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Development And Validation Of HPLC Method For Determination Of Bortezomib In Pharmaceutical Preparation

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ABSTRACT

In this work an assay method for Bortezomib and its validation by using HPLC was carried out. Bortezomib is a new drug not much analytical work is done over it. And it is an anticancer drug and is very toxic too. So an adequate estimation of drug in dosage forms is very essential. So here we will develop and validate HPLC method to estimate Bortezomib available in injection form. We will develop an accurate, reliable and economical method to estimate Bortezomib.

Key-word : Bortezomib, HPLC validation, UVspectrophotometer, precision, accuracy.

INTRODUCTION

In recent years the international conference on harmonization of technical requirement for registration of pharmaceuticals for human use (ICH) has adopted Scientific standards for quality control monitoring[1] .These standards are the basis for most regulatory guidelines ,including those published by the food and drug administration[2] .Key steps on the path include pharmaceutical analysis and stability studies that are required to determine and assure the identity ,potency and purity of ingredients ,as well as those formulated products. Impurities are the unwanted chemicals that remain with active pharmaceutical ingredients. The presence of these chemicals may influence the efficacy and safety of the pharmaceutical products. So impurity profiling is now getting important critical attention from regulatory authorities.

MATERIALS AND METHODS

Materials

Bortezomib injection is used for the validation process. Main equipments used are HPLC,UV spectrophotometer, column of 250×4.6mm 10µ,packing L11.acetonitrile,THF,water (double distilled),methanol (HPLC grade) are used as solvents.

Methods

Standard testing procedure[3]

Examined by liquid chromatography, column hypersil of 250×4.6mm 10µ packing L 11,flow rate 1

ml per minute, load 20µl,detect at 280nm,run time 10 minutes, solvent HPLC grade water.

Standard preparation: 35 mg of Bortezomib standard in 100 ml water.

Sample preparation: vial make up to 10 ml with water.

Validation

A sample solution shall be prepared and injected as described under method of analysis. System suitability parameters shall be calculated and recorded in table.

Acceptance criteria

Tailing factor; tailing factor of Bortezomib peak should not be less than 1.5.

Theoretical plate; theoretical plate for Bortezomib peak should not be less than 2500.

Acceptance criteria; relative standard deviation of Bortezomib peak should not be more than 2.0%

Precision[4]

Method precision shall be established by determining the assay in six different preparations of a homogenous batch of Bortezomib. The average standard deviation and relative standard deviation shall be calculated and tabulated in the table.

Acceptance criteria; relative standard deviation of assay obtained for 6 different preparations should not be more than 2%.

System precision shall be evaluated by performing 6 replicate injections of sample solution of bortezomib at its target concentration .The individual peak responses shall be recorded and the average ,standard deviation and relative standard shall be calculated and recorded in table.

Acceptance criteria; relative standard deviation of retention times and areas of peak shall not be more than 2%.

Accuracy[5]

Accuracy can be determined by adding known amount of standard solution in the range 75-125 % of the target concentration.

Acceptance criteria; the percent recovery should be within 98-102%of the theoretical value.

Limit detection[6]

$$\text{Limit detection (conc.ppm)} = \frac{3.3 \times \text{solution B conc (PPM)}}{\text{S/N}}$$

Acceptance criteria; RSD % for area of Bortezomib is not more than 2.0%.

Limit quantification

$$\text{Limit quantification (conc.PPM)} = \frac{10.0 \times \text{solution B conc. (PPM)}}{\text{S/N}}$$

Acceptance criteria; RSD % of area of Bortezomib is not more than 10.0%

Robustness[7]

Stability study of solution shall be performed by injecting a solution of stability at its target 0,6,12 and 24 hrs at room temperature and refrigerator. The variation areas observed shall as studied and recorded in table.

Acceptance criteria ; % RSD for area should not be more than 2.0 % .percent relative difference for average area with respect to initial average area should not be more than 2.0%.

Flow rate[8]

Stabilize the instrument with 1.1 ml/min flow rate. Inject six times sample preparation .and record chromatograms .Observe the theorettical plates, tailing factor, and RSD % for bortezomib peak.

Acceptance criteria; the method shall be considered to be validated when results obtained in validation comply with the acceptance criteria describe each test and related documents are checked and verified.

RESULT AND DISCUSSION

System Suitability

Table 1

Name	**RT**	**Tailing Factor**	**Theoretical Plate**
BORTEZOMIB	6.560	1.04283	5028

Table 2

Replicates	**Area**
Replicate 1	56774183
Replicate 2	55568983
Replicate 3	56269534
Replicate 4	56167745
Replicate 5	56498127
Replicate 6	56539954
Average	56303087.67

Symmetry factor: Tailing factor for BORTEZOMIB injection peak should not be more than 1.5

Theoretical Plate: Theoretical plate for BORTEZOMIB injection peak should not be les than2500

Acceptance Criteria: Relative Standard Deviation for BORTEZOMIB injection peak should not be more than 2.0%

Table 3 **System Precision**

Replicates	**RT**	**Area**
1	3.378	56765898
2	3.377	56987651
3	3.381	56687456
4	3.380	56777754
5	3.386	56672485
6	3.376	56172584
Average	3.380	56677304.67
Standard deviation	0.004	271706.79
Relative standard deviation(%)	0.107	0.479

Acceptance criteria : Relative standard deviation of retention times and areas of peak shall not be more than 2.0%

Table 4 **Method Precision**

Replicates	**Assay (%)**
1	100.037
2	99.995
3	100.068
Average	100.033333
Standard deviation	0.03663787
Relative Standard Deviation	0.03662566

Acceptance criteria: RSD% of assay obtained for 6 different preparations should not be more than 2.0%

$$\textbf{Calculation Potency} = \frac{\text{Test solution Average area}}{\text{Standard solution average area}} \times \frac{\text{Standard solution wt. (mg)}}{\text{Test Solution (mg)}} \times \text{Std}$$

$$= \frac{56850248 \times 35.2 \times 99.6 \times 10}{56907517 \times 100 \times 100}$$

Assay- =100.068%

Assay-2

$$\frac{56808571 \times 35.2 \times 99.6 \times 10}{56907517 \times 100 \times 100} = \textbf{99.995\%}$$

Assay-3

$$\frac{56832305 \times 35.2 \times 99.6 \times 10}{56907517 \times 100 \times 100} = \textbf{100.068\%}$$

Table 5 **Intermediate Precision**

Sl. No.	**Date**	**Equipment**	**Analyst**	**Results**
1		HPLC003		99.99
2		HPLC005		100.04
Average				100.02
Standard deviation				0.04
Relative standard deviation (%)				0.04

Acceptance criteria : Relative standard deviation of assay obtained should not be more than 2.0%

$$\textbf{Calculation} = \frac{\text{Test solution Average area}}{\text{Standard solution average area}} \times \frac{\text{Standard solution wt. (mg)}}{\text{Test Solution (mg)}}$$

Std Potency

Table 6 **Accuracy / Recovery**

Sl. No.	**Injection**	**Proposed concentration to be spiked (% of specification limit)**	**Spiked quantity of BORTEZOMIB**	
			Area Observed	**Recovery (%)**
1.0	Replicate 1	75.0%	42879388	100.286%
	Replicate 2		42588440	
	Replicate 3		42709511	
2.0	Replicate 1	100.0%	56799565	101.154%
	Replicate 2		57405609	
	Replicate 3		56776009	
3.0	Replicate 1	125.0%	71165873	100.229%
	Replicate 2		70899871	
	Replicate 3		70989547	

Average Accuracy: 100.55%

Acceptance criteria : The percent recovery should be within 98-102% of the theoretical value.

Table 7 **Linearity & Range**

Sl. No.	**Concentration (%)**	**Area**
1.0	25%	16044
2.0	50%	32145
3.0	75%	49009
4.0	100%	65050
5.0	125%	81263

6.0	150%	97243

Acceptance Criteria : Correlation coefficient should not be less than 0.98

Limit of detection

Table 8 Blank Solution

Sl. No.	**Noise at RT (N) 2.5 to 5 min**
01	190
02	235
03	210
04	245
Average	220

Table 9 Sample solution

Sl. No.	**Signal of BORTEZOMIB (S)**
01	179204
02	157833
03	160929
04	180167
05	170983
06	155082
Average	167366.333

BORTEZOMIB S/N = 760.756061

LOD concentration of BORTEZOMIB (PPM) = 0.022

LOD Sol.- 10mg in 100ml then 1ml of this in 100ml again 2.2ml diluted to 100ml with Water

Table 10

Sl. No.	**BORTEZOMIB**		
	Concentration (PPM)	**Retention Time**	**Area**
1	0.022	3.389	9105
2	0.022	3.394	9386
3	0.022	3.399	9249
4	0.022	3.359	9527
5	0.022	3.371	9647
6	0.022	3.366	9689
Average	-	3.380	9433.833333
Standard Deviation	-	0.016	229.7010
% of RSD	-	0.487	2.435

Acceptance criteria: RSD % for area of BORTEZOMIB not more than 10.0%

Table 11 **Limit of quantification Blank Solution**

Sl. No.	**Noise at RT (N) 2.5 to 5 min**
01	190
02	235
03	210
04	245
05	220
06	190
Average	0.8616085

Table 12 **Sample solution**

Sl. No.	**Signal of BORTEZOMIB (S)**
01	179204
02	157833
03	160929
04	180167
05	170983
06	155082
Average	167366.333

BORTEZOMIB S/N = 760.756061

LOQ concentration of BORTEZOMIB (PPM) = 0.066 PPM

LOQ Sol. 10mg in 100ml then 1ml of this in 100ml again 6.6ml diluted to 100ml with Water

Table13

Sl. No.	BORTEZOMIB		
	Concentration (PPM)	Retention Time	Area
1	0.066 PPM	3.392	16044
2		3.377	16190
3		3.395	16342
4		3.369	16489
5		3.383	16575
6		3.401	16799
Average		3.386	16406.5
Standard Deviation		0.012	272.6512

% of RSD		0.355	1.662

Acceptance criteria: RSD% for area of BORTEZOMIB not more than 10.0%

Stability of analytical solutions

Table 14 **Room Temperature**

Time of Injection	**Area**	**Average Area**
Initial	56799565	57102587
	57405609	
6 hours	56729878	56757222
	56784565	
12 hours	56974291	56875590
	56776888	
24 hours	56498127	56519041
	56539954	
% RSD		0.43

Table 15 **Refrigerator**

Time of Injection	**Average Area**
Initial	56587456
6 hours	56733754
12 hours	56662485
24 hours	56172584
% RSD = 0.385	

Acceptance criteria : % RSD for area should not be more than 2.0%

Table 16 **Different column**

STANDARD PARAMETER				
Sl. No.	**Retention Time**	**Area**	**Theoretical Plates**	**Tailing Factor**
1	3.382	56774183	7325	1.20286
2	3.387	55568983	7335	1.21956
3	3.379	56269534	7392	1.20251
4	3.381	56167745	7389	1.20144
5	3.383	56498127	7393	1.20164
6	3.380	56539954	7388	1.20158
Average	3.382	56303087.7	7370.333333	1.204765
Standard Deviation	0.003	418193.7259	31.4558	0.0073
% of RSD	0.084	0.743	0.427	0.603

Table 17

Column No.: 0383962Q

Sl. No.	**Retention Time**	**Area**	**Theoretical Plates**	**Tailing Factor**
1	3.378	56765898	7441	1.20430
2	3.377	56987651	7405	1.20199
3	3.381	56687456	7399	1.20201
4	3.380	56777754	7397	1.20159
5	3.386	56672485	7388	1.20211
6	3.376	56172584	7379	1.20183
Average	3.380	56677304.67	7401.5	1.202305

Standard Deviation	0.004	271706.7993	21.3892	0.0010
% of RSD	0.107	0.479	0.289	0.083

Table 18

Column No.: 0186698Q

Sl. No.	**Retention Time**	**Area**	**Theoretical Plates**	**Tailing Factor**
1	3.382	56774183	7325	1.20286
2	3.387	55568983	7335	1.21956
3	3.379	56269534	7392	1.20251
4	3.381	56167745	7389	1.20144
5	3.383	56498127	7393	1.20164
6	3.380	56539954	7388	1.20158
Average	3.382	56303087.7	7370.333333	1.204765
Standard Deviation	0.003	418193.7259	31.4558	0.0073
% of RSD	0.084	0.743	0.427	0.603

Variation in Flow Rate

Table 19 CHANGED PARAMETER: 1.1 ml Flow

Sl. No.	**Retention Time**	**Area**
1	3.066	2693736
2	3.059	2677588
3	3.056	2679136
4	3.070	2694555
Average	3.063	2686253.75

Standard Deviation	0.006	9140.61
% of RSD	0.209	0.340

Table 20 0.9ml flow

Sl. No.	**Retention Time**	**Area**
1	3.751	3271642
2	3.759	3260736
3	3.745	3298334
4	3.761	3225567
Average	3.754	3264069.75
Standard Deviation	0.007	30138.31
% of RSD	0.197	0.923

Table 21 pH Increase

Sl. No.	**Retention Time**	**Area**
1	3.737	3487000
2	3.741	3412360
3	3.731	3462008
4	3.749	3449087
Average	3.740	3452613.75
Standard Deviation	0.008	31109.85
% of RSD	0.202	0.901

Table 22 pH Decrease

Sl. No.	**Retention Time**	**Area**
1	3.066	2794283
2	3.061	2769027
3	3.069	2774663
4	3.070	2780150
Average	3.067	2779530.75
Standard Deviation	0.004	10832.60
% of RSD	0.132	0.404

CONCLUSION

As we know Bortezomib is an anticancer drug and it is very toxic, so to control and estimate the accurate amount of drug in injection form we developed and validate HPLC method.In this method we used acetonitrile and water in major quantity and tetrahydrofuran in minor quantity. This method is quite reliable because it justifies all the validation parameters.It is economical method because it consumes chemicals that are not so expensive and also consumes fewer amounts of chemicals due to short run time.It is also a beneficial method because is consumes mixtures of acetonitrile and water .This mixture is very useful in increasing the life of column.In this way we can say that this method is quite reliable, accurate, economical and beneficial too.

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