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Analytical Method Development and Validation for Quantification of Bortezomib by RP-HPLC

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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 **[1].**

Bortezomib is a first-in-class proteasome inhibitor drug which has been approved for first-line treatment of multiple myeloma. It is associated with thrombocytopenia and neutropenia that follow a cyclical pattern with nadirs occurring following the last dose of each cycle and typically recovering prior to initiation of the subsequent cycle [**2**].

Mechanism of Action

Bortezomib is a reversible inhibitor of the chymotrypsin-like activity of the 26S proteasome in mammalian cells. The 26S proteasome is a large protein complex that degrades ubiquitinated proteins. The ubiquitin-proteasome pathway plays an essential role in regulating the intracellular concentration of specific proteins, thereby maintaining homeostasis within cells. Inhibition of the 26S proteasome prevents this targeted proteolysis, which can affect multiple signaling cascades within the cell. This disruption of normal homeostatic mechanisms can lead to cell death. Experiments have demonstrated that Bortezomib is cytotoxic to a variety of cancer cell types *in vitro*. Bortezomib causes a delay in tumor growth *in vivo* in nonclinical tumor models, including multiple myeloma [**3**].

There was no chromatographic method available for Bortezomib in literature. So development of the method was started by taking references of similar drugs reported in literature **[4, 5]**

Preparation of standard solution:-

Accurately weighed and transferred about 10 mg of Bortezomib WS in a 50 mL volumetric flask. About 30 mL of diluent was added and sonicated and volume was made up.

Preparation of Sample Solution

Accurately weighed and transferred about 10 mg of Bortezomib sample in a 50 mL volumetric flask. About 30 mL of diluent was added and sonicated and volume was made up.

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Preparation of mobile Phase:-

Prepare a mixture of methanol and water (1:1) containing 1.1 gm of sodium octane sulphonic acid per liter and pH was adjusted to 3.0 with ortho phosphoric acid. The resulting solution was filtered and degassed with .45µm filter.

Chromatographic conditions

The mobile phase used in this study was a mixture of methanol and water (1:1) containing 1.1 gm of sodium octane sulphonic acid per liter and pH 3.0, then the content was sonicated for 45 min for degassing purpose and then filtered through 0.45 µ (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 **270 nm**. The column temperature was maintained ambient throughout the experiment.

Inference:

One sharp peak at 6.99 min with optimum peak area was observed.

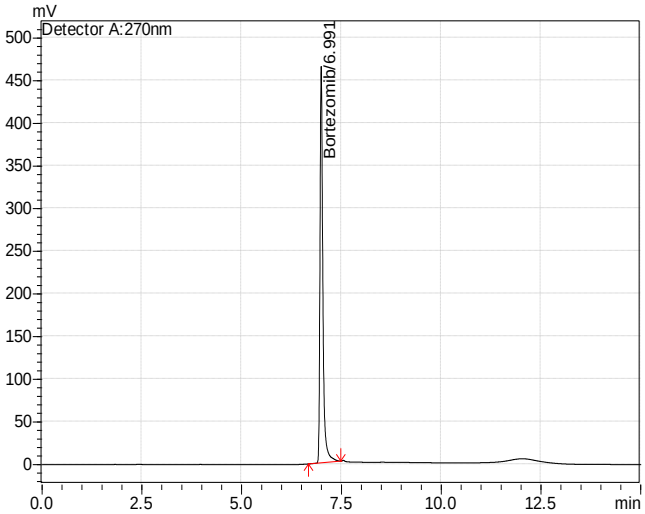

Fig. 1

Method Validation

Method validation is the process of demonstrating that analytical procedures are suitable for their intended use .The method validation process for analytical procedures begins with the planned and systematic collection by the applicant of the validation data to support analytical procedures.

- **Accuracy**

Accuracy is calculated as the percentage of recovery by the assay of the known added amount of analyte in the sample, or as the difference between the mean and the accepted true value shown in Table 1.

- **Linearity and Range**

The linearity of an analytical procedure is its ability (within given range) to obtain test results which are directly proportional to the concentration of analyte in the sample. [Finished Product]. The range of an analytical procedure

is the interval between the upper and lower levels of analyte (including these levels) that have been demonstrated to be determined with a suitable level of precision, accuracy and linearity using the procedure as written. The range is normally expressed in the same units as test results (e.g. percent, parts per million) obtained by the analytical procedure.

Determination of Linearity and Range –

Linearity should be established across the range of the analytical procedure. It should be established initially by visual examination of a plot of signals as a function of analyte concentration of content. For the estimation of linearity a minimum of 5 concentrations is recommended.

Wt of Standard Taken : 50.2 mg (Diluted to 100 ml)

Assay 99.00 %

Level	Wt of API Added (mg)	Area Count 1	Area Count 2	Mean Area Count	Actual Amount Added (mg)	Amount Recovered	% Recovery
80 % -1	80.1	1123167	1127945	1125556	79.3	78.6	99.1
80 % -2	79.7	1134232	1129648	1131940	78.9	79.1	100.3
80 % -3	79.8	1140971	1135864	1138418	79.0	79.5	100.6
100 % -1	100.7	1409104	1422143	1415624	99.7	98.9	100.3
100 % -2	100.6	1418863	1424592	1421728	99.6	99.3	99.7
100 % -3	98.2	1376297	1389178	1382738	97.2	96.6	99.4
120 % -1	120.1	1688263	1683317	1685790	118.9	117.7	99.0
120 %-2	120.0	1697421	1701908	1699665	118.8	118.7	99.9
120 % -3	120.1	1693565	1691987	1692776	118.9	118.2	99.4
						Mean	99.7
						SD	0.57
						% RSD	0.57

Table 1

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Level	Vol. of Stock (ml)	Final Vol. (ml)	Conc. (ppm)	Injection 1	Injection 2	Mean Area Counts
70 %	7	100	348.2	977507	978521	978521
80 %	8	100	397.9	1123032	1132691	1132691
90 %	9	100	447.6	1251467	1261626	1261626
100 %	10	100	497.4	1403054	1404253	1404253
110 %	11	100	547.1	1484621	1484942	1484942
120 %	12	100	596.9	1689916	1691067	1691067
130 %	13	100	646.6	1799426	1804225	1804225
					Slope	2741
					Intercept	30753
					R^2	0.99718857

Table 2 Calculation for determining Linearity of Bortezomib

➢ **CHROMATOGRAPHIC PURITY**

The chromatographic purity of Bortezomib was carried out for the separation of the Bortezomib peak and any impurities peak. Peak purity parameter was used to check that no impurity peaks was merged with peak of Bortezomib.

Acceptance criteria:

1. The purity line and noise+solvent line should be parallel with each other.
2. The purity angle should be less than purity threshold.

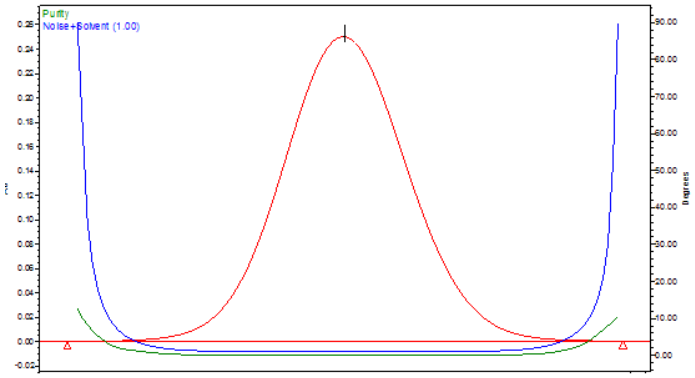

Fig. 2: Peak purity chromatogram for Bortezomib

	Name	Purity angle	Purity threshold
1	**Bortezomib**	0.014	0.258

Results & Discussion

A convenient and rapid RP- HPLC method has been developed for estimation of bortezomib in injection dosage form. The assay provides a linear response across a wide range of concentrations. Low intra-day and inter-day % RSD coupled with excellent recoveries. The pro-posed method is simple, fast, accurate and precise for the simultaneous quantification of bortezomib in dosage form, bulk drugs as well as for routine analysis in quality control.

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