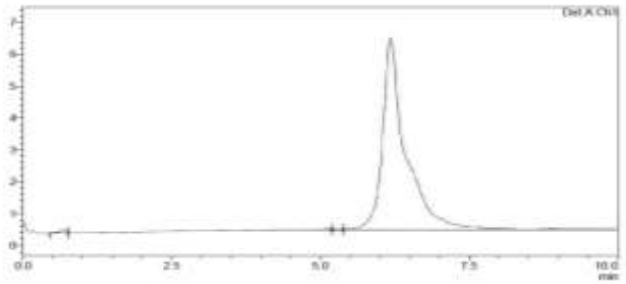

Figure 2: CMG showing trial-2 chromatogram

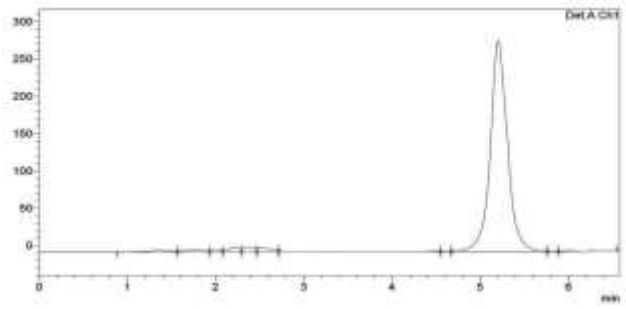

Figure 3: CMG showing Optimized chromatogram

Preparation of the optimized mobile phase:

Preparation of 0.1% formic acid

Weigh 0.1ml of formic acid and transferred to 100 ml volumetric flask, dissolved in sufficient quantity of water and then diluted to the mark with water [3].

Preparation of mobile phase

Preparation of mobile phase by using Methanol and water in the ratio of Acetonitrile : 0.1% formic acid (50: 50 v/v). The mobile phase was filtered through 0.45 um membrane filter paper. After filtration it was ultra sonicated for 10 minute on ultra sonicator [4].

Preparation of stock solution of Bortezomib

API of Bortezomib (10mg) accurately weighed and transferred to 10 ml volumetric flask, dissolved in mobile phase. The solution contains 1000ug/ml of Bortezomib .The solution was filtered through 0.45 um membrane filter paper and firs few drops of filtrate were discarded [5].

Preparation of sample solution:

Take 25 mg equivalent tablet powder of GSF and dilute up to 25 ml with Acetonitrile : 0.1% formic acid (50: 50 v/v). Sonicate it for 10 minutes. Filter the solution through Whatmann filter paper no. 41. This solution was used as sample solution [6].

Procedure: 20 µL of the blank, standard and sample was injected in to the chromatographic system and areas for the Bortezomib peaks were used for calculating the % assay by using the formulae [7].

System suitability

- Tailing factor for the peaks due to Bortezomib in standard solution should not be more than 1.5.
- Theoritical plates for the Bortezomib peaks in standard solution should not be less than 1.5.

Analytical Method Validation

Validation parameters

1. Specificity
2. Linearity
3. Range
4. Accuracy
5. Precision

i. Repeatability

ii. Intermediate precision

6. Limit of Detection
7. Limit of Quantitation
8. Robustness

1. Specificity [8]: In the case of assay, demonstration of assay specificity is required to show that the procedure is unaffected by the impurities or exipients. Specificity of an analytical method indicates that the analytical method is its able to measure accurately and specifically the analyte of interest without any interference from blank. So here, the specificity was determined by the comparision of the chromatograms of

a. Blank (mobile phase)

b. Standard sample solutions of Bortezomib

c. Sample solution of Bortezomib

Acceptance criteria:

Chromatogram of standard and sample should be identical with near Retention time.

2. Linearity [9]

Preparation of solution:

API of Bortezomib (10mg) accurately weighed and transferred to 10 ml volumetric flask, dissolved in sufficient quantity of methanol and then diluted to the mark with mobile phase.

Preparation of level – 1 (20µg/ml of Bortezomib)

From the standard solution (SS) 0.2 ml was taken in to 10 ml volumetric flask and diluted up to mark with methanol.

Preparation of level – 2 (40µg/ml of Bortezomib)

From the standard solution (SS) 0.4 ml was taken in to 10 ml volumetric flask and diluted up to mark with diluents.

Procedure:

Each level was injected in to the chromatographic system and peak area was measured. Plot a graph of peak area versus concentration (on x –axis concentration and on y axis peak area) and the correlation was calculated.

Acceptance criteria

Correlation coefficient should be not less than 0.9994.

3. Range [10]

Based on precision, linearity and accuracy data it can be concluded that the assay method is precise, linear and accurate in the range of 20-120 µg/ml of Bortezomib respectively.

4. Accuracy [11]

The accuracy of the test method is demonstrated by % of recovery. Accuracy was performed in three different three concentration levels and injected three times (Like 50%, 100%, and 150%). The observations are mentioned below.

Preparation of sample solutions

Preparation of 50% solution (with respect to target assay concentration)

From the stock solution 0.4ml was taken in to 10ml volumetric flask and diluted up to the mark with diluents.

Preparation of 100% solution (with respect to target assay concentration)

From the stock solution 0.8 ml was taken in to 10ml volumetric flask and diluted up to the mark with diluents.

Procedure:

The standard solutions of accuracy 50%, 100% and 150% were injected in to chromatographic system. Calculate the amount found and amount added for Bortezomib and calculate the individual % recovery and mean % recovery values.

Acceptance criteria

% Recovery at each spike level shall be not less than 98.0 and not more than 102.0.

5. Precision [12]

i. Repeatability

Preparation of solution

From the stock solution 0.8 ml was taken in to 10ml volumetric flask and diluted up to the mark with diluents

Procedure

The standard solution was injected for five times and measured the area for all five injections in hplc. The % RSD for the area of five replicate injections was found to be within the specified limits.

Acceptance criteria

The % RSD for the area of five standard injections results should not be more than 2.

ii. Intermediate precision/Ruggedness

To evaluate the intermediate precision (also known as ruggedness) of the method, precision was performed on different day by using different make column of same dimensions.

Preparation of solution

From the stock solution 0.8 ml was taken in to 10ml volumetric flask and diluted up to the mark with methanol.

Procedure

The standard solution was injected for five times and measured the area for all five injections in HPLC. The % RSD for the area of five replicate injections was found to be within the specified limits.

Acceptance criteria

The % RSD for the area of five sample injections results should not be more than 2%.

6. Limit of detection (LOD)

LOD's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (s) at levels approximating the LOD according to the formula. The standard deviation of the response can be determined based on the standard deviation of y- intercepts of regression lines [13].

Formula: LOD = 3.3 X $\frac{SD}{S}$

Where

SD – Standard deviation (SD) S – Slope

Acceptance criteria

The values should not more than 3 for LOD solution.

7. Limit of quantification (LOQ)

LOQ's can be calculated based on the standard deviation of the response (SD) and the slope of the calibration curve (s) according to the formula. Again, the standard deviation of the response can be determined based on the standard deviation of y- intercepts of regression lines [14].

Formula: LOD = 10 X $\frac{SD}{S}$

Where

SD – Standard deviation (SD) S – Slope

Acceptance criteria

The values should not more than 10 for LOQ solution.

8. Robustness [15]

As Part of the robustness, deliberate change in the flow rate, mobile phase composition was made to evaluate the impact on the method

a) The flow rate was varied at **±** 10%. Standard solution 40 µg/ml of Bortezomib was prepared and analysed using the varied flow rates along with method flow rate.

b) The Temperature was varied (± 5°C) Standard solution 40 µg/ml of Bortezomib was prepared and analysed using the varied flow rates along with method flow rate.

RESULTS AND DISCUSSION

Validation Report:

Specificity:

Specificity by Direct comparison method

There is no interference of mobile phase, and placebo with the analyte peak and also the peak purity of analyte peak which indicate that the method is specific for the analysis of analytes in their dosage form [Table 3].

Table 3: Specificity Data

S.No	Peak Name	Observation	
1	Blank	Nil	
2	Placebo	Nil	
3	Standard	**R_t:** 5.3 min	**λ max:** nm

System suitability: System suitability test was an integral part of method development and has been used to ensure adequate performance of the chromatographic system [Table 4].

Table 4: Results of System Suitability

Parameter	Result	Acceptance Limit
Retention time (Rt)*	5.3 min	More than 2
Resolution factor*	NA	--
Number of theoretical plates (N)*	3652	More than 2000
Tailing factor (T)*	1.32	Less than 2
* Number of injections: 6 replicates		

Results for intraday and inter day precision

Table 5: Intraday and Inter day precision

S.No.	Intraday precision Area	Inter day precision Area
1	244125	242145
2	242451	241245
3	244512	248596
4	242010	245487
5	241201	242403
6	254125	251593
Mean	244737.3	245244.8
Std Dev	4353.645	3769.877
%RSD	1.778905	1.537189

Table 6: Linearity

S. No	Concentration (µg/mL)	Peak Area
1	20	68451
2	40	121021
3	60	184241
4	80	241520
5	100	304512
6	120	365412

Linearity and range

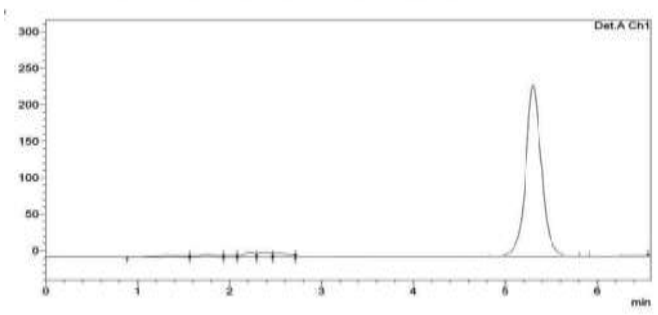

Figure 4: Chromatogram showing Linearity 20 µg/mL

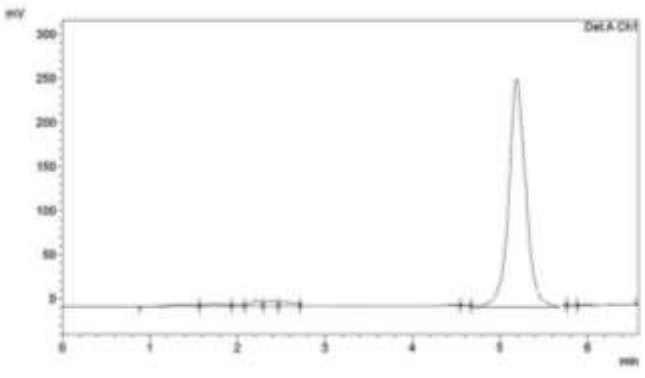

Figure 5: Chromatogram showing Linearity 40 µg/mL

Accuracy

Accuracy of the method was determined by Recovery studies [Table 7]. To the formulation (pre analyzed sample), the reference standards of the drugs were added at the level of 50%, 100% [Figure 6,7].

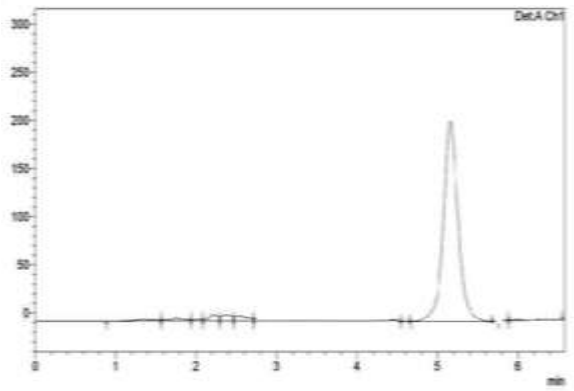

Figure 6: Chromatogram showing Accuracy 50% injection-1

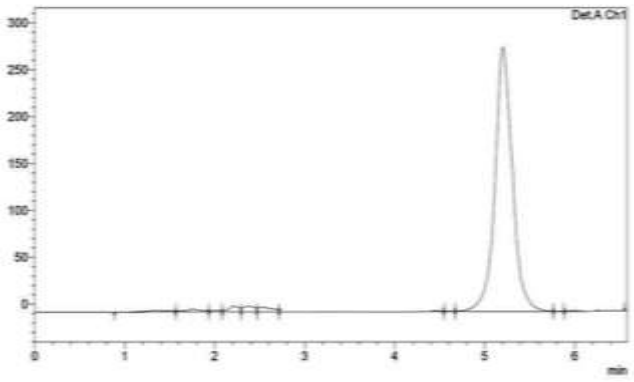

Figure 7: Chromatogram showing Accuracy 100% injection-2

Table 7: Results of accuracy

Spiked Concentration (µg/mL)	Peak area	Amount added (µg/mL)	Amount Found (µg/mL)	Recovery	% Mean Recovery
50%	121021	40.01	40.2496	100.5989	101.54
	120041		39.92367	99.78423	
	125412		41.70998	104.2489	
100%	241520	80.02	80.3256	100.3819	101.37
	248745		82.72852	103.3848	
	241452		80.30298	100.3536	

LOD & LOQ:

Table 8: Results of LOD & LOQ:

S.NO	Parameter	Slope	Standard Deviation	Value (µg/mL)
1	Limit of Detection	5978	4353	**2.402**
2	Limit of Quantification			**7.281**

Robustness:

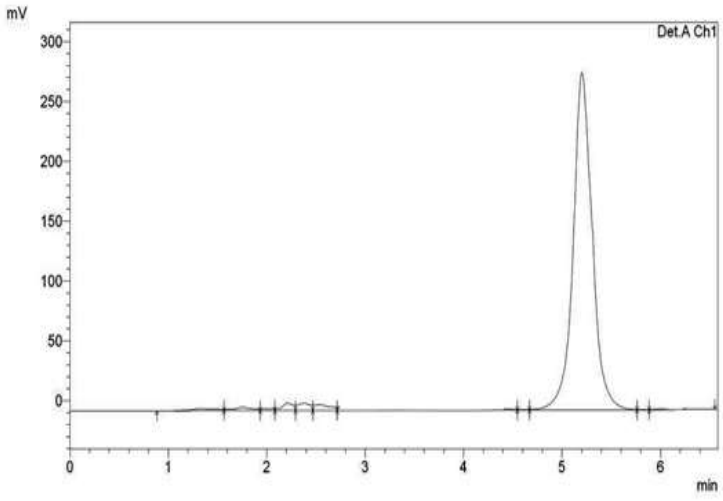

Figure 8: Chromatogram showing flow rate 0.9mL/min

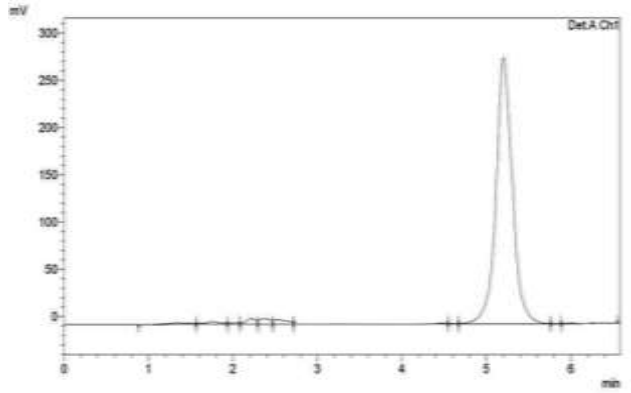

Figure 9: Chromatogram showing flow rate 1 mL/min

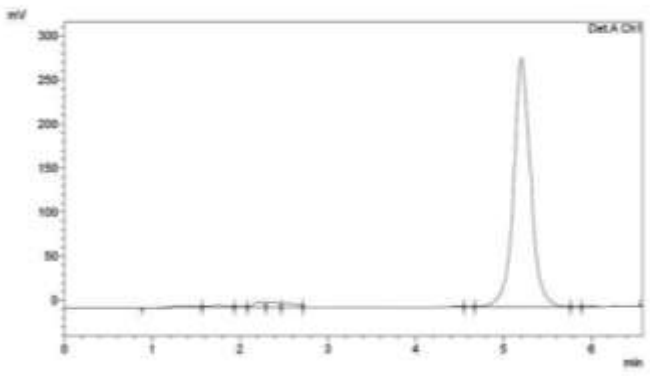

Figure 10: Chromatogram showing flow rate 1.1 mL/min

Change of Flow rate (± 0.1mL)

Table 9: Robustness

S.No	Flow Rate	0.9mL/min	1mL/min	1.1mL/min
1		249875	247851	241782
2		241578	246987	249514
3		239874	238745	237891
4	Mean	243775.6667	244527.7	243062.3
5	Std dev	4368.623302	4104.148	4830.664
6	% RSD	1.792067011	1.678398	1.987418

Change in Temperature (± 5°C)

Table 10: Robustness

S.No	Temperature	30 °C	35 °C	40 °C
1		231457	234154	241598
2		241547	231451	246587
3		239874	241548	239852
4	Mean	237626	235717.7	242679
5	Std dev	4415.288062	4267.797	2853.825
6	% RSD	1.858082896	1.810555	1.175967

CONCLUSION:

The analytical method was validated according to ICH guidelines (ICH, Q2 (R1). The linearity study for Bortezomib was found in concentration range of 20µg-120µg/ml and correlation coefficient (r^2) was found to be 0.9994, % recovery was found to be 101.37%, % RSD for repeatability was 1.778, % RSD for intermediate precision was 1.537 respectively. The precision study was precise, robust, and repeatable. LOD value was 2.6402, and LOQ value was 7.281 respectively. Hence the suggested RP-HPLC method can be used for routine analysis of Bortezomib in API and pharmaceutical dosage form.

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Conflict of Interest: The authors attest that they have no conflict of interest in this study.

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