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Determination of enantiomer impurity in Bortezomib lyo injection formulation by using normal-phase liquid chromatography

Sanni Babu Najana* and Hari Babu Bollikolla

Abstract

Background: A highly stereo-specific liquid chromatographic technique was built up and authenticated to quantify the (1S,2R-enantiomer) impurity in Bortezomib lyo injection formulation. The separation was achieved on Chiral Pak ID-3 (3 µm, 4.6 × 250 mm) column ("amylose-based 3-chlorophenylcarbamate" chiral stationary phase) through a movable segment consisting of n-heptane, 2-propanol, ethyl alcohol, and TFA (82:15:3:0.1, v/v/v/v) at a flow rate of 0.6 mL/min. Column temperature preserved 25 °C, injection level 20 µL, sample cooler temperature ambient, and detection wavelength 270 nm.

Results: The retention time of (1S,2R-enantiomer) impurity and Bortezomib was determined 10.57 and 17.98 min, respectively. The resolution between (1S,2R-enantiomer) impurity and Bortezomib was found to be 4.2. The acceptance limit of the (1S,2R-enantiomer) impurity is 0.5%. The established method was authenticated as per ICH guidelines in respect of precision, accuracy, sensitivity, linearity, specificity, ruggedness, and robustness. The minimum quantity of the sample required for detection (LOD) was observed at 0.282 µg per mL and similarly the quantifying sample (LOQ) was observed to be 0.896 µg per mL.

Conclusion: The proposed normal phase-HPLC method that can quantify (1S,2R-enantiomer) impurity in Bortezomib lyo injection formulation at trace level concentration has been urbanized and authenticated as per ICH guidelines. The effectiveness of the technique was ensured by the specificity, exactitude, linearity, and accuracy. Hence, the method well suit for their intended purposes and can be successfully useful for regular analysis in laboratories and is suitable for the quality control.

Keywords: (1S,2R-enantiomer) Impurity, Bortezomib, Validation, Limit of quantitation

Background

Bortezomib (M.F. $C_{19}H_{25}BN_4O_4$) is an anti-cancer medication used to treat multiple myeloma and mantle cell lymphoma and is marketed with the brand name Velcade [1]. Moreover, this includes multiple myeloma in those humans who have and have not previously received treatment [2]. Chemically, Bortezomib is (1R)-3-methyl-1-[[(2S)-1-oxo-3-phenyl-2-[(pyrazinylcarbonyl) amino]propyl]amino]-butyl]boronic acid, a potent first-in-class dipeptidyl boronic acid proteasome inhibitor [3–8] (Figs. 1 and 2). It was approved in the year 2003 in the USA for the treatment of relapsed multiple myeloma where the disease is refractory to conventional lines of therapy. Further, it is also mostly used along with other medications. It is given in the form of injection. Bortezomib, formerly known as PS-341, it binds to the proteasome via the boronic acid moiety, and therefore, the presence of this moiety is necessary to achieve proteasome inhibition. The proteasome is an interesting new target for cancer therapy, and the proteasome inhibitor PS-341 warrants continued investigation in cancer therapy.

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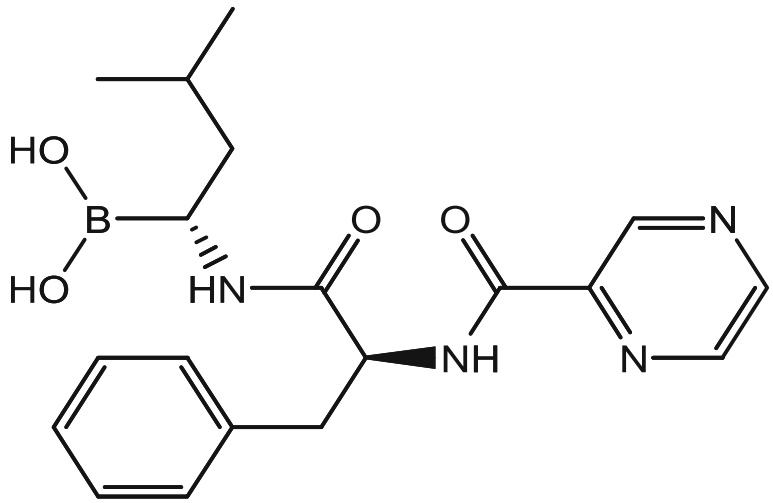


Fig. 1 Chemical structure of Bortezomib

Bortezomib has two diastereomers and two enantiomers. RP-HPLC methods are available for the determination of diastereomers (S,S Isomer and R,R Isomer) [9] and stereoisomer -I (1R,2S)-enantiomer [10] in Bortezomib API as well as finished product, whereas to determine the enantiomer (1S,2R Isomer) in Bortezomib finished product, *normal phase-HPLC method are available for Bortezomib drug substance*. In drug substance analysis, no extraction procedure required for extraction of the impurities and as well as main analyte. Previously existing method (drug substance) was not suitable for finished product. Due to finished product formulations, extraction procedure is applicable for the extraction of the impurities as well as main analyte.

Stereoisomers are distinguished by biological systems and can have different pharmacokinetic properties (absorption, distribution, biotransformation, and excretion) and quantitatively or qualitatively different pharmacologic or toxicological effects.

(1S,2R-enantiomer) Impurity is an inactive form in the drug. When stereoisomers are biologically distinguishable, they might seem to be different drugs. Bortezomib and other related substances of Bortezomib are determined in reversed-phase liquid chromatography.

A review on literature revealed that several analytical methods, based on RP-HPLC [9, 11–14], UV-Vis [15], and normal phase HPLC (NP-HPLC) [16] were available for the determination of Bortezomib.

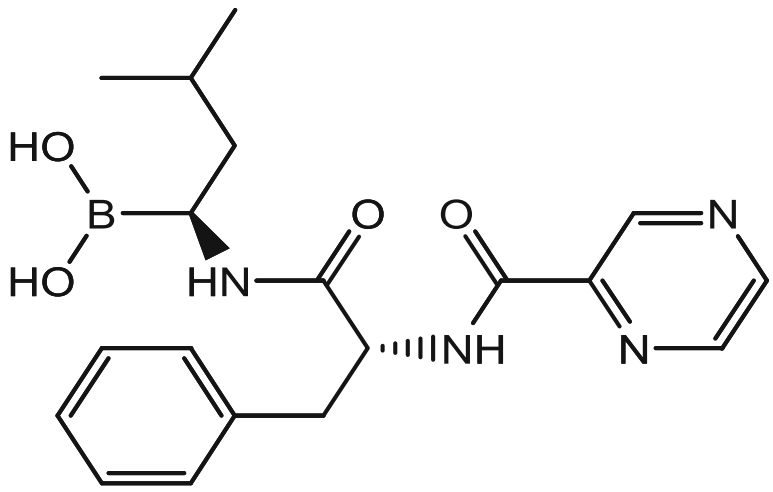


Fig. 2 Chemical structure of Bortezomib (1S,2R)-enantiomer

Table 1 Extraction for the use of different solvent details

S. no.	Solvent name	Appearance of solution, clear solution (yes/no)	Observations
1	Methanol	No	Observed Bortezomib peak response was very low.
2	Ethanol	No	Observed Bortezomib peak response was very low.
3	Dimethyl formamide	Yes	Bortezomib peak response was not detected.
4	Dimethyl sulfoxide	Yes	Bortezomib peak response was not detected.
5	Chloroform	No	Observed Bortezomib peak response was very low.
6	Ethyl acetate	No	Observed Bortezomib peak response was very low.
7	Water	Yes	Further, chloroform added to the aqueous clear solution and extracted the Bortezomib. Observed Bortezomib peak response was found not satisfactory.
8	Water	Yes	Further, ethyl acetate added to the aqueous clear solution and extracted the Bortezomib. Observed Bortezomib peak response was found satisfactory.

In this work, a new stereo-selective isocratic NP-high performance liquid chromatography technique was established and validated for the direct parting of enantiomers of Bortezomib and determination of (1S,2R)-enantiomer impurity in the Bortezomib lyo injection formulation. In this method, remaining isomeric impurities are also well separated.

Methods

Chemicals and reagents

n-Heptane, isopropyl alcohol, ethyl alcohol, trifluro acetic acid, and ethyl acetate (AR grade) was procured from Merck, India. Bortezomib (1S,2R)-enantiomer impurity was procured from Sisco Research Laboratories (SRL), Hyderabad, India. The drug substances and Bortezomib finished dosage form (lyo injection) "Bortezomib" for research obtained from Jodas Expoim Pvt. Ltd, Hyderabad, India.

Mobile phase

Prepare a mixture of 820 mL n-heptane, 150 mL isopropyl alcohol, 30 mL of ethyl alcohol, and 1 mL trifluoroacetic acid. Sonicate to degas for 5 min.

Preparation of diluent

Prepare a mixture of 500 mL ethyl acetate and 500 mL of mobile phase in the proportion of (50:50 volume/volume). Sonicate to degas for 5 min.

Chromatographic conditions

Normal phase-LC analysis was carried out on Agilent-1260 Infinity series (Agilent Corporation, USA) Chiral Pak ID-3 (250 × 4.6 mm, 3 μm) column was utilized as immobile segment, movable segment consisting of n-heptane, 2-propanol, ethyl alcohol, and TFA (82:15:3:0.1, v/v/v/v). The flow rate of the movable segment be reserved at 0.6 mL/min. The injection volume was set as 20 μL. Column heater temperature 25 °C and auto

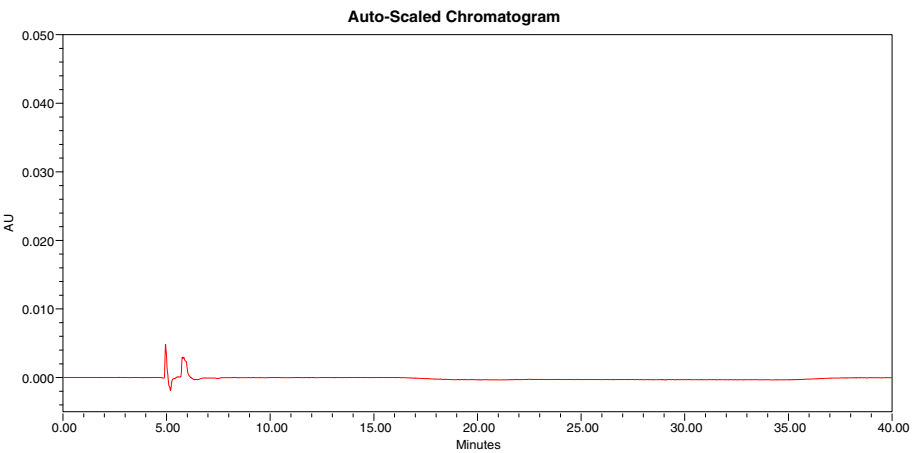


Fig. 3 Typical chromatogram of blank

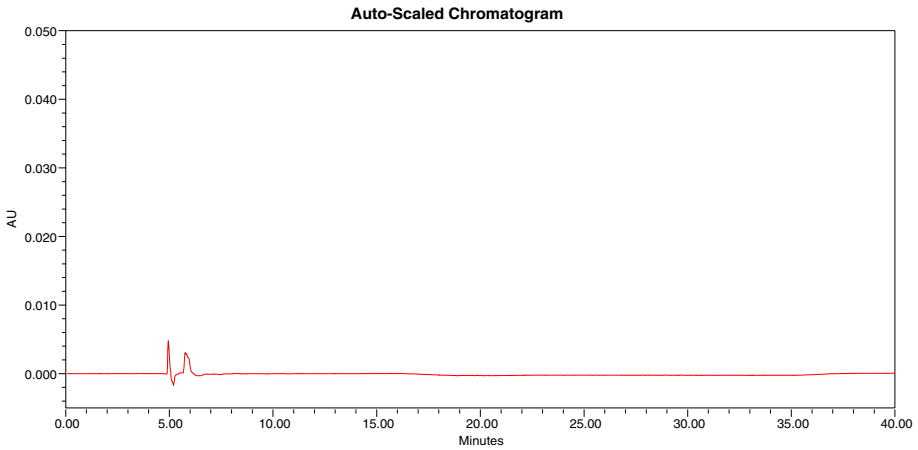


Fig. 4 Typical chromatogram of placebo

sampler temperature ambient and detection wavelength 270 nm were used.

Preparation of blank

Diluent used as blank.

(1S,2R-enantiomer) Impurity stock solution preparation

Precisely weighed and transferred 2.5 mg of (1S,2R-enantiomer) impurity, into a 50-mL volumetric flask. Later, added ethyl acetate (5 mL) and dissolved the contents. Finally, diluted to the volume with ethyl acetate and blended well.

Preparation of system suitability solution

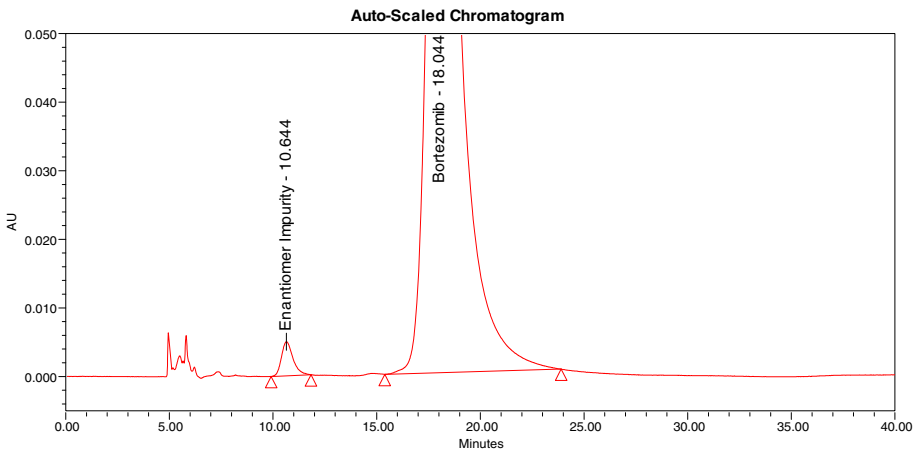
Precisely weighed and transferred 5 mg of Bortezomib standard into a 10-mL Borosil volumetric flask. Added 4 mL of ethyl acetate and dissolved the contents. Added (1S, 2R-enantiomer) impurity stock solution (1 mL) and diluted to the volume with same mobile phase and blended well.

Preparation of standard solution

Transferred 1 mL of (1S,2R-enantiomer) impurity stock solution into 10 mL volumetric flask. Added 4 mL of ethyl acetate and diluted the remaining volume with mobile phase and blended well.

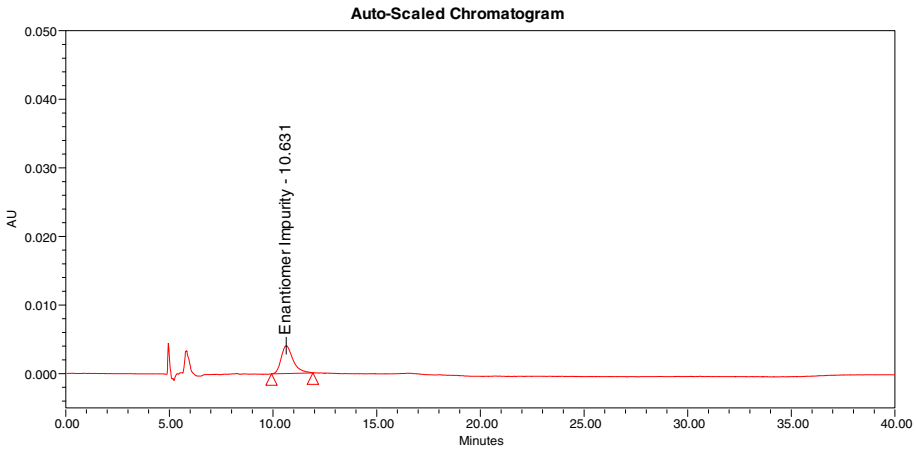
Preparation of placebo solution

Taken 1 vial of Bortezomib for injection placebo. Added 0.5 mL of water and dissolve the contents. Added 3.5 mL of ethyl acetate. Shake the contents vigorously for 5 min and settled the contents for 5 min. Separated the ethyl acetate layer and transferred 1 mL of ethylacetate layer



	Peak Name	Retention Time (min)	Area	% Area	USP Resolution	RT Ratio	USP Tailing	USP Plate Count	Int Type
1	Enantiomer Impurity	10.644	192457	1.39		0.59	1.30	1820	BB
2	Bortezomib	18.044	13651128	98.61	4.2	1.00	1.57	909	BB

Fig. 5 Typical chromatogram of System suitability solution



	Peak Name	Retention Time (min)	Area	% Area	USP Tailing	USP Plate Count	Int Type
1	Enantiomer Impurity	10.631	162030	100.00	1.39	1717	BB

Fig. 6 Typical chromatogram of standard

into 5 mL volumetric flask and added 1 mL of mobile phase and blended well.

Preparation of sample solution

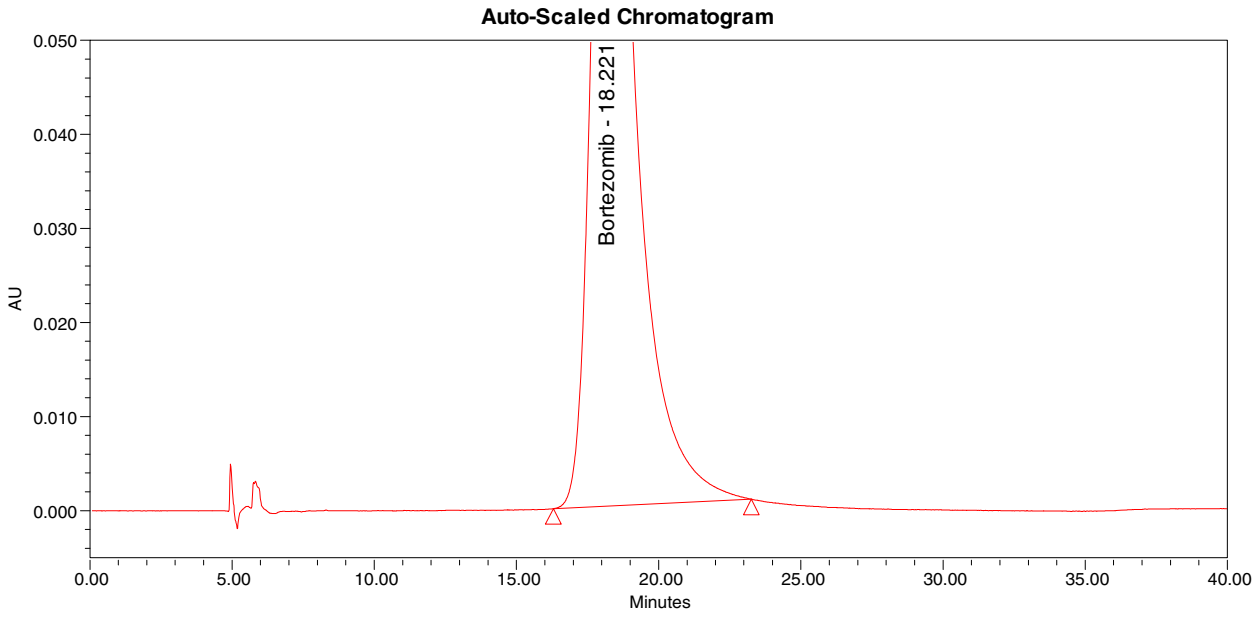
Taken 1 vial of Bortezomib for injection sample. Added 0.5 mL of water and dissolve the contents. Added 3.5 mL of ethyl acetate. Shake the contents vigorously for 5 min and settled the contents for 5 min. Separated the ethyl acetate layer, and transferred 1 mL of ethyl acetate layer into a 5-mL volumetric flask and added 1 mL of mobile phase, and blended well.

Results

Method development

Experiment 1

To develop the method for the determination of enantiomer (1S, 2R Isomer) impurity in Bortezomib lyo



	Peak Name	Retention Time (min)	Area	% Area	RT Ratio	USP Tailing	USP Plate Count	Int Type
1	Bortezomib	18.221	10495091	100.00	1.00	1.59	909	BB

Fig. 7 Typical chromatogram of As such sample

Table 2 System suitability results

Component name	Retention time (min)	Relative retention time (RRT)	Resolution	Tailing factor	Theoretical plates
(1S,2R-enantiomer) impurity	10.57	0.59	4.20	1.36	1784
Bortezomib	17.98	1.00		1.54	891

injection formulation by using column stationary phase Chiralpak AD-H (5 µm, 4.6 × 250 mm) HPLC column, with n-hexane, ethanol, 2-propanol, methanol, and TFA (82:8:8:2:0.5, v/v/v/v) mobile phase. Separation between enantiomer impurity and Bortezomib was found very less.

Experiment 2

For next trial, Chiralpak ID-3 (3 µm, 4.6 × 250 mm) HPLC column, with n-heptane, 2-propanol, ethanol, and TFA (82:15:3:0.1, v/v/v/v) mobile phase respectively. Separation between enantiomer impurity and Bortezomib peaks found satisfactory as well as no interference was observed with impurity peaks of Bortezomib.

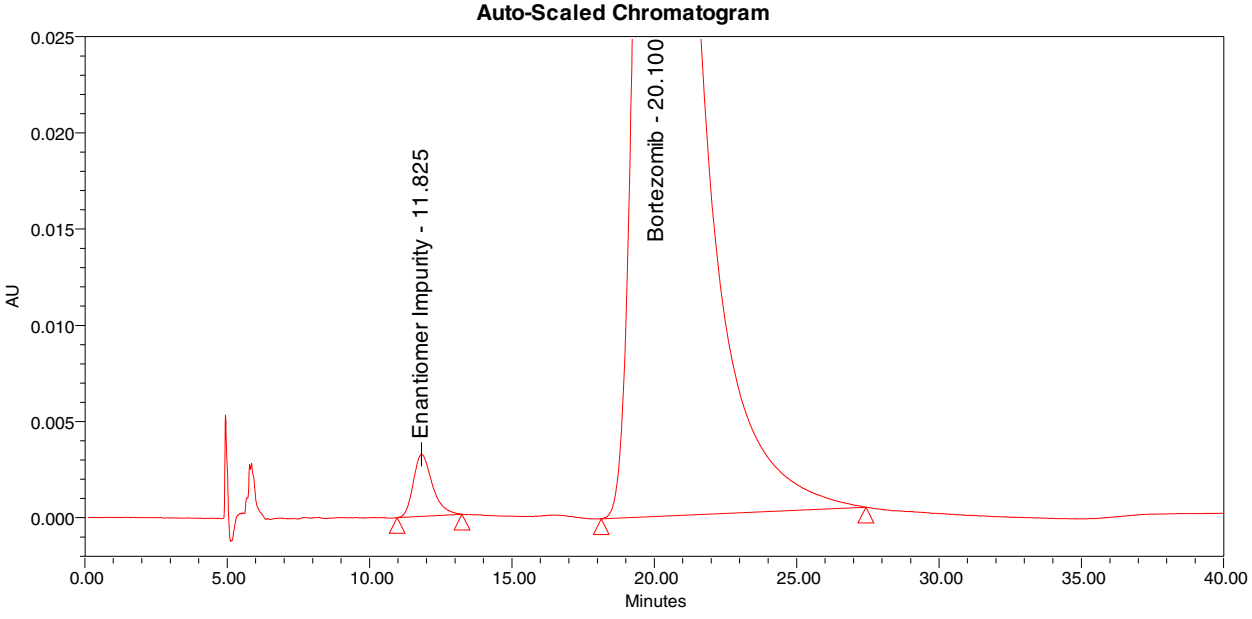
Experiment 3

For extraction of Bortezomib from its lyo injection formulation, the Bortezomib finished product dissolved in water. As aqueous phase should not be injected into normal phase, it should be extracted with other solvents like chloroform and ethyl acetate. Initial tried with chloroform added to the aqueous clear solution and extracted the Bortezomib. Observed Bortezomib peak response was found not satisfactory.

Further extraction with ethyl acetate was found satisfactory as the Bortezomib peak response meeting the expectation. So, ethyl acetate can be used for extraction of Bortezomib from its finished product. Extraction of enantiomer impurity and Bortezomib use different solvent details mentioned in Table 1. Hence, the elution order was observed from the chromatograms (Figs. 3, 4, 5, and 6).

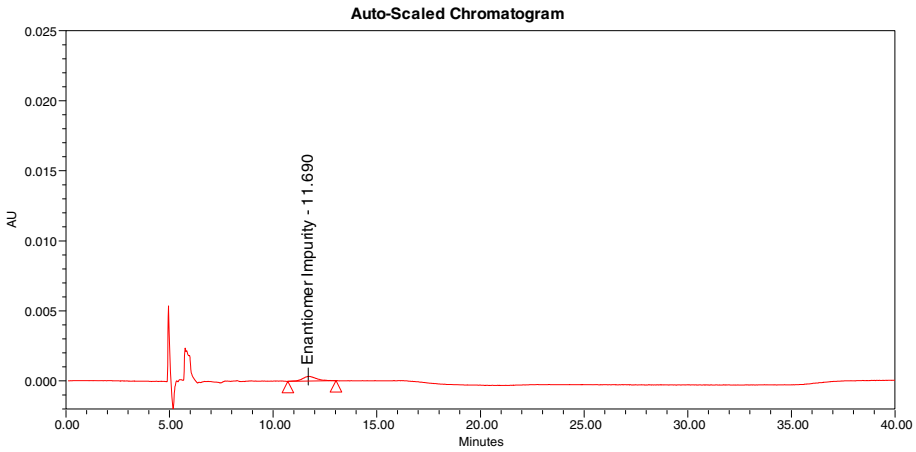
Analytical method validation

Analytical technique corroboration is the methodology of exhibiting that scientific techniques are suitable for their expected use. As per ICH guidelines, the method was validated using the following parameters.



	Peak Name	Retention Time (min)	Area	% Area	USP Resolution	USP Tailing	USP Plate Count	Int Type	RT Ratio
1	Enantiomer Impurity	11.825	149849	1.38		1.29	1559	BB	0.59
2	Bortezomib	20.100	10721125	98.62	3.9	1.78	774	BB	

Fig. 8 Typical chromatogram of spiked sample



	Peak Name	Retention Time (min)	Area	% Area	USP Tailing	USP Plate Count	Int Type
1	Enantiomer Impurity	11.690	15722	100.00	1.10	1462	BB

Fig. 9 Typical chromatogram of LOQ precision sample

System suitability testing

Equilibrated the chromatographic system with mobile phase until constant baseline is observed and solutions were injected as per sequence and parameters required for system suitability were recorded. System suitability test was performed each day before starting the parameter (Fig. 7). Results obtained are tabulated in Table 2.

Specificity

Blank and placebo interference A study to ascertain the meddling of blank and placebo was performed. Diluent and placebo were injected into the column to identify the above chromatographic conditions and recorded the blank and placebo chromatograms. The chromatogram obtained for the blank solution (Fig. 8) showed no peak at the retention time of (1S,2R-enantiomer) impurity and Bortezomib analyte peak. This has indicated that the diluent solution used in sample preparation does not interfere in estimation of (1S,2R-enantiomer) impurity in Bortezomib lyo injection formulation. Similarly, chromatogram obtained for the placebo solution (Fig. 9) showed no peaks at the retention time of (1S,2R-enantiomer) impurity and Bortezomib analyte peak. This was also indicated that the placebo used in the preparation sample solution does not interfere in estimation of (1S,2R-enantiomer) impurity in Bortezomib lyo injection formulation.

Precision

System precision System precision was exhibited by preparing blank, sensitivity solution, system appropriateness solution, and standard solution as per test method and chromatographed the same into HPLC system. The pinnacle regions and retention time of analyte were recorded for these system appropriateness injections. The system precision was assessed by processing the % RSD

Table 3 System precision results

S. no.	(1S,2R-enantiomer) impurity	
	Retention time	Area response
1	11.731	145160
2	11.795	142381
3	11.807	144684
4	11.801	143904
5	11.782	143112
6	11.756	145429
Average	**11.779**	**144112**
STD DEV.	**0.029548**	**1199.9668**
% RSD	**0.3**	**0.8**

Table 4 Results of method precision

Preparation	(1S,2R-enantiomer) impurity (% recovery)
1	104.0
2	104.4
3	103.8
4	104.5
5	104.4
6	105.7
Average	**104.5**
STD DEV.	**0.6623**
% RSD	**0.6**

Table 5 LOD for (1S,2R-enantiomer) impurity

Component name	Inj-1		Inj-2		Inj-3		Mean area	Mean S/N
	Area	S/N	Area	S/N	Area	S/N		
(1S,2R-enantiomer) impurity	4645	4.5	4701	3.9	4678	5.1	**4675**	**4.5**

for the peak area and retention time of these system suitability injections. The observations are tabulated in Table 3.

Method precision At the specification level, the precision of the impurity was calculated by injecting six sample solutions spiked with impurities (1S,2R-enantiomer) impurity. The samples were prepared as per the method and the results of the precision study were tabulated in Table 4. The % RSD of method precision was found to be 0.6% for (1S,2R-enantiomer) impurity.

Limit of detection and limit of quantitation

A solution containing 0.282 µg/mL of (1S,2R-enantiomer) impurity standard was injected three times. The poorest value of signal to noise ratio for each peak was greater than 3 in each injection and all the peaks were detected in all the three injections.

A solution containing 0.896 µg/mL of (1S,2R-enantiomer) impurity standard was injected six times. The relative SD of areas, deviations of each six replicates from the linear regression curve, and average deviation for each standard were calculated.

The limit of quantitation and limit of detection values obtained for (1S,2R-enantiomer) impurity were within the acceptable range and results are tabulated in Tables 5 and 6.

Linearity

Linearity was determined by injecting the solutions in duplicate containing (1S,2R-enantiomer) impurity ranging from LOQ to 150% of the specified limit. Performed the regression analysis and determined the correlation co-efficient and residual summation of squares. Determined the response factor for (1S,2R-enantiomer) impurity with respect to Bortezomib. Reported the linearity range as the range for determining the impurity. Results obtained are in the table and figure

Table 6 LOQ for (1S,2R-enantiomer) impurity

Component name	Inj-1	Inj-2	Inj-3	Inj-4	Inj-5	Inj-6	Avg.	% RSD
(1S,2R-enantiomer) impurity	15722	16328	16204	15039	15727	15002	**15670**	**3.6**

Table 7 Linearity of detector response (1S,2R-enantiomer) impurity

Level (%)	Concentration (µg/mL)	Mean area
LOQ	0.896	10596
50	4.975	80048
75	7.463	117207
100	9.950	152141
125	12.438	200140
150	14.925	235214
R^2 value		0.9986
% *Y*-intercept		1.62
Slope		15.995.7106
Intercept		2464.7928

shows the line of best fit for peak area versus concentration and results were tabulated in Table 7.

Accuracy

Recovery of (1S,2R-enantiomer) impurity in Bortezomib was calculated. The sample was taken and varying amounts of enantiomer impurity representing LOQ to 150% of specification level were added to the analytical flasks. The spiked examples were set up according to the strategy and the outcomes are organized in Table 8.

Solution stability

The sample solution was injected into HPLC initially; after 24 h and after 48 h at each interval, the % area of (1S,2R-enantiomer) impurity in spiked solution was

Table 8 Recovery results of (1S,2R-enantiomer) impurity

(1S,2R-enantiomer) impurity			
% Level	**% Recovery**	**% Mean recovery**	**% RSD**
LOQ	85.6	**85.9**	**2.0**
	87.8		
	84.4		
50%	101.4	**101.3**	**1.7**
	99.6		
	103		
100%	104.3	**105.8**	**1.8**
	105.2		
	108		
150%	103.5	**104.3**	**1.4**
	103.3		
	106		

Table 9 Results of standard solution stability

Time interval	% Recovery for standard solution	
	Bench top	Refrigerator condition (2-8 °C)
0 h	NA	NA
48 h	101.5	100

recorded and the difference in % area with respect to % area obtained at initial day interval was calculated.

Solution stability parameter was established and standard and sample solutions were consistent for 48 h on bench top and in cooler (2-8 °C) condition and results are tabulated in Tables 9 and 10.

Robustness

Heftiness of test technique was established by preparing all system appropriateness solutions as per test technique and chromatographed same into the HPLC system. Carrying out system appropriateness under normal circumstances and a piece of the changed conditions mentioned below. The heftiness was evaluated by report in the system appropriateness parameters as per test technique in Table 11.

Discussion

An easy, economic, accurate, and precise normal phase-HPLC method was established successfully. The parting was achieved on Chiral Pak ID-3 (3 µm, 4.6 × 250 mm) column (amylose-based chiral stationary phase) [17] using a movable segment consisting of n-heptane, 2-propanol, ethyl alcohol, and TFA (82:10:30:0.1, volume/volume/volume/volume) at a flow velocity of 0.6 mL/min. Column temperature preserved 25 °C, injection level 20 µL, sample cooler temperature ambient, and detection wavelength 270 nm [18]. The results got were found to be accurate and reproducible. The technique developed was statistically authenticated in stipulations of selectivity, accuracy, linearity, precision, robustness, and stability of solution as per ICH [19] and USP [20] guidelines.

For knowing the selectivity, the chromatograms were recorded for standard and sample solutions of (1S,2R-enantiomer) impurity and Bortezomib. This study revealed that the peaks were well separated from each other. Therefore, the method was selective for the determination of (1S,2R-enantiomer) impurity in Bortezomib lyo injection formulation. There is no interference of diluent and placebo at (1S,2R-enantiomer) impurity and Bortezomib peaks.

Table 10 Results of spiked test sample solution stability

Time interval	% Recovery for (1S,2R-enantiomer) impurity	
	Bench top	Refrigerator condition (2-8 °C)
Initial	104	
24 h	100.3	99.8
48 h	104.6	103.4

Table 11 Robustness results for (1S,2R-enantiomer) impurity

Parameter	Altered Condition	S/N	Resolution	Tailing factor	% RSD
As such condition (method precision)		**69**	**3.9**	**1.33**	**0.8**
Flow variation (0.6 mL/min)	Low flow (0.5 mL/min)	51	3.5	1.39	0.6
	High flow (0.7 mL/min)	52	3.8	1.32	0.8
Temperature variation (25 °C)	Low Temperature 20 °C	60	3.5	1.35	0.6
	High Temperature 30 °C	73	4.7	1.33	0.6
Mobile phase Composition variation **(IPA)**	**Low variation** n-Heptane: isopropyl alcohol: ethanol: trifluoroacetic acid-(835:135:30:1) v/v/v/v	53	3.8	1.35	0.3
n-Heptane: isopropyl alcohol: ethanol: trifluoroacetic acid-(820:150:30:1) v/v/v/v					
	High variation n-Heptane: isopropyl alcohol: ethanol: trifluoroacetic acid-(805:165:30:1) v/v/v/v	79	4.2	1.36	0.4
Mobile phase Composition variation **(ethanol)**	**Low variation** n-Heptane: isopropyl alcohol: ethanol: trifluoroacetic acid-(823:150:27:1) v/v/v/v	73	4.1	1.39	0.8
	High variation n-Heptane: isopropyl alcohol: ethanol: trifluoroacetic acid-(817:150:33:1) v/v/v/v	65	4.3	1.41	0.6

The LOD and LOQ for (1S,2R-enantiomer) impurity standard were 0.282 and 0.896 µg/mL respectively. The linearity results for (1S,2R-enantiomer) impurity in the determined or specified concentration range were found satisfactory, with a correlation coefficient value greater than 0.99 (Fig. 10). A calibration curve was plotted and the correlation co-efficient for enantiomeric impurity was found to be 0.9986. The accuracy studies were found as % recovery for (1S,2R-enantiomer) impurity at specification level. The limit of % recovered shown was in the range of 80 to 120%, and the results obtained were found to be within the limits. Hence, the method was found to be accurate. Further, the relative SD values of recoveries obtained for enantiomeric impurity are in the range of 1.4-2.0%.

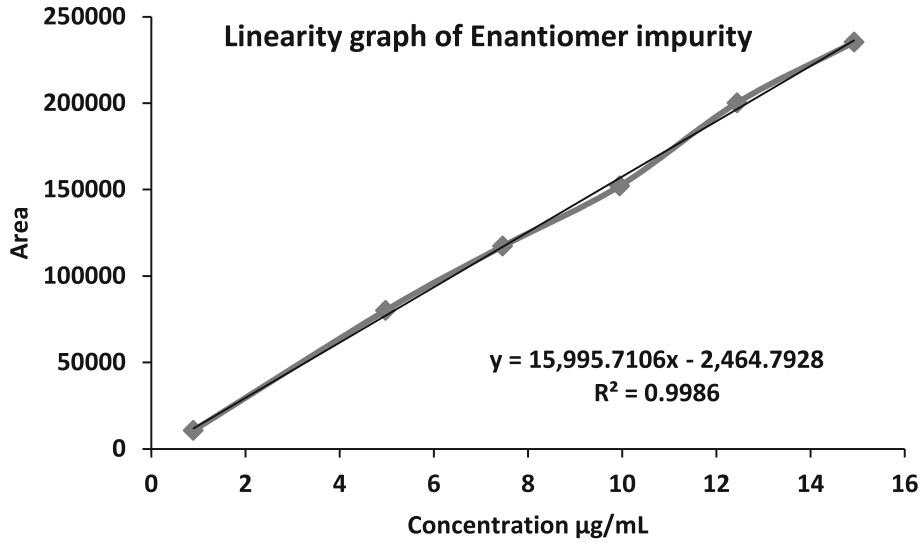


Fig. 10 Linearity of detector response for (1S,2R-enantiomer) impurity

To determine precision studies, six (6) replicate injections were carried out. The % RSD was determined from the peak areas of (1S,2R-enantiomer) impurity found to be 0.60%. The acceptance limit should not be > 10, and the results were originated to be within the acceptance limits.

Hence, the chromatographic technique developed for (1S,2R-enantiomer) impurity in Bortezomib lyo injection formulation was rapid, simple, precise, sensitive, and accurate. Therefore, the proposed technique is useful for the routine analysis of the active pharmaceutical ingredients for the declaration of its quality during its formulation.

Conclusion

The proposed normal phase-HPLC method that can quantify (1S,2R-enantiomer) impurity in Bortezomib lyo injection formulation at trace level concentration has been urbanized and authenticated as per ICH guidelines. The effectiveness of the technique was ensured by the specificity, exactitude, linearity, and accuracy. Hence, the method well suit for their intended purposes and can be successfully useful for regular analysis in laboratories and is suitable for the quality control.

Abbreviations

NP-HPLC: Normal phase high-performance liquid chromatography; ICH: International Conference on Harmonization; LOD: Limit of detection; LOQ: Limit of quantification; RSD: Relative standard deviation

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Authors' contributions

We have assured that "all authors have read and approved the manuscript." All the authors have equal contribution and participation in this research work. SB has analyzed all samples on NPLC instrument and completed the experimental work and was a major contributor in writing the manuscript. He had completed his work under the supervision of HB who help him to elaborate the methodology as well as theoretical approach.

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Ethics approval and consent to participate

Not applicable

Consent for publication

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Competing interests

No competing interests to declare.

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References

1. "Bortezomib monograph for professionals". Drugs.com. Retrieved 13 October 2019.
2. "Velcade". European Medicines Agency. 17 September 2018. Retrieved 13 October 2019.
3. Adams J, Stein R (1996) Novel inhibitors of the proteasome and their therapeutic use in inflammation. Annu Rep Med Chem 31(C):279–288
4. Adams J (2004) The proteasome: a suitable antineoplastic target. Nat Rev Cancer 4(5):349–360
5. Adams J, Ma Y, Stein R, Baevsky M, Grenier L, Plamondon L (1996) Boronic ester and acid compounds, synthesis and uses. US, 1448.012TW01
6. Richardson PG, Hideshima T, Anderson KC (2003) Bortezomib (PS-341): a novel, first-in-class proteasome inhibitor for the treatment of multiple myeloma and other cancers. Cancer Control 10(5):361–369
7. Snow RJ, Bachovchin WW (1995) Boronic acid inhibitors of dipeptidyl peptidase IV. A new class of immunosuppressive agents. Adv Med Chem 3(C):149–177
8. European Medicines Agency (2004) European public assessment report: scientific discussion. The committee for medicinal products for human use.
9. Rambabu C, Venkatrao S, Ramu G, Ganesh M (2011) Estimation of bortezomib in bulk and its pharmaceutical dosage forms by using a novel

validated accurate reverse phase high performance liquid chromatography. Int J Pharm Pharm Sci 3:303–305
10. Srinivasulu K, Naidu MN, Rajasekhar K, Veerender M, Suryanarayana MV (2012) Development and validation of a stability indicating LC method for the assay and related substances determination of a proteasome inhibitor bortezomib. Chromatogr Res Int:1–13
11. Utage M, Swamy BMV (2013) Analytical method development and validation of related substance method for bortezomib for injection 3.5 mg/vial by RP-HPLC method. Int J Pharm Res Sch 2:27–32
12. Leveque D, Carvalho MCM, Maloisel F (2007) Clinical pharmacokinetics of bortezomib. In vivo (Athens, Greece) 21:273–278
13. Bisht AK, Bhushan B, Dhiman V, Dhawan RK, Baghel US, Gupta AK et al (2013) Gradient RP-HPLC method development for bortezomib in parenteral dosage form. J Biomed Pharm Res 2:10–15
14. Raju VVSSA, Mohamud AB, Pathi PJ, Raju NA (2014) The estimation of bortezomib in powder for injection dosage forms by RP-HPLC. Int J Pharm Arch 3:269–272
15. Rao SV, Srinivasarao M, Ramu G, Rambabu C (2012) UV visible spectrophotometric determination of bortezomib in its bulk and formulation dosage forms. Pharm Lett 4:720–727
16. Kamalzadeh Z, Babanezhad E, Ghaffari S, Ezhiyeh AM, Mohammadnejad M, Naghibfar M, Bararjanian M, Attar H (2017) Determination of bortezomib in API samples using HPLC: assessment of enantiomeric and diastereomeric impurities. J Chromatogr Sci 55(7):697–705
17. Ahmed M, Gwairgi M, Ghanem A (2014) Conventional Chiralpak ID vs. Capillary Chiralpak ID-3 amylose tris-(3-chlorophenylcarbamate)-based chiral stationary phase columns for the enantioselective HPLC separation of pharmaceutical racemates. Chirality 26(11):677–682
18. Chandra Sekhar K, Sudhakar P, Ramesh Reddy G, Vijaya Babu P, Linga Swamy N (2013) A new UV-method for determination of bortezomib in bulk and pharmaceutical dosage form. Int J Pharm Bio Sci 3(1):623–627
19. ICH guidelines (2005) Validation of analytical procedures: text and methodology, Q2A (R1).
20. United State Pharmacopoeia (2007) The U.S. pharmacopeia convention, 30th edn. United State Pharmacopoeia, Rockville

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