(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
21 April 2016 (21.04.2016)

WIPO | PCT

(10) International Publication Number
WO 2016/059515 Al

(51) **International Patent Classification:**
A61K 31/4965 (2006.01)

(21) **International Application Number:**
PCT/IB2015/057695

(22) **International Filing Date:**
8 October 2015 (08. 10.2015)

(25) **Filing Language:** English

(26) **Publication Language:** English

(30) **Priority Data:**
5218/CHE/2014 18 October 2014 (18.10.2014) IN

(71) **Applicant: SHILPA MEDICARE LIMITED** [IN/IN]; 10/80, Second Floor, Rajendra Gunj, Raichur, Karnataka 584102 (IN).

(72) **Inventors: SHIVAKUMAR, Pradeep;** SHILPA MEDICARE LIMITED: R&D Centre, Survey No.207, Modavalasa Village, Vizianagaram Distric, Andhra Pradesh, Vizianagaram 531162 (IN). **DASARI, Nagaraju;** SHILPA MEDICARE LIMITED: R&D Centre, Survey No.207, Modavalasa Village, Vizianagaram Distric, Andhra Pradesh, Vizianagaram 531162 (IN). **KULKARNI, Raghavendra;** SHILPA MEDICARE LIMITED: R&D CENTRE, Survey No.207, Modavalasa Village, Vizianagaram Distric, Andhra Pradesh, Vizianagaram 531162 (IN). **AKSHAY KANT, Chaturvedi;** 10/80, Second Floor, Rajendra Gunj, Raichur, Karnataka 584102 (IN).

(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available):* AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available):* ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to the identity of the inventor (Rule 4.17(i))*

— *of inventorship (Rule 4.17(iv))*

Published:

— *with international search report (Art. 21(3))*

WO 2016/059515 A1

(54) **Title:** BORTEZOMIB FORMULATIONS

(57) **Abstract:** The present invention relates to stable pharmaceutical compositions comprising bortezomib or pharmaceutically acceptable salt or solvate thereof, and Orthophosphoric acid. The present invention also relates to use of stable pharmaceutical compositions comprising bortezomib or pharmaceutically acceptable salt or solvate thereof, for treating cancer in mammals.

BORTEZOMIB FORMULATIONS

FIELD OF THE INVENTION

The present invention relates to stable pharmaceutical compositions comprising bortezomib or pharmaceutically acceptable salt or solvate thereof, and Orthophosphoric acid.

The present invention also relates to use of stable pharmaceutical compositions comprising bortezomib or pharmaceutically acceptable salt or solvate thereof, for treating cancer in mammals.

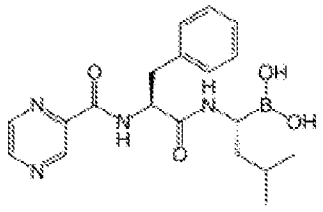
BACK GROUND OF THE INVENTION

Boronic acid and ester compounds display a variety of pharmaceutically useful biological activities. Shenvi et al., U.S. Pat. No. 4,499,082 (1985), discloses that peptide boronic acids are inhibitors of certain proteolytic enzymes. Kettner and Shenvi, U.S. Pat. No. 5,187,157 (1993); U.S. Pat. No. 5,242,904 (1993); and U.S. Pat. No. 5,250,720 (1993), describe a class of peptide boronic acids that inhibit trypsin-like proteases. Kleeman et al., U.S. Pat. No. 5,169,841 (1992), discloses N-terminally modified peptide boronic acids that inhibit the action of renin. Kinder et al., U.S. Pat. No. 5,106,948 (1992), discloses that certain tripeptide boronic acid compounds inhibit the growth of cancer cells.

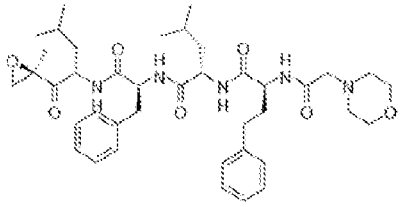
Unfortunately, alkyl boronic acids are relatively difficult to obtain in analytically pure form. Snyderet al., *J. Am. Chern. Soc,* 3611(1958), teaches that alkyl boronic acid compounds readily form boroxines (anhydrides) under dehydrating conditions. Also, alkyl boronic acids and their boroxines are often air-sensitive. Korcek et al., *J. Chern. Soc, Perkin Trans.* 2242 (1972), teaches that butyl boronic acid is readily oxidized by air to generate I-butanol and boric acid. These difficulties limit the pharmaceutical utility of boronic acid compounds, complicating the characterization of pharmaceutical agents comprising boronic acid compounds and limiting their shelf life.

In normal course of cellular functioning, it requires processing of proteins regulating Cell cycle, Growth, and Apoptosis. The ubiquitin-proteasome pathway (UBP) modulates intracellular protein degradation. Specifically, the 26S proteasome is a multi-enzyme protease that degrades misfolded or redundant proteins; conversely, blockade of the proteasomal degradation pathways results in accumulation of unwanted proteins and cell death.

Cancer cells are more highly proliferative than normal cells and their rate of protein translation and degradation is also faster. This typical behavior led to the development of various proteasome inhibitors as useful therapeutics in cancer. The FDA approved the first proteasome inhibitor bortezomib (Velcade) for the treatment of newly diagnosed and relapsed/refractory multiple myeloma. Other improved and II generation proteasome inhibitor approved recently includes Carfilzomib. Further on-going studies are examining other novel proteasome inhibitors, in addition to bortezomib, for the treatment of multiple myeloma and other cancers. Well known Proteasome inhibitors known today are Bortezomib (A), Carfilzomib (B), Marizomib (C)



(A)



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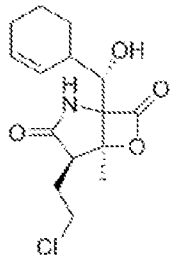


(C)

Bortezomib chemically known as ((N-(2-pyrazine) carbonyl-L-phenylalanine-L-leucine boronic acid) is a 26S proteasome inhibitor that is approved for use in treatment of relapsed multiple myeloma and mantle cell lymphoma. It is believed that the boron atom in bortezomib binds to the catalytic site of the proteasome, ultimately leading to proteasome inhibition and reduced degradation of pro-apoptotic factors, which in turn triggers apoptosis in treated cells.

Stability of aminoalkyl boronic acids (including bortezomib) has remained a concern as they often undergo a spontaneous 1,3-rearrangement to give the homologous amines, owing to the instability of free a-amino groups. These compounds yield boric acids and alcohols by degradation and undergo oxidative reactions that easily destroy the C-B bond which is longer and weaker than the corresponding C-C bond (see e.g., Adele Bolognese, Anna Esposito, MicheleManfra, Lucio Catalano, Fara Petruzziello, Maria CarmenMartorelli, Raffaella Pagliuca, VittoriaMazzarelli, Maria Ottiero, Melania Scalfaro, and Bruno Rotoli. Advances in

Hematology, 2009 (2009) 1-5). Such instability is borne out in stress testing and accelerated stability studies of bortezomib that has established that bortezomib in aqueous solution for injection is intrinsically unstable. For example, in an ethanol: normal saline solution (2:98, pH 2.8), Bortezomib (0.5 mg/mL) degraded 20% at 25°C in 1 month, and in propylene glycol: ethanol: water (50:10:40), the stability of the compound improved, but still degraded 20% in 8 months when stored at 25°C. Among other factors, it was speculated that the degradation of Bortezomib observed in PEG300 : EtOH : H20 (40:10:50) solvent might be due to the presence of peroxides, as PEG300 is known to undergo auto-oxidation with concomitant peroxide generation. (Journal of Pharmaceutical Sciences, 89, 2000 758-765).

In other studies, bortezomib was reported to be susceptible to oxidative degradation under a number of experimental conditions, and that the oxidation of alkyl boranes (which yields the ester of boric acid) can also be due to reaction with alkyl peracids, alkyl peroxides, or oxygen radical species. (Brown H C. 1972. Boranes in organic chemistry. Ithaca, N.Y.: Cornell University Press.). The initial oxidation can be attributed to peroxides or molecular oxygen and its radicals and as light, metal ions, and alkaline conditions normally facilitate oxidation. These conditions are therefore not considered favorable to the stability of bortezomib or any other alkyl boronic acid derivative. (Hussain M A, Knabb R, Aungust B J, Kettner C.1991. Anticoagulant activity of a peptide boronic acid thrombin inhibitor by various routes of administration in rats. Peptides 12: 1153-1 154).

Formation of boronic esters from diol and polyols was reported by Kuivila et al. reporting the preparation of several esters of phenyl boronic acid by reaction with sugars like mannitol and sorbitol, and 1, 2-diols like catechol and pinacal. (J. Org. Chem. 1954, 8, 780-783), and reversible formation of boronic ester by the interaction of boronic acids and polyols in water was first noted by Lorand and Edwards. (J. Org. Chem. 1959, 24, 769-774).

Adams et al in US5780454 discloses Bortezomib, its pharmaceutically acceptable salts, pharmaceutical composition and use in inhibiting the proteosome function in a mammal. Further, it discloses a process for the preparation of Bortezomib and its analogues.

Gupta et al in US67 13446 discloses lyophilized formulation of Bortezomib esters.

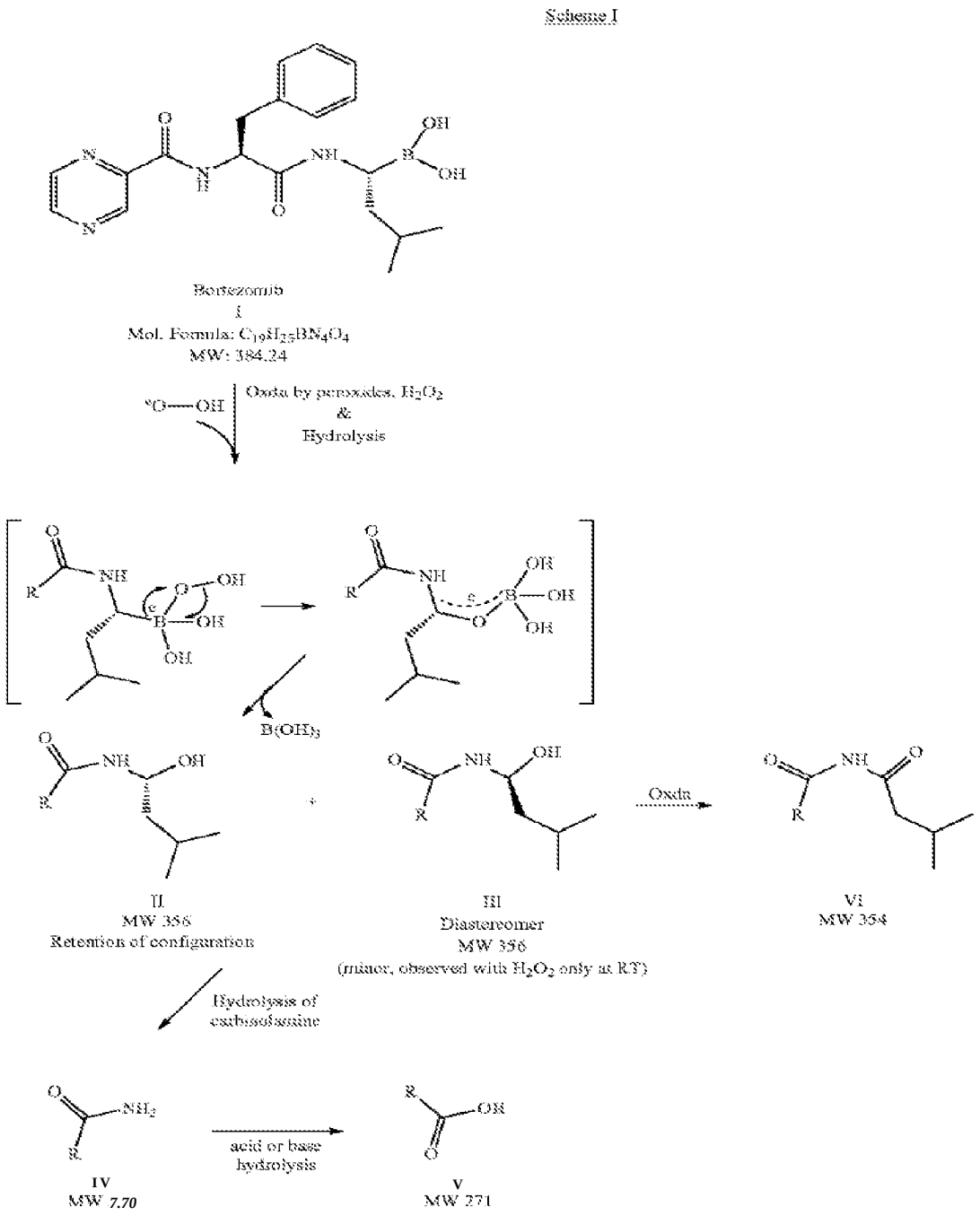
Attempts to form the ester of boronic acid with alpha-hydroxy and beta-carboxylic acids like citric acid and with buffers were disclosed in WO 2009/154737.

Namdeo et al in WO20 10089768 discloses parenteral pharmaceutical composition comprising therapeutically effective amounts of N-(2-pyrazine) carbonyl-L-phenylalanine-L-leucine boronic acid or its salts or its derivatives and tromethamine wherein the composition is stable. Kocherlakota et al in WO2010039762 disclose pharmaceutical compositions comprising bortezomib for oral or parenteral administration that are sugar free pharmaceutical compositions of bortezomib, including its pharmaceutically acceptable salts or solvates, in the form of ready-to-use solutions, lyophilized forms, or physical admixtures.

Bricout et al in WO20101 14982 disclose a lyophilized cake comprising bortezomib or a pharmaceutically acceptable salt or ester, a cyclodextrin and atleast one member selected from the group of bulking agents and surfactants.

Soppimath et al in US8263578 disclose a storage-stable liquid pharmaceutical composition that includes bortezomib in a therapeutically effective amount, the composition comprising: a single-phase liquid formulation comprising a substantially non-aqueous solvent system suitable for injection, an aqueous acetate buffer, and bortezomib, wherein the bortezomib is present in the formulation at a therapeutically effective concentration; wherein the solvent system comprises as a predominant component propylene glycol, and wherein the buffer has a pH of 3; and wherein the solvent system, the buffer, and the pH are selected such as to be effective to suppress formation of at least one of an amide degradation product, a first carbinolamide degradation product, and a second carbinolamide degradation product when the liquid formulation is stored under storage conditions. Soppimath et al describes degradation of bortezomib in solution as a well-known phenomenon and an exemplary degradation scheme is depicted in Scheme I below.

Scheme I



Here, compound II is a first carbinolamide degradation product, compound III is a second carbinolamide degradation product (which is a stereo isomer of II). Hydrolysis of II or III will lead to the formation of the corresponding amide IV, which can be further hydrolyzed to the carboxylic acid product V.

Usayapant et al in WO2012047845 disclose a bortezomib composition includes bortezomib and boric acid in a mass ratio of boric acid to bortezomib is from 1:1 to 10:1.

Anderson et al in WO2012148799 disclose a non-aqueous, homogeneous solution comprising a solubilized lipophilic pharmaceutical agent and an amphiphilic liquid polymeric solvent, the formulation being essentially free of non-polymeric organic solvents, water and non-solubilized particles, wherein the solubilized lipophilic pharmaceutical agent has a concentration of at least about 0.5 mg/mL, and further wherein the solution remains stable and essentially free of non-solubilized particles for at least 40 days when stored at room temperature.

Navin et al in WO2013 128419 disclose a pharmaceutical composition comprising Bortezomib or pharmaceutically acceptable salt or solvate thereof, tromethamine and an organic carboxylic acid, wherein the pH of the composition is from about 3.0 to 6.0.

Dhiraj et al in 3324/DEL/2012 disclose a lyophilized composition comprising Bortezomib, polyvinyl pyrrolidone and optionally sodium chloride. However, the teachings provided under this prior art reference suffers from several drawbacks, viz; these compositions do not produce stable pre-lyophilized and/or lyophilized compositions as specified impurity-2 show alarming levels of more than 1.0% at initial stage, and also there is a considerable delay in reconstitution time of lyophilized cake in 0.9% sodium chloride which is between 5 to 10 min.

Pradeep et al in WO2014102755 disclose a lyophilized composition comprising Bortezomib, polyvinyl pyrrolidone, lactic acid, and an antioxidant. The drawbacks of 3324/DEL/2012 were solved to a considerable extent under this prior art reference with the incorporation of lactic acid as this ensured quicker reconstitution time of lyophilized cake in 0.9% sodium chloride which is below 2 min, and also incorporation of antioxidant has reduced the levels of specified impurity-2 {((N-(l-[((R)-l-Hydroxy-3-Methylbutyl) amino)-l-oxo-3-Phenylpropan-2yl] Pyrazine-2-carboxamide)} in pre-lyophilized solutions, within the acceptable limits prescribed by various drug regulatory authorities.

Hence, despite there are many disclosures on lyophilized formulations for bortezomib, many of them suffer from atleast one of the drawbacks like delay in reconstitution time, limited inuse solution stability when bortezomib is in isotonic solution, particularly over extended time periods, and therefore, there is a need for a simple bortezomib lyophilized formulations having excipients which are well acceptable to regulatory authorities, and also such formulations address the existing drawbacks and exhibit good reconstitution time and have acceptable inuse

drug stability in isotonic solutions like 0.9% Sodium chloride, so as to elicit good therapeutic efficacy to patients in need thereof.

SUMMARY OF THE INVENTION

In accordance with the illustrated objectives, the present invention encompasses stable pharmaceutical compositions of bortezomib.

In accordance with one embodiment, the present invention provides a stable pharmaceutical composition comprising of Bortezomib or pharmaceutically acceptable salt or solvate, and Orthophosphoric acid.

In accordance with second embodiment, the present invention provides a process of preparing a stable pharmaceutical composition comprising of Bortezomib or pharmaceutically acceptable salt or solvate, and Orthophosphoric acid.

One of the aspects of the present invention relates to a stable pharmaceutical composition consisting of Bortezomib Form-SB, Orthophosphoric acid, polyvinylpyrrolidone.

In accordance with third embodiment, the present invention provides a stable pharmaceutical composition consisting of Bortezomib, Orthophosphoric acid, polyvinylpyrrolidone and antioxidant.

One of the aspects of the present invention relates to a stable pharmaceutical composition consisting of Bortezomib Form-SB, Orthophosphoric acid, polyvinylpyrrolidone and Monothioglycerol.

In accordance with fourth embodiment, the present invention provides a process of preparing a stable pharmaceutical composition consisting of Bortezomib, Orthophosphoric acid, polyvinylpyrrolidone and antioxidant.

In accordance with fifth embodiment, the present invention provides stable pharmaceutical compositions comprising bortezomib or pharmaceutically acceptable salt or solvate thereof, and their use for treating various types of cancers in mammals.

DETAILED DESCRIPTION OF THE INVENTION

The term "pharmaceutical composition" as used herein shall mean a composition that is made under conditions such that it is suitable for administration to humans, e.g., it is made under GMP conditions and contains pharmaceutically acceptable excipients, e.g., without limitation, bulking agents and antioxidants. As used herein pharmaceutical composition includes but is not limited to a pre-lyophilization solution or dispersion, lyophilized composition, or a reconstituted solution of a lyophilized composition, meant for IV bolus and/or for subcutaneous administration.

According to the context of the discussion under the specification, "pharmaceutical composition" or "lyophilized composition" or "lyophilized formulation" are used interchangeably, and is meant for any composition having sufficient stability to have utility as a pharmaceutical agent. Preferably, the formulation has sufficient stability to allow storage at a convenient temperature, preferably between 0°C and 40°C, for a reasonable period of time, preferably longer than one month, more preferably longer than three months, even more preferably longer than six months, and most preferably longer than one year. Also, the term "lyophilized composition" as used herein means that the pharmaceutical composition when in the form of a lyophilized cake or powder that is the composition is not reconstituted, remains unaltered in terms of physical and chemical parameters for a prolonged period of time when packed in container which are either protected or unprotected against light, under various storage conditions. For instance, when the containers such as vials are not opened and are stored at controlled room temperature 25°C (77°F) with variation to a range of about 15 to 30°C (59 °F to 86°F) the pharmaceutical composition of the present invention remains stable for 6 months. The pharmaceutical composition when reconstituted with a suitable reconstitution medium such as water for injection or physiological saline (0.9% NaCl), the reconstituted solution is said to be stable when there is no significant chemical degradation for at least 8 hours and there are no signs of precipitation or appearance of particles in the clear solution on storage at room temperature for the said time.

The term "pharmaceutically acceptable" refers to an ingredient that is useful in preparing a pharmaceutical composition that is generally safe, non-toxic, and neither biologically nor

otherwise undesirable, and includes those acceptable for veterinary use as well as human pharmaceutical use.

The term "Specified impurities" refer to any of impurity-1, impurity-2 and impurity-3.

Specified impurity-1: (S)-3-Phenyl-2-(Pyrazine-2-Carboxamido) Propanoic acid.

Specified impurity-2: (N-(l-[((R)-l-Hydroxy-3-Methylbutyl) amino)-l-oxo-3-Phenylpropan-2yl] Pyrazine-2-carboxamide).

Specified impurity-3: N-(l-amino-l-oxo-3-phenylpropan-2-yl) pyrazine-2-carboxamide.

The term "Total impurities" as referred in the specification relates to sum of all Specified impurities and unspecified impurities.

The term "BLD" as referred in the specification relates to below the limit of detection.

The term "LOD" as referred in the specification relates to the limit of detection.

The term "LOQ" as referred in the specification relates to the limit of quantification.

The term "water for injections or WFI" as referred in the specification relates to distilled or sterile water for injection.

The term "PPM" as referred in the specification relates to Parts Per Million.

By "stable pharmaceutical composition" is meant to include pharmaceutical composition having sufficient inuse stability to have utility as a pharmaceutical product. For example, when the pharmaceutical composition is reconstituted with different volumes of 0.9% sodium chloride, such reconstituted product may be stored for up to 8 hours and can be used at different concentrations for various routes of administration, like the concentration of bortezomib which is used for subcutaneous administration (2.5 mg/mL) is greater than the concentration of bortezomib which is used for intravenous administration (1 mg/mL).

In an embodiment of the present invention, it provides a stable pharmaceutical composition comprising of Bortezomib or pharmaceutically acceptable salt or solvate, and Orthophosphoric acid.

In one of the particular embodiments of the present invention, it provides a stable pharmaceutical composition comprising of Bortezomib Form-SB, and Orthophosphoric acid.

Bortezomib as monohydrate Form-SB is discussed in detail under WO2014076713A2 and WO2014102755A1. XRPD, DSC and FTIR spectrums of Bortezomib Form-SB are shown in Figures 1, 2 & 3 respectively. Bortezomib Form-SB is a monohydrate form characterized by X-ray powder diffraction pattern comprising characteristic 20° peaks selected from the XRPD peak set of 5.6, 7.5, 9.8, 10.2, 11.3, 15.1, 18.0, 20.5, 21.5and 23.6 ± 0.20 20°, wherein peaks at 9.8 and 11.39 ± 0.20 20° are un-split and 100 % intensity peak is present at 5.6± 0.20 20°, DSC isotherm comprising the endothermic peaks ranging between 45 to 60°C (Peak -1) and 175 to 185°C (Peak -2) and IR absorption characteristic peaks approximately at 3387 cm^{-1}, 3304 cm^{-1}, 2953 cm^{-1},2927 cm^{-1}, 2868 cm^{-1}, 1627 cm^{-1}, 1455 cm^{-1}, 1400 cm^{-1}, 1201 cm^{-1}, 1150 cm^{-1}, 1020 cm^{-1}, 747 cm^{-1} and 702 cm^{-1} and Raman absorption spectra having characteristic peaks approximately at 3066 cm^{-1}, 1583 cm^{-1}, 1528 cm^{-1},1281 cm^{-1}, 1213 cm^{-1}, 1035 cm^{-1}, 1022 cm^{-1} and 1004 cm^{-1}.

In one of the embodiments of the present invention, it provides a stable pharmaceutical composition comprising of Bortezomib or pharmaceutically acceptable salt or solvate, Orthophosphoric acid and a bulking agent and optionally, an antioxidant.

The bulking agents, are "generally regarded as safe" (GRAS) status from the United States Food and Drug Administration (FDA) are well known in the art of pharmaceutical lyophilization and tend to strengthen the structure of the resulting lyophilized cake. More specifically, bulking agents used in the present invention include polyvinylpyrrolidone and glycine. A most preferred bulking agent is polyvinylpyrrolidone.

In one of the particular embodiments of the present invention, it provides a stable pharmaceutical composition consisting of Bortezomib Form-SB, Orthophosphoric acid and polyvinylpyrrolidone.

The antioxidants are selected from Monothioglycerol, sodium metabisulphite, butylated hydroxytoluene, butylated hydroxyanisole, propyl gallate, DL-tocopherol, tocopherol acetate, tocopherol polyethylene glycol Succinate, L-cysteine, or mixtures thereof. A most preferred antioxidant is Monothioglycerol.

In one of the particular embodiments of the present invention, it provides a stable pharmaceutical composition consisting of Bortezomib Form-SB, Orthophosphoric acid, polyvinylpyrrolidone and Monothioglycerol.

For the Bortezomib pharmaceutical compositions of the present invention, Polyvinylpyrrolidone is used in the range of 20 to 200 mg, for 3.5mg of bortezomib, Orthophosphoric acid is used in the range of 0.2 to 4.0 mg, for 3.5mg of bortezomib and Monothioglycerol is used in the range of 1.0 to 10.0 mg, for 3.5mg of bortezomib.

In one of the embodiments of the present invention, it provides a stable pharmaceutical composition comprising of Bortezomib or pharmaceutically acceptable salt or solvate, Orthophosphoric acid, Polyvinylpyrrolidone, and Monothioglycerol, and the invention compositions are reconstituted in 0.9% Sodium chloride, wherein total impurities is not more than 2.0%.

In one of the embodiments of the present invention, it provides stable pharmaceutical compositions comprising bortezomib or pharmaceutically acceptable salt or solvate thereof, and their use for treating various types of cancers in mammals.

In one of the embodiments of the present invention, it provides a process of preparing a stable pharmaceutical composition comprising of Bortezomib or pharmaceutically acceptable salt or solvate, and Orthophosphoric acid. The stepwise details of the process are given below -

Step (a) -

Dissolving Orthophosphoric acid in water for injection, to get clear solution. Usually Orthophosphoric acid is easily soluble in WFI with mild stirring, and the usual time taken is less than about 10 min and mostly below 5 min. The volume of WFI needed for step (a) is usually 50%v/v to 80%v/v of total volume of WFI, and preferrably 60%v/v.

Step (b) -

Dispersing Bortezomib in required quantity of tertiary butanol. Usually the drug may be added gradually into the tertiary butanol under stirring so as uniform milky dispersion can be obtained, and the usual time taken is less than about 30 min and preferrably below 15 min. The quantity of tertiary butanol required for the batch is usually in the range of 20%v/v to 80%v/v, and most preferrably, 30%v/v of total volume of solvents required for preparing pre-lyophilization copositions.

Step (c) -

Adding Bortezomib dispersion of step (b) in to the step (a), and mixed gently to get a clear solution. The addition step usually takes not more than 45 min, preferrably 30 min, and the step (c) solution is made upto final volume to 100%v/v water for injection. The pH of the final bulk solution to be measured and it is in the range of 2.0 to 3.0.

Step (d) -

The pre-lyophilized solution of step (c) is subjected to lyophilization. Optionally, if required the bulk solution is filtered through 0.45 μιη PVDF filter, and the filtrate is collected and filled in to 10 mL vials with fill volume of 2 mL and half stoppered with dark grey Bromobutyl rubber stopper, and loaded into lyophilizer.

In one of the particular embodiments of the present invention, it provides a process of preparing stable pharmaceutical composition comprising of Bortezomib or pharmaceutically acceptable salt or solvate, Orthophosphoric acid, polyvinylpyrrolidone and an antioxidant. The stepwise details of the process are given below -

Step (a) -

Dissolving antioxidant in water for injection, to get clear solution. Usually Monthioglycerol is a preferred one which is easily soluble in WFI with mild stirring, and the time taken is less than about 10 min and mostly below 5 min. The volume of WFI needed for step (a) is usually 50%v/v to 80%v/v of total volume of WFI, and most preferrably 60%v/v. To the above

solution to that measured quantity of Ortho phosphoric acid was added and stirred for 5min, and then dissolving polyvinylpyrrolidone to the above solution with gentle stirring for less than 10 min, preferrably 5 min.

Step (b) -

Dispersing Bortezomib in required quantity of tertiary butanol. Usually the drug may be added gradually into the tertiary butanol under stirring so as milky dispersion can be obtained, and the usual time taken is less than about 30 min and preferrably below 15 min. The quantity of tertiary butanol required for the batch is usually in the range of 20%v/v to 80%v/v, and most preferrably, 30%v/v of total volume of solvents required for preparing pre-lyophilization copositions.

Step (c) -

Adding Bortezomib dispersion of step (b) in to the step (a), and mixed gently to get a clear solution. The addition step usually takes not more than 45 min, preferrably 30 min, and the step (c) solution is made upto final volume to 100%v/v water for injection. The pH of the final bulk solution to be measured and it is in the range of 2.0 to 3.0.

Step (d) -

The pre-lyophilized solution of step (c) is subjected to lyophilization. Optionally, the above bulk solution was filtered through 0.22 μιη PVDF filter, and the filtrate was collected and filled in to 10 mL vials with fill volume of 2 mL and half stoppered with dark grey Bromobutyl rubber stopper, and loaded into lyophilizer under the following lyophilization cycle:

The technique known as lyophilization is sometimes employed to process injectable pharmaceuticals that exhibit poor active ingredient stability in aqueous solutions. Lyophilization processing is suitable for injectables because it can be conducted under sterile conditions, which is a primary requirement for parenteral dosage forms. Lyophilization or freeze-drying is a process in which water is removed from a product after it is frozen and placed under a vacuum, allowing the ice to change directly from a solid to a vapor, without passing through a liquid phase. The process consists of three separate, unique,

and interdependent processes; a freezing phase, a primary drying phase (sublimation), and a secondary drying phase (desorption). These processes may be optimized to enhance the product stability as well as decrease the manufacturing costs.

Freezing Phase:

A primary function of the freezing phase is to ensure that the entire container having the complex solution is completely frozen, prior to proceeding to a subsequent phase. Additionally, it is usually desired that these containers freeze in a uniform manner. While there are different ways that this can be accomplished, one option is to chill the containers after they are loaded onto the lyophilizer shelves and holding for 30-60 min prior to initiation of the freezing cycle. It is generally not practical to equilibrate the shelves to a freezing temperature, because of frost accumulation during the filling and loading of the containers.

Primary Drying Phase:

Once the formulation is brought to the desired frozen state, primary drying via sublimation can proceed. The primary drying phase involves the removal of bulk water or solvent, at a product temperature below the ice transition temperature under a vacuum (pressures typically between 50-300 mTorr). This phase can be a critical one for stabilizing an active. The goal is to identify the glass transition temperature (Tg') for the formulation. The Tg' is the temperature at which there is a reversible change of state between a viscous liquid and a rigid, amorphous glassy state. One can measure the Tg' of candidate formulations using a differential scanning calorimeter (DSC), in particular with modulated DSC. Generally, the collapse temperature is observed to be about 2-5°C greater than the Tg'. Hence, the shelf temperature is set such that the target product temperature is maintained near or below the Tg' of the formulation throughout the removal of solvent during the primary dry phase.

As the solvent is progressively removed from the formulation containers, the product temperature will approach and reach the shelf temperature since it is no longer cooled by water sublimation. To optimize the duration of the primary dry phase, the removal of solvent vapor can be tracked using a moisture detector, or by monitoring the decrease in pressure difference between a capacitance manometer and a thermocouple pressure gauge or by a pressure drop measurement. The optimization of the primary dry cycle involves a removal of solvent as

quickly as possible without causing cake collapse and subsequent product instability.

Secondary Drying Phase:

The secondary drying phase is the final segment of the lyophilization cycle, where residual moisture is removed from a formulation's interstitial matrix by desorption with elevated temperatures and/or reduced pressures. The final moisture content of a lyophilized formulation, which can be measured by Karl Fischer or other methods, is important because if the solid cake contains too much residual moisture, the stability of the active can be compromised. Hence, it is imperative that one achieves a moisture level as low as possible.

To accomplish a low residual moisture, the shelf temperature is typically elevated to accelerate desorption of water molecules. The duration of the secondary drying phase is usually short. When microstructure collapse occurs, the residual moisture is generally significantly greater than desired. One alternative is to purge the sample chamber of the lyophilizer with alternating cycles of an inert gas such as nitrogen, to facilitate displacement of bound water. However, another solution is to properly formulate the drug product and run an optimal lyophilization cycle.

Example 1

S.no	Ingredients	Qty (mg/ml)	Qty per batch
1	Bortezomib Form-SB	1.75mg	0.04726g
2	Ortho phosphoric acid	0.89mg	0.0222g
3	Tert-butyl alcohol (30%)	0.3mL	7.5 mL
4	Milliq water	q.s to 1mL	q.s to 25 mL

Note: The batch quantity of Bortezomib is calculated based on reported potency of Bortezomib on as is basis and its water content.

Brief method of preparation:-

1. Approximately 110% of desired quantity of water for injection was collected in clean Duran bottle.

2. About 60% of the total batch quantity of WFI was collected from the above step in to the Duran bottle and weighed batch quantity of Ortho phosphoric acid was added and stirred for 5 min to get a clear solution.

3. Dispensed quantity of Tertiary Butanol was taken in a separate duran bottle and weighed quantity of Bortezomib Form-SB, was added, and stirred for 15 min to get Milky dispersion.

4. The solution from the above step 3 was transferred to solution of step 2 and stirred, for 20 min to get clear solution.

5. The volume of the above solution was made up to 100% using WFI and stirred for 5 min. The pH of the solution was found to be 2.58.

6. The above bulk solution was filtered through 0.45 μιη PVDF filter and the filtrate was collected and filled in to 10 mL vials with fill volume of 2 mL and half stoppered with dark grey Bromobutyl rubber stopper and loaded into lyophilizer under the following lyophilization cycle:

Precooling at -5°C

Step	Temperature °C	Ramp duration (min)	Soak duration (min)	Pressure (m torr)
Freezing				
1	-45	30	150	-
2	-20	60	60	-
3	-45	120	60	-
Primary Drying				
4	-45	-	20	150
5	-30	45	500	150
6	0	60	100	150
7	25	60	600	50
Secondary Drying				
11	40	45	400	50

7. After completion of the cycle, the vials are stoppered under Nitrogen and unloaded from lyophilizer. The unloaded vials were sealed using flip off aluminium seals.

Example 2

S.no.	Ingredients	Qty (mg/ml)	Qty per batch
1	Bortezomib Form-SB	1.75mg	0.04726g
2	Ortho phosphoric acid	0.89mg	0.0222g
3	Povidone K12	17.5mg	0.437g
4	Tert-butyl alcohol (30%)	0.3mL	7.5 mL
5	Milliq water	q.s to 1mL	q.s to 25 mL

Note: The batch quantity of Bortezomib is calculated based on reported potency of Bortezomib on as is basis and its water content.

Brief method of preparation:-

1. Approximately 110% of desired quantity of water for injection was collected in clean Duran bottle.

2. About 60% of the total batch quantity of WFI was collected from the above step in to the Duran bottle and weighed batch quantity of Povidone K12 was added and stirred for 5 min to get a clear solution, and to that measured quantity of Ortho phosphoric acid was added and stirred for 5 min to get a clear solution.

3. Dispensed quantity of Tertiary Butanol was taken in a separate duran bottle and weighed quantity of Bortezomib Form-SB, was added, stirred for 15 min to get Milky dispersion.

4. The solution from the above step 3 was transferred to solution of step 2 and stirred, for 30 min to get clear solution.

5. The volume of the above solution was made up to 100% using WFI and stirred for 5 min. The pH of the solution was found to be 2.70.

6. The above bulk solution was filtered through 0.22 μιη PVDF filter and the filtrate was collected and filled in to 10 mL vials with fill volume of 2 mL and half stoppered with dark grey Bromobutyl rubber stopper and loaded in to lyophilizer following lyophilization cycle of Example 1.

7. After completion of the cycle, the vials are stoppered under Nitrogen and unloaded from lyophilizer. The unloaded vials were sealed using flip off aluminium seals.

Example 3

S.no.	Ingredients	Qty (mg/ml)	Qty per batch
1	Bortezomib	1.75mg	94.52mg
2	Monothioglycerol	5mg	0.25g
3	Ortho phosphoric acid	1.75mg	87.5mg
4	Povidone K12	20mg	1g
5	Tert-butyl alcohol (30%)	0.3mL	15 mL
6	Milliq water	q.s to 1mL	q.s to 50 mL

Note: The batch quantity of Bortezomib is calculated based on reported potency of Bortezomib on as is basis and its water content.

Brief method of preparation:-

1. Approximately 110% of Milli-Q water was collected in clean and Duran bottle and nitrogen purged for 30 min.

2. 60% of the total batch quantity of Milli Q water was collected from the above step 1 in to the Duran bottle and weighed quantity of Monthioglycerol was added and stirred for 5min to get a clear solution, to that measured quantity of Ortho phosphoric acid was added and stirred for 5min.

3. To the above step 2, weighed quantity of povidone K12 was added and stirred for 5min to get a clear solution.

4. Dispensed quantity of Tertiary butyl alcohol was taken in a separate duran bottle and weighed quantity of Bortezomib was added and stirred. Time taken to get Milky dispersion was 15min.

5. The solution from the step 3 was transferred to the step 4 solution and stirred. Time taken to get clear solution was 15min.

6. The volume of the above bulk solution from step 5 was made up to 100% by using water and stirred for 5 min. The pH of the solution was found to be 2.36.

7. The above bulk solution was filtered through 0.22μιη PVDF filter and the filtrate was collected and filled in to 10 mL vials with fill volume of 2 mL and half stoppered with dark

grey Bromobutyl rubber stopper and loaded to lyophilizer and the lyophilization cycle carried as per the below recipe:

Precooling at -5°C

Step	Temperature °C	Ramp duration (min)	Soak duration (min)	Pressure (m torr)
Freezing				
1	-45	60	150	-
2	-20	60	60	-
3	-45	120	120	-
Primary Drying				
4	-45	-	20	150
5	-30	30	500	150
6	-25	30	200	150
7	-20	30	150	150
8	-10	30	150	150
9	0	30	240	150
10	25	60	300	50
Secondary Drying				
11	40	30	300	50

8. After completion of the cycle, the vials are stoppered under Nitrogen and unloaded from lyophilizer. The unloaded vials were sealed using flip off aluminium seals.

Bortezomib lyophilized compositions of Examples 1, 2 & 3 are characterized for Reconstitution time in 0.9% sodium chloride and the levels of Related substances are determined in the reconstituted product.

The results are given below:

Bortezomib lyophilized cake	Reconstitution time
Example 1	5 min 50 sec
Example 2	2 min 30 sec

Example 3	2 min 10 sec

Reconstituted Bortezomib solution of Examples 1, 2 & 3 (lmg/ml Bortezomib concentration - 3.5mg Bortezomib vial / 3.5 ml of 0.9%sodium chloride) are determined for the levels of Related substances at different time intervals based on the test method described below:

Test method: Related Substances by HPLC:

Reagent preparation:

Preparation of Mobile phase: A

Prepare a filtered and degassed mixture of water, Acetonitrile, Formic acid in the ratio of 715:285:1.

Preparation of Mobile phase: B

Transfer 200 ml of Water to a 1000 ml volumetric flask. Added 800ml of methanol to the volumetric flask and Sonicate and degassed.

Diluent 1: Weigh and transfer 9.0gm of NaCl into a 1000ml volumetric flask and dilute to volume with water.

Diluent 2: Prepare a filtered and degassed mixture of 250 ml of water and 750 ml of Acetonitrile.

Chromatographic conditions:

Column: Waters symmetry shield RP-18; 250 mm X 4.6mm, 5μιη

Wavelength: 270nm

Flow rate: 1.0 ml / minute

Injection volume: 20 μL

Diluent 1: 0.9% NaCl

Diluent 2: Water: ACN

Run Time: 60 min for Blank, System sensitivity solution Sample & Placebo; 20 min for Diluted standard.

Column Temperature: 30°C

Sampler Temperature: 5°C

Retention time of Bortezomib: 14.5 -15.5 min.

Gradient Programme: for Samples, Blank & Placebos

Time (Min)	Mobile Phase A (%)	Mobile Phase B (%)
0.0	100	0
20.0	100	0
35.0	0	100
50.0	0	100
52.0	100	0
60.0	100	0

Gradient Programme: for Diluted standard: Time (Minutes)	Mobile Phase A (%)	Mobile Phase B (%)
0.0	100	0
20.0	100	0

Bortezomib Standard Stock solution:

Weighed and transferred 5.0 mg of Bortezomib Working Standard in to 25 ml volumetric flask dissolve and dilute to volume with Diluent 2(water : ACN).

Diluted Standard Solution (lOppm):

Transfer 1 mL of Standard stock solution to 20 mL volumetric flask and dilute with diluent-1 (0.9%NaCl).

S.no	Bortezomib	Specified impurity-1	Specified impurity-2	Specified impurity-3
LOD (PPM)	0.112	0.071	0.084	0.071
LOQ (PPM)	0.370	0.200	0.265	0.213
LOD (%)	0.011	0.006	0.008	0.007
LOQ (%)	0.038	0.020	0.026	0.022

Related substances results of Example 1, 2 & 3 formulations are given below:

Example 1			
Name of impurity	% of impurity		
	Initial	4hrs	8hrs
Bortezomib	95.95	95.87	95.78
SP-1	0.00	0.00	0.00
SP-2	0.08	0.11	0.12
SP-3	0.08	0.08	0.08
Chiral Impurity	0.00	0.00	0.00
Unknown- 1	0.01	0.01	0.01
Unknown-2	0.01	0.01	0.01
Unknown-3	0.01	0.01	0.01
Unknown-4	0.04	0.05	0.05
Unknown-5	0.13	0.01	0.23
Unknown-6	0.03	0.15	0.04
Unknown-7	0.05	0.03	0.06
Unknown- 8	0.04	0.06	0.04
Unknown -9	-	0.04	0.00
Unknown- 10	-	-	-
Maximum unknown	0.13	0.15	0.23
Total Known	0.16	0.19	0.20
Total Unknown	0.32	0.37	0.45
Total Impurities	0.48	0.56	0.65

Example 2			
Name of Impurity	% of impurity		
	Initial	4hrs	8hrs
Bortezomib	97.72	97.45	97.35
SP-1	0.01	0.01	0.01
SP-2	0.35	0.34	0.35
SP-3	0.03	0.03	0.03

Chiral Impurity	0.00	0.00	0.00
Unknown- 1	0.09	0.09	0.08
Unknown-2	0.07	0.07	0.06
Unknown- 3	0.05	0.05	0.05
Unknown-4	0.01	0.01	0.01
Unknown- 5	0.01	0.01	0.01
Unknown- 6	0.01	0.01	0.01
Unknown-7	0.01	0.01	0.01
Unknown- 8	0.06	0.02	0.01
Unknown- 9	0.52	0.07	0.02
Unknown- 10	0.08	0.61	0.08
Unknown- 11	0.15	0.14	0.65
Unknown- 12	0.04	0.25	0.15
Unknown- 13	-	0.05	0.29
Unknown- 14	-	-	0.05
Maximum unknown	0.52	0.61	0.65
Total Known	0.39	0.38	0.39
Total Unknown	1.10	1.39	1.48
Total Impurities	1.49	1.77	1.87

Example 3			
Name of impurity	% of impurity		
	Initial	4Hrs	8Hrs
Bortezomib	98.64	98.13	97.79
SP-1	0.00	0.00	0.01
SP-2	0.44	0.24	0.36
SP-3	0.07	0.04	0.07
Chiral Impurity	0.01	0.00	0.00
Unknown- 1	0.00	0.00	0.01
Unknown-2	0.01	0.01	0.01
Unknown- 3	0.00	0.00	0.00

Unknown-4	0.00	0.00	0.01
Unknown- 5	0.01	0.00	0.01
Unknown- 6	0.00	0	0
Unknown-7	0.02	0.23	0.34
Unknown- 8	0.02	0.01	0.03
Unknown -9	0.39	0.40	0.61
UnKnown-10	0.00	0.00	0.04
Unknown- 11	0.01	0.00	0.02
Unknown- 12	0.05	0.18	0.01
Unknown- 13	0.04	0.23	0.01
Unknown- 14	0.01	0.00	0.00
Unknown- 15	0.03	0.30	0.43
Unknown- 16	0.07	0.00	0.00
Unknown- 17	0.02	0.10	0.07
Unknown- 18	0.02	0.00	0.00
Unknown- 19	0.01	0.00	0.01
Unknown-20	0.00	0.00	0.00
Maximum unknown	0.39	0.40	0.61
Total Known	0.51	0.28	0.44
Total Unknown	0.72	1.46	1.61
Total Impurities	1.06	0.76	1.92

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Claims:

1. A stable pharmaceutical composition consisting of Bortezomib or pharmaceutically acceptable salt or solvate, Orthophosphoric acid, polyvinylpyrrolidone and Monothioglycerol.

2. A process of preparing a stable pharmaceutical composition comprising:

a) Dissolving Monothioglycerol, Orthophosphoric acid and Polyvinylpyrrolidone in water for injection.

b) Dispersing Bortezomib in Tertiary Butanol and added to the step (a).

c) Step (b) solution is made upto final volume with water for injection.

d) Lyophilizing the solution of step (c).

3. A stable pharmaceutical composition according to claim 1, wherein polyvinylpyrrolidone is in the range of 20 to 200 mg, for 3.5mg of bortezomib.

4. A stable pharmaceutical composition according to claim 1, wherein Orthophosphoric acid is in the range of 0.2 to 4.0 mg, for 3.5mg of bortezomib.

5. A stable pharmaceutical composition according to claim 1, wherein Monothioglycerol is in the range of 1.0 to 10.0 mg, for 3.5mg of bortezomib.

6. A stable pharmaceutical composition according to claim 1, wherein total impurities is not more than 2.0%.

7. A stable pharmaceutical composition comprising of Bortezomib or pharmaceutically acceptable salt or solvate, and Orthophosphoric acid.

8. A stable pharmaceutical composition according to claim 7, comprising a bulking agents selected from polyvinylpyrrolidone and glycine.

9. A stable pharmaceutical composition according to claim 8, comprising an antioxidant selected from Monothioglycerol, sodium metabisulphite, butylated hydroxytoluene, butylated hydroxyanisole, propyl gallate, DL-tocopherol, tocopherol acetate, tocopherol polyethylene glycol Succinate, L-cysteine, or mixtures thereof.

10. A process of preparing a stable pharmaceutical composition of claim 7 comprising:

a) Dissolving Orthophosphoric acid in water for injection, to get clear solution.

b) Dispersing Bortezomib in Tertiary Butanol and added to the step (a).

c) Step (b) solution is made upto final volume with water for injection.

d) Lyophilizing the solution of step (c).

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2015/057695

A. CLASSIFICATION OF SUBJECT MATTER
A61K31/4965 Version=2015.01

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K, C07F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Patseer and IPO internal database

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2012/0172808 Al; 05 July 2012 (05/07/2012); INNOPHARMA, LLC para 31, 32 & 41	claim 1 and 7-9
Y	US 2012/0172808 Al; July 5 2012 (05/07/2012); INNOPHARMA, LLC para 31, 32 & 41	claim 1 and 3-9
X	WO 2014102755 Al; 3 July 2014 (03/07/2014); SHILPA MEDICARE LIMITED Claim 6	claim 2 and 10
Y	WO 2014102755 Al; 3 July 2014 (03/07/2014); SHILPA MEDICARE LIMITED claim 7, 8 & 10	claim 1 and 3-9

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
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"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
09-12-2015	09-12-2015

Name and mailing address of the ISA/	Authorized officer
Indian Patent Office Plot No. 32, Sector 14,Dwarka, New Delhi-110075 Facsimile No.	Sudipta Dey Telephone No. +91-1125300200

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/IB2015/057695

Citation	Pub.Date	Family	Pub.Date
US 20120172808 A1	05-07-2012	US 9180093 B2	10-11-2015
WO 2014102755 A1	03-07-2014	WO 2014102755 A4	21-08-2014