



## ESTIMATION OF BORTEZOMIB IN BULK AND ITS PHARMACEUTICAL DOSAGE FORMS BY USING A NOVEL VALIDATED ACCURATE REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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### ABSTRACT:

A Novel rapid, precise, economical and Accurate HPLC method for estimation of Bortezomib in bulk and formulations was developed and validated. The Chromatographic resolution of Bortezomib was achieved using Acetonitrile: 0.1M Ammonium phosphate (acetic acid) Buffer, (60: 40 V/V) as a mobile phase UV detection at 230 nm and BDS Hypersil C8 column. flow rate 1ml/min. the extraction Recovery of Bortezomib from its formulation dosage form (tablets) was >99.59% And the calibration curve was linear ( $r^2 = 0.999$ ) over Bortezomib concentration ranging from 20 to 120  $\mu\text{g/ml}$ . the method has an accuracy of > 99% and LOD and LOQ of 0.51900  $\mu\text{g/ml}$  and 2.43450  $\mu\text{g/ml}$  respectively. A result of the present method was validated statistically and by recovery studies were found to be satisfactory.

**Keywords:** Bortezomib, Multiple Myelogenous Leukemia (MML), RP-HPLC, CC, LOD AND LOQ

### INTRODUCTION

Bortezomib is a highly selective, reversible inhibitor for the 26S protease. This drug thought to inhibit many proteins (known as proteasomers) that cancer cells need to survive and multiply. It has been shown to have anti-tumor activity in B cell malignancies.

Bortezomib is indicated (recommended) for single-agent use in the treatment of proteins with multiple myeloma that have received at least two prior therapies and are progressing on their most recent therapy. Clinical investigations have been completed or are under way to evaluate the safety and efficacy of Bortezomib alone or in combination with chemotherapy in multiple Myeloma, both at relapse and presentation, as well as in other cancer types.

Bortezomib (VALCADE®, formerly PS-341) was approved for the treatment of patients with relapsed or refractory multiple myeloma in May 2003 by the US foods and drugs Administration and in April 2004 by the Committee for treatment of mantle cell lymphoma.

A few publications are available for Bortezomib, some of are available on (1) characterization of Bortezomib and metabolites observed in human plasma with the help of MDS sciex API 3000 triple quadruple LC MS using turbo ion spray interface set at 325°C and (2) Enhanced Delivery of cisplatin to international Ovarian Carcinomas mediated by the effects of bortezomib on human copper transporter And (3) one of it is in Human plasma using LC MS and another one is on (4)

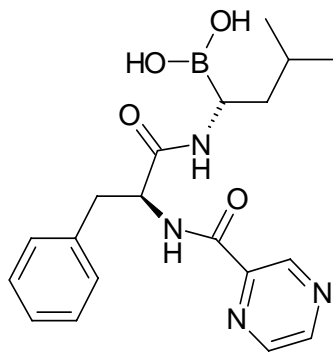


Fig. 1: Chemical structure for Bortezomib

Modulation of gemcitabine Pharmacokinetics and Pharmacodynamics in non lung cell cancer and blood mononuclear cells are reported, But none of them are employed an economical, precise and accurate RPHPLC method, so we here present a new method for determination of Bortezomib in bulk and Pharmaceutical dosage forms which utilizes a very cheap solvent system **On Hypersil BDS C 8** analytical columns with UV detector uv maximum 230nm. This kind of method effective to produce Better retentions, very sharp and symmetrical peak shapes and exhibit very good Sensitivity for Bortezomib in its Bulk and formulation dosage forms.

### MATERIALS AND METHODS

#### Instrumentation

Quantitative HPLC was performed on a binary gradient HPLC with Shimadzu LC20AT and LC20AT VP series HPLC pumps, with a 20 $\mu\text{l}$  injection of sample loop (manual), and SPD20 A VP UV -visible Detector. The out put signal was monitored and integrated using ShimadzuClass VP version 6.12 SP1 software. BDS Hypersil C8 (250 x 4.6 $\mu\text{m}$ , 5 $\mu\text{m}$ ) column was used for Separation.

#### Standards and chemicals

Bortezomib and its formulation capsules were purchased from Pfizer Pharmacy and standard sample was gifted by Chandra labs. Acetonitrile HPLC grade, Potassium dihydrogen Phosphate Purchased from Merck chemicals. Which are highly purified and their purities not less than 99.8% purity

#### Preparation of standard drug solution

50mg each of Bortezomib and an amount of its formulation equivalent to 50mg, accurately weighed and transferred in two separate 50 ml of volumetric flask containing 25ml of mobile phase Sonicated for 15 min, Diluted with mobile phase up to the lower meniscus mark and filtered it through 0.45 $\mu\text{m}$  memberane to get this stock solution (1mg per ml)

#### Chromatographic conditions

The mobile phase used in this study was a mixture of Acetonitrile and Ammonium phosphate (buffer PH~4 with acetic acid) 60:40 V/V, then the content was solicited for 45 min for degassing purpose and then filtered through 0.45  $\mu\text{m}$  (pore diameter) Whitman filter paper. the mobile phase was pumped from the solvent reservoir to the column at a flow rate of 1.0ml/min. the eluents were monitored at UV max 230 nm. The column temperature was maintained ambient through out the experiment.

### Selection of mobile phase

Based on sample solubility, stability and suitability. Various mobile phases and compositions were tried to get a good resolution and sharp peak. The standard solution was run in different mobile phases.

The following mobile phases were tried

1. Water :Methanol (10:90% v/v)
2. Buffer(PA):Methanol (30:70 v/v)
3. Buffer(0.1%AA): ACN(50:50 v/v)
4. Buffer(0.1% AA,pH-5): ACN (50:50 v/v)
5. Buffer(0.01%SP,pH-4):ACN (20:80 v/v)
6. Buffer(0.1%SP,pH-3.5):ACN (40:60 v/v)

### Calibration of standards

Calibration standards were prepared by spiking working standard into Mobile phase containing 25ml volumetric flask to yield concentrations of 20, 40, 60, 80, 100, and 120  $\mu\text{g}/\text{mL}$  to the above 20  $\mu\text{g}/\text{mL}$ . The final volume was made up to the mark. The represented data was shown in table 1. A 20  $\mu\text{L}$  aliquot was

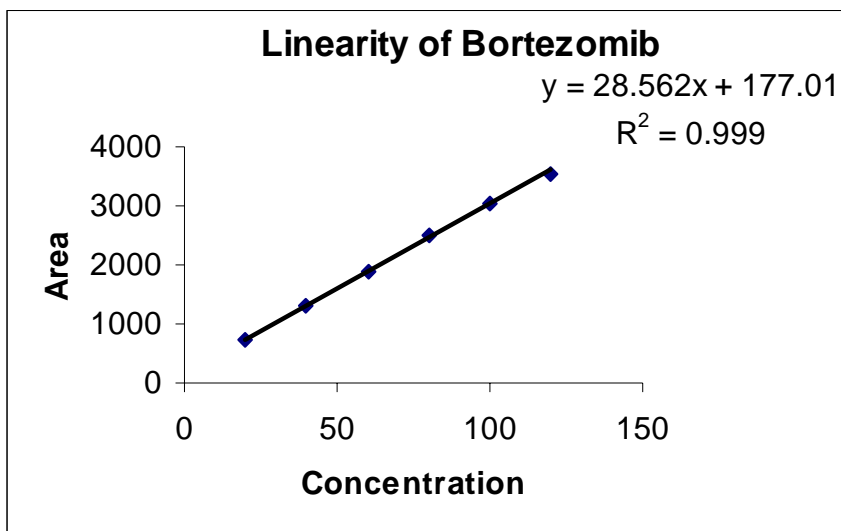
injected into the analytical column. The resultant peak areas of the drug were measured. Calibration curve was plotted between peak areas of drug against concentration of the drug.

**Table1: Linearity range of Bortezomib**

Concentration ( $\mu\text{g}/\text{ml}$ )	Peak area ratio	Statistical analysis
20	727.540	slope : 28.562
40	1306.695	
60	1902.340	Intercept : 177.01
80	2512.490	
100	3052.449	Correlation coefficient: 0.999
120	3556.803	
		Asymmetric factor : 1.348

### Recovery of Bortezomib from its Formulation

The finely powdered formulation dosage and accurately weighed sample of formulation equivalent to 50 mg Bortezomib was extracted with Acetonitrile in a 50ml volumetric flask using ultra sonicator. This solution was diluted with mobile phase, so as to obtain a concentration in the range of linearity previously determined. All determinations were carried out in five replicates. The represented data was shown in table 2.



**Fig. 2: Graph for linearity**

**Table 2: Amount of Bortezomib in formulation tablet By HPLC Method**

Formulation Tabs in Mg	Labeled Amount in mg	Recovered amount in mg	% Recovery
3.5 mg Tablet	3.5	3.483 mg	99.59%

\*each value is the average of five determinations

## RESULTS AND DISCUSSION

### Method validation

Specificity and selectivity of the method was assessed by preparing a drug concentration of 100  $\mu\text{g}/\text{mL}$  from pure drug stock and commercial sample stock in selected mobile phase and analyzed. The HPLC chromatograms recorded for the drug matrix showed almost no other peaks within a retention time range of 6 min (figure 3). Thus the HPLC method developed in this study is selective for Bortezomib. The method is linear in the concentration range 20 to 120  $\mu\text{g}/\text{mL}$ .

intra day precision was studied by five replicate measurements at three different concentration levels over a period of 3 consecutive days. Accuracy of the method was determined by calculating recovery studies. Statistical evaluation revealed that relative standard deviation (%RSD) of the drug at different concentration levels for five injections was less than 0.2. Precision and accuracy data were shown in table 3 And 4 respectively.

For system suitability, five replicates of standard sample were injected and different parameters were studied (table 5). The tailing factor for Bortezomib was always less than 2.0

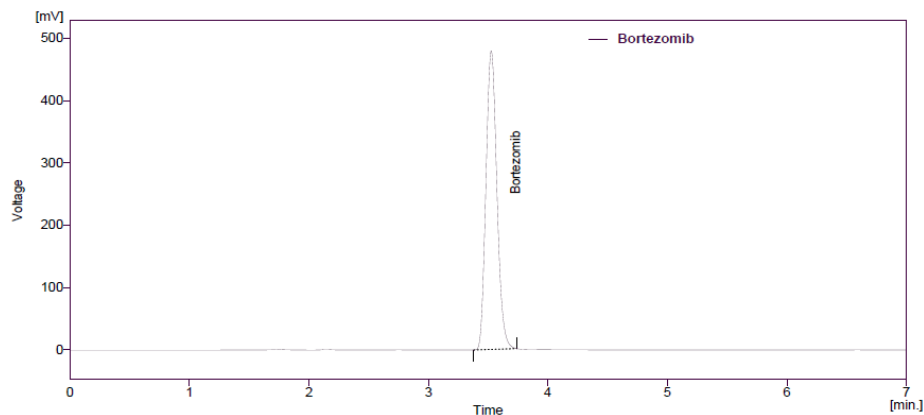


Fig. 3: HPLC chromatogram of Bortezomib (standard)

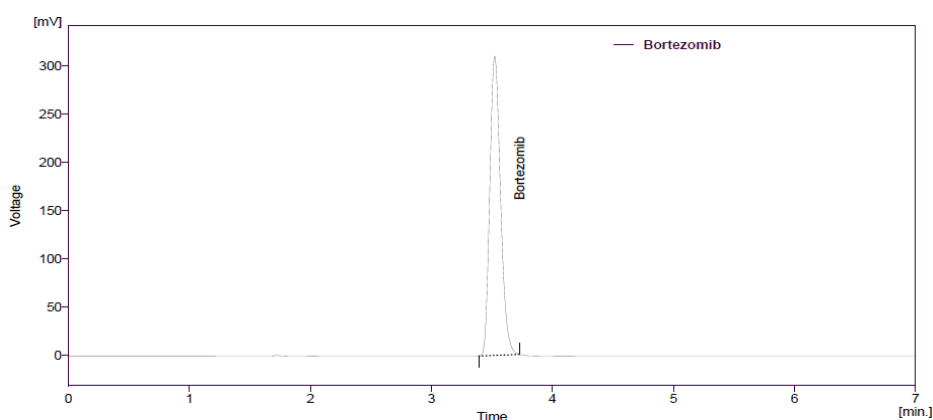


Fig. 4: HPLC chromatogram of Bortezomib (formulation)

Table 3: Precision studies

Concentration in ( $\mu\text{g/ml}$ )	Peak area	%RSD
100 $\mu\text{g/ml}$	3114.386	0.06434

\*each value is the average of five determinations

Table 4: Accuracy studies

Mixture of pure and formulation	concentration of formulation in ( $\mu\text{g/ml}$ )	% of Recovery of pure drug	%RSD
80%	79.61	99.52	0.39041
100%	99.92	99.92	0.02469
120%	119.55	99.63	0.47910

\*each value is the average of five determinations

Table 5: System suitability

S.NO	Parameters	Values
1.	Theoretical Plates (N)	6625.00
2.	LOD, $\mu\text{g/ml}$	0.51900
3.	LOQ, $\mu\text{g/ml}$	2.43450

## CONCLUSIONS

The results obtained from these studies are well fit into the standard specifications stipulated by the regulatory agencies. The method is able to reproduce the results consistently and the recovery studies of Bortezomib are found to be **99.59%**. This indicates that commonly used excipients in pharmaceutical formulation were not interfering in the proposed method. The observation of **% C.C less than 2.0** for intra day measurements also indicates high degree of precision. In the present method, we have established a linearity range of **20-120  $\mu\text{g/mL}$** ; this linearity range covers all the strengths of Bortezomib, hence this can be conveniently used in the pharmaceutical manufacturing and formulation environment.

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