Determination of force degradation and solution stability studies of (bortezomib) proteasome inhibitor by HPLC method

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ABSTRACT

A forced degradation study is an essential step in the design of a regulatory compliant stability program for both drug substances and products, and formalized as a regulatory requirement in ICH Guideline Q1A in 1993. In designing forced degradation studies of Bortezomib, remembered that more strenuous conditions than those used for accelerated studies (25°C/60% RH or 40°C/75% RH) are used. Investigation was performed in conditions acid like base hydrolysis, hydrolysis at various pH, thermal degradation, photolysis, and oxidation. Forced degradation is a degradation of new drug substance and drug product at conditions more severe than accelerated conditions. Solution stability showing that more than 98 % of the Bortezomib remained unchanged after storage at room temperature for 12 h, on the basis of comparison of peak areas with those obtained from a freshly prepared solution of Bortezomib (200 ppm). This research paper showing solution stability and forced degradation studies of Bortezomib substances and Bortezomib products by providing a strategy for conducting studies on degradation mechanisms.

KEY WORDS: HPLC, Proteasome inhibitor, Bortezomib, Force degradation studies, solution stability.

1. INTRODUCTION

Bortezomib is the first therapeutic proteasome inhibitor to be tested in humans. It is approved in the U.S. for treating relapsed multiple myeloma and mantle cell lymphoma. Bortezomib was originally synthesized in 1995 and in May 2003 Bortezomib was approved in the United States by the Food and Drug Administration (FDA) for use in multiple myeloma. Forced degradation studies are also known as stress testing or stress studies and is a process that involves degradation of drug products and drug substances at conditions more severe than accelerated conditions. This degradation generates degradation products that can be studied to determine the stability of the molecule.

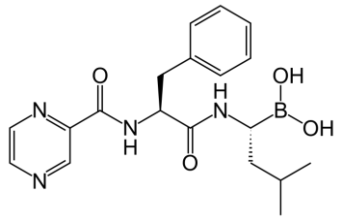


Figure.1.Chemical structure of Bortezomib

IUPAC Name: [(1R)-3-methyl-1-({(2S)-3-phenyl-2-[(pyrazin-2-ylcarbonyl) amino] propanoyl} amino) butyl] boronic acid. The ICH guideline states that stress testing is intended to identify the likely degradation products which further helps in determination of the intrinsic stability of the molecule and establishing degradation pathways, and to validate the stability indicating procedures used.

Force degradation studies are described various International guidelines. The International committees for Harmonization of Technical Requirements for Registration of pharmaceutical Human use (ICH) has published a set of guidelines which have been discussed, agreed upon and adopted by the American, European and Japanese regulatory authorities. In the majority of cases, the ICH guidelines only apply to the marketing applications for new products, i.e they do not apply during clinical development. However, since the conditions used for forced degradation are only defined in general terms, it is possible to apply them for developing stability indicating methods during clinical development. The same forced degradation conditions can then be applied to the drug substance during development and commercialization.

The question of how much stressing is enough has been the subject of much discussion amongst pharmaceutical scientists. In general, values anywhere between 5% to 20% degradation of the drug substance have been considered as reasonable and acceptable for validation of chromatographic assays. However, for small pharmaceutical molecules for which acceptable stability limits of 90% of label claim is common, pharmaceutical scientists have agreed that approximately 10% degradation is optimal for use in analytical validation. In the event that the experimental conditions generate little or no degradant due to the exceptional stability of the molecule, an evaluation should be made to verify if the drug substance has been exposed to energy in excess of the energy provided by accelerated storage (ie, 40°C for 6 months). It must be noted that a forced degradation study is a "living

process" and should be done along the developmental time line as long as changes in the stability-indicating methods, manufacturing processes, or formulation changes are ongoing. Forced degradation is only considered complete after the manufacturing process is finalized, formulations established, and test procedures developed and qualified.

2. MATERIALS AND METHODS

HPLC grade acetonitrile and AR grade formic acid and other reagents were procured from S.D. Fine chemicals Ltd. High pure standard and market samples were used for this study. Hypersil BDS packed with Octadecylsilyl silica gel column was used for this product

2.1 Chromatographic conditions:

Instrumentation: Shimadzu LC 2010A HT system was used for chromatographic analysis and equipment operated with Class VP software.

Mobile Phase: Acetonitrile: Water: Formic Acid (35:65:0.1)

Diluent: Acetonitrile: Water (40:60)

Column: Hypersil BDS packed with Octadecylsilyl silica gel (100 mm x 4.6mm x 3µm)

Flow rate: 0.8 mL per minute

Wavelength : 270 nm

Inj. Volume : 20 µL

Run time: 20 minutes

Column Temperature: 25 0C

Blank Solution: Diluent

Standard Preparation (200 ppm): Transferred accurately weighed 20 mg of Bortezomib WS into 100 ml volumetric flask containing 70 ml of diluent, shake well to dissolve. Sonicate

if necessary for 5-10 minutes. Make up the volume with diluents and mixed.

Sample Preparation (200 ppm): Prepared the all dosage forms and Bortezomib API equivalent to 0.2 mg/ml of Bortezomib with diluent.

System suitability: USP theoretical plates for standard solution should be not less than 2000.

Calculation: % of Assay: (Area of test sample x Concentration of Standard x Potency of Standard) / (Area

of Standard x Concentration of sample)

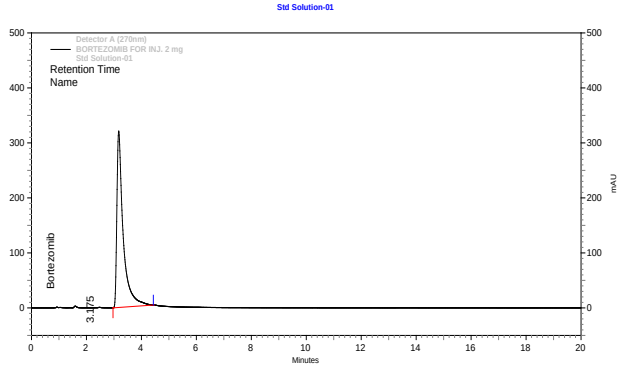


Figure.2.Chromatograph of Bortazomib by RP-HPLC

Table.1.Results of chromatographs

Name	Retention Time	Area	Theoretical plates	Tailing Factor
Bortezomib	3.175	6113608	2480.20	1.81

3. RESULTS AND DISCUSSION

3.1. Degradation conditions of Bortezomib: In designing forced degradation studies, it must be remembered that more strenuous conditions than those used for accelerated studies (25°C/60% RH or 40°C/75% RH) should be used. At a minimum, the following conditions should be investigated: (1) acid and base hydrolysis, (2) hydrolysis at various pH, (3) thermal degradation, (4) photolysis, and (5) oxidation.

3.2. Hydrolytic conditions: Hydrolysis is one of the most common degradation pathways that include decomposition of a chemical compound by reaction with water and with wide range of pH solution. Hydrolytic study under acidic and basic condition involves catalysis of ionisable functional groups present in the molecule. Acid or base stress testing involves forced degradation of a drug substance by exposure to acidic or basic conditions which generates primary degradant in desirable range. The selection of the type and concentrations of acid or base depends on the stability of the drug substance.

3.3. Acidic Hydrolysis: For acidic hydrolysis studies 0.1 N HCl, and 0.2 N HCl Solution was prepared and used 1 ml to 2 ml of above solution for injection and 10

ml to 20 ml for API and kept room temperature for 6 hrs and kept also on heat at 70 ^{0}C for 30 min. Using 2 ml of acidic solution showing more significant effects in condition at 70 ^{0}C for 30 min. Best results showing in Tables of optimum results of stress studies.

3.4. Basic Hydrolysis: For basic hydrolysis studies 0.1 N NaOH and 0.2N NaOH Solution was prepared and used 1 ml to 2 ml of above solution for injection and 10 ml to 20 ml for API and kept room temperature for 6 hrs and kept also heat at 70 ^{0}C for 30 min. Using 2 ml of basic solution showing more significant effects in condition at 70 ^{0}C for 30 min. Best results showing in tables of optimum results of stress studies.

3.5. Oxidation conditions: Hydrogen peroxide is used for oxidation of drug substances in forced degradation studies. I used diluted solution of 50 % hydrogen Peroxide. I used 1% - 10% H_2O_2 diluted solution about 1-2 ml for injection and 10 ml to 20 ml for API and kept sample for room temperature at 6 hrs and kept also heat at 70 ^{0}C for 60 min. In 10 % H_2O_2 solution showed some significant effect at room temperature and oxidation condition at 70 ^{0}C for 60 min, 5% and 10 % showed prominent degradation is observed. Best results showing in tables of optimum results of stress studies.

3.6. Photolytic conditions: Photo stability studies is performed by exposure of drug substances to UV conditions and I kept sample at wavelength UV 254 for 1-3 days. UV light showed negligible changes. Best results showing in tables of optimum results of stress studies.

3.7. Thermal conditions: Thermal degradation (dry heat) is carried out at more strenuous conditions than recommended ICH Q1A accelerated testing conditions. Thermal degradation study is carried out at 60 – 80 ^{o}C for 24 hrs. Thermal condition at 60 ^{0}C showed significant changes and condition at 80 ^{0}C showed prominent significant changes. Best results showing in tables of optimum results of stress studies.

Table.2.Optimum results of stress studies of Bortezomib API

	Experimental Condition	**Degradation Observed in API**
Acidic Hydrolysis	2ml solution of 0.2 HCl at room temperature for 6 Hrs	3.8 %
	2ml solution of 0.2 HCl at 70°C for 30 min	13.6 %
Alkaline Hydrolysis	2ml solution of 0.2 N NaOH at Room Temperature at 6 hrs	5.1 %
	2ml solution of 0.2 N NaOH at 70°C for 30 min	17.4 %
Oxidation	2ml solution of 10 % v/v H_2O_2 at room temperature for 6 hrs	4.4 %
	2ml solution of 10 % v/v H_2O_2 at at 70°C for 60 min	18.3 %
Thermal (Dry heat)	At 60°C for 24 hrs	6.8 %
	At 80°C for 24 hrs	9.2%
Photolytic	UV 254 for 3 days	2.1 %

Table.3.Optimum results of stress studies of Bortezomib Injection:

Standard	**Condition**	**Degradation in Pemetrexed Injection**
Acidic Hydrolysis	2ml solution of 0.2 HCl at room temperature for 6 Hrs	2.6 %
	2ml solution of 0.2 HCl at 70°C for 30 min	11.2 %
Alkaline Hydrolysis	2ml solution of 0.2 N NaOH at Room Temperature at 6 hrs	4.6 %
	2ml solution of 0.2 N NaOH at 70°C for 30 min	15.8%
Oxidation	2ml solution of 10 % v/v H_2O_2 at room temperature for 6 hrs	5.2%
	2ml solution of 10 % v/v H_2O_2 at at 70°C for 60 min	21.4 %
Thermal (Dry heat)	At 60°C for 24 hrs	7.1%
	At 80°C for 24 hrs	10.2 %
Photolytic	UV 254 for 3 days	2.9 %

3.8. Solution Stability study of analytical solution of Bortezomib: To ensure the reliability of the results in relation to handling and storage standard solutions are performed at 4, 8, 12 hrs time periods at room temperature. A solution containing 200 µg mL−1 is analyzed. After storage at room temperature for 12 h, more than 98 % of the Bortezomib remained unchanged, on the basis of comparison of peak areas with those obtained from a freshly prepared solution of Bortezomib (200 µg mL−1). This suggests Bortezomib standard solution is stable for at least 12 hrs when stored at room temperature. Solution stability Initial at 25°C:

Table.4.Results of solution stability of Bortezomib WS at initial at 25°C

Name	No. of injection	RT	Area
Bortezomib WS	1	3.233	5527901
	2	3.242	5531862
	3	3.242	5544250
	4	3.242	5527708
	5	3.25	5532872
	6	3.267	5526537
	Average	3.246	5531855
	SD	0.012	6569.37
	% RSD	0.358	0.119

Table.5.Results of solution stability of Bortezomib for injection initial at 25°C

Name	No. of injection	RT	Area
Bortezomib for Injection	1	3.342	5514016
	2	3.300	5496379
	3	3.325	5479949
	4	3.325	5503698
	5	3.333	5493909
	6	3.342	5500577
	Average	3.328	5498088
	SD	0.016	11320.57
	% RSD	0.469	0.206

Table.6.Compilation Results of Solution stability up to 12 hours at room temperature

Sr. No.	Initial	4 hrs	8 hrs	12 hrs	% Differences
	% Assay	% Assay	% Assay	% Assay	(Initial-12 hrs)
Assay -1	100.29	100.17	99.90	99.68	0.61
Assay -2	99.97	99.90	99.66	99.37	0.60
Assay -3	99.67	99.51	99.31	98.73	0.95
Assay -4	100.11	100.06	99.97	99.92	0.18
Assay -5	99.93	99.86	99.65	99.54	0.39
Assay -6	100.05	100.01	99.76	99.51	0.54

4. CONCLUSION

Force degradation studies performed to identify the likely degradation products which further helps in determination of the intrinsic stability of the molecule and establishing degradation pathways. For degradation studies optimize after many trails by using Acidic Hydrolysis, Basic Hydrolysis, Oxidation, Photolytic conditions, Thermal conditions, to obtained 5 to 30 % degradant. In acidic hydrolysis 0.1N HCl solution used for (70°C for 30 min) to obtain 10 to 14 % degradation products of Bortezomib API and Bortezomib injection. In Basic hydrolysis 0.1N NaOH solution used for (70°C for 30 min) to obtain 15 to 17 % degradation products of Bortezomib API and Bortezomib injection. Oxidation showed 18 to 22 % degradation at 10 % v/v H_2O_2 at 70°C for 60 min condition. 8 to 11 % degradation showed when Bortezomib API and Bortezomib injection kept on dry heat at 80°C for 24 hrs. After storage at room temperature for 12 h, more than 98 % of the Bortezomib remained unchanged, on the basis of comparison of peak areas with those obtained from a freshly prepared solution of Bortezomib (200 µg mL−1). This suggests Bortezomib standard solution is stable for at least 12 hrs when stored at room temperature.

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