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- (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, 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.
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- Published:**
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(54) **Title:** NOVEL CRYSTALLINE FORM OF BORTEZOMIB AND PREPARATION METHOD THEREOF

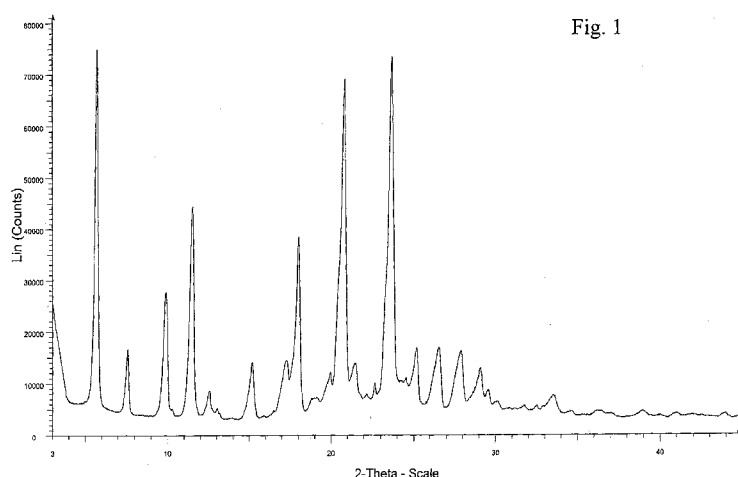


Fig. 1

(57) **Abstract:** The present invention relates to a novel crystalline form of bortezomib and a preparation method thereof. The novel crystalline form of bortezomib is prepared by crystallizing bortezomib from a mixed solvent of methanol and acetonitrile and filtering the formed crystal. The novel crystalline form of bortezomib according to the present invention has substantially improved stability compared to known crystalline forms.

Novel Crystalline Form of Bortezomib and Preparation Method Thereof

Technical Field

5 The present invention relates to a novel crystalline form of bortezomib, which has improved stability, and a preparation method thereof.

Background Art

 Bortezomib (CAS No. 179324-69-7) is an anticancer compound having a chemical
10 name of [(1R)-3-methyl-1[[[(2S)-1-oxo-3-phenyl-2-
[(pyrazinylcarbonyl)amino]propyl]amino]butyl]boronic acid.

 It is commercially available under the trade name of Velcade[®], which is a
proteasome inhibitor approved for use in the treatment of multiple myeloma or mantle cell
lymphoma and contains 3.5 mg of sterilized and lyophilized bortezomib per vial for
15 intravenous injection.

 US Patent No. 5,780,454 discloses bortezomib, a pharmaceutically acceptable salt
thereof, a pharmaceutical composition containing the same, and the use thereof for
inhibiting proteasome function in a mammal. In addition, it discloses methods for
preparing bortezomib (referred to as MG-341 compound in the patent document) and its
20 derivatives.

 Polymorphism is the occurrence of different crystalline forms of a single compound,
and crystalline polymorphs are regarded as different solids having the same molecular
formula and have unique physical and chemical properties such as solubility, melting point,
X-ray diffraction peaks or IR absorption spectra. Such physiological and chemical
25 properties offer an opportunity to increase the stability, solubility and flowability of an
active pharmaceutical ingredient (API) to thereby increase the safety and efficacy of the
pharmaceutical drug.

 International Patent Publication No. WO 08/075376 discloses crystalline form I and
crystalline form II of bortezomib and a preparation process thereof. As disclosed therein,
30 crystalline form I of bortezomib is prepared using a acetone or a mixture of chloroform and
isopropyl ether or a mixture of acetonitrile and isopropyl ether, has 2 θ values of 5.643,
9.785, 11.435, 12.952, 15.275, 20.467, 20.762, 21.486, 22.079, 23.632 and 26.264 $\pm$ 0.2 $^\circ$,

and has a melting point of 160.44 to 180.75°C as measured by differential scanning calorimetry (DSC). Crystalline form II of bortezomib is prepared using ethyl acetate, has 20 values of 4.532, 6.066, 8.497, 9.374, 11.869, 12.270, 14.539, 16.355, 22.585 and 23.420 $\pm 0.2^\circ$, and has melting points of 137.50 to 153.57 °C and 172.32 to 182.14 °C as measured
5 by differential scanning calorimetry (DSC).

International Patent Publication No. WO 09/036281 discloses crystalline form A of bortezomib, crystalline form B of bortezomib, and preparation processes thereof. As disclosed therein, crystalline form A of bortezomib is prepared using a mixture of methanol and purified water, was identified as a monomer in mass spectral analysis, and
10 has a moisture content of 5% or less. Also, it was identified to have the melting point at 75.2°C and 179.73°C in differential scanning calorimetry (DSC). Crystalline form A of bortezomib is characterized in that the purity thereof is maintained for 90 days during storage at 2-8°C. Crystalline form B of bortezomib is prepared using a mixture of ethyl acetate or dichloromethane and toluene.

15 International Patent Publication No. WO 11/099018 discloses bortezomib crystalline form HI, bortezomib amorphous form, and preparation processes thereof. As disclosed therein, bortezomib crystalline form HI is prepared using acetonitrile, and has 20 values of 6.8, 13.2, 18.2, 19.1 and 19.5 $\pm 0.2^\circ$.

International Patent Publication No. WO 2011/107912 discloses crystalline form
20 A1 of bortezomib, crystalline form A2 of bortezomib, and preparation processes thereof. As disclosed therein, crystalline form A1 of bortezomib is prepared using acetonitrile, has 20 values of 3.52, 4.16, 4.55, 4.69, 4.83, 5.31, 5.59, 6.08, 6.69, 7.25, 8.72, 10.39 and 12.37 $\pm 0.2^\circ$, and exhibits a melting point between 195 and 205°C in differential scanning calorimetry (DSC). Crystalline form A2 of bortezomib is prepared using a mixture of
25 dioxane and purified water, has 20 values of 3.37, 3.50, 4.15, 4.54, 4.66, 4.84, 5.36, 5.54, 6.05, 6.67, 7.26, 8.30, 8.79, 10.38, 12.27 and 12.86 $\pm 0.2^\circ$, and exhibits melting points between 170 and 185 °C and between 195 and 210 °C in differential scanning calorimetry (DSC).

Bortezomib, an active pharmaceutical ingredient, is generally freeze-stored (about -
30 20°C). If it is cold-stored (about 5°C), problems can arise during the storage and distribution thereof, because the appearance thereof changes, related compounds increase, and the content thereof decreases (see the bortezomib interview form of the Japanese Pharmaceutical Prescription).

Accordingly, there is an urgent need for the development of a technology for preparing a novel crystalline form of bortezomib which is stable even at room temperature.

Disclosure

5 Technical Problem

It is an object of the present invention to provide a novel crystalline form of bortezomib having improved stability, and a preparation method thereof.

Technical Solution

10 The present invention provides a crystalline form of bortezomib, characterized by X-ray powder diffraction with peaks at 2θ of 5.7, 7.5, 9.9, 11.5, 18.0, 20.8 and $23.7 \pm 0.2^\circ$.

In an embodiment of the present invention, there is provided a crystalline form of bortezomib, characterized by X-ray powder diffraction with peaks at 2θ of 5.7, 7.5, 9.9, 11.5, 15.2, 17.2, 18.0, 20.4, 20.8, 23.7, 25.2, 26.6 and $27.9 \pm 0.2^\circ$.

15 In another embodiment of the present invention, there is provided a crystalline form of bortezomib, which characterized by X-ray powder diffraction with peaks at 2θ of 5.7, 7.5, 9.9, 11.5, 18.0, 20.8 and $23.7 \pm 0.2^\circ$, and shows endothermic peaks at 69°C and 180°C in differential scanning calorimetry (DSC; heating from 30°C to 200°C at a rate of $10^\circ\text{C}/\text{min}$).

20 In another embodiment of the present invention, there is provided a crystalline form of bortezomib, which characterized by X-ray powder diffraction with peaks at 2θ of 5.7, 7.5, 9.9, 11.5, 15.2, 17.2, 18.0, 20.4, 20.8, 23.7, 25.2, 26.6 and $27.9 \pm 0.2^\circ$, and shows endothermic peaks at 69°C and 180°C in differential scanning calorimetry (DSC; heating from 30°C to 200°C at a rate of $10^\circ\text{C}/\text{min}$). More specifically, the crystalline form of
25 bortezomib has a moisture content of 6-9%.

Advantageous Effects

The novel crystalline form of bortezomib according to the present invention has excellent physical and chemical stability, and thus has high storage stability and can be
30 advantageously used in pharmaceutical compositions.

Description of Drawings

FIG. 1 shows the powder X-ray diffraction (PXRD) peaks of a crystalline form of

bortezomib prepared in Example 1.

FIG. 2 shows a differential scanning calorimetry (DSC) thermogram of a crystalline form of bortezomib prepared in Example 1.

FIG. 3 shows the Fourier transform infrared (FT-IR) spectrum of a crystalline form
5 of bortezomib prepared in Example 1.

Mode for Invention

A novel crystalline form of bortezomib according to the present invention is prepared by crystallizing bortezomib from a mixed solvent of methanol and acetonitrile and filtering the formed crystal. Specifically, bortezomib is dissolved in methanol and
10 concentrated in a vacuum, after which acetonitrile is added thereto, followed by stirring at 15~30°C. After about 30 minutes to 2 hours of stirring, a crystal is formed. The crystal is completely precipitated when the suspension is maintained at 0~20°C for 2 hours or more. The obtained crystal is filtered and washed with acetonitrile. Then, it is filtered for 2-5
15 hours while being purged with nitrogen, and the filtrate is dried in a vacuum at 50°C for 1-2 hours, thereby obtaining a crystalline form of bortezomib. The remaining solvent is within the range given by the ICH (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use) guideline. More specifically, 200 ppm or less of acetonitrile remains, and the crystalline form of
20 bortezomib is characterized by a moisture content of 6.0-9.0% as measured by the Karl Fischer water content determination method. Also, it has melting points of about 69°C and 180°C as measured by differential scanning calorimetry (DSC). The novel crystalline form of bortezomib exhibited an X-ray powder diffraction with peaks at 2θ of 5.7, 7.5, 9.9, 11.5, 18.0, 20.8 and $23.7 \pm 0.2^\circ$. More specifically, the present invention provides a crystalline
25 form of bortezomib characterized by having peaks at 2θ of 5.7, 7.5, 9.9, 11.5, 15.2, 17.2, 18.0, 20.4, 20.8, 23.7, 25.2, 26.6 and $27.9 \pm 0.2^\circ$, as shown in Table 1 below.

Table 1.

Diffraction angle (2-Theta)	d value (Angstrom)	Intensity (%)
5.7	15.583	99.2
7.5	11.708	22.6
9.9	8.962	37.3
11.5	7.709	59.9
15.2	5.839	19.0
17.2	5.137	19.5
18.0	4.929	52.0
20.8	4.266	92.3
23.7	3.755	100.0
25.2	3.537	22.4
26.6	3.355	22.9
27.9	3.195	21.9

Experimental and analysis conditions

5 Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) was performed on a Mettler Toledo DSC1 calorimeter. The temperature of the sample was programmed for heating from 30°C to 200°C at a rate of 10°C/min. The product was placed in a crimped aluminum capsule, and the amount of the analyzed product was 2-5 mg.

10 Powder X-ray diffraction (PXRD)

Powder X-ray diffraction (PXRD) analysis was performed on a Bruker D8 Advance diffractometer with reflection-mode Bragg-Brentano focusing (θ -2 θ) geometry. The analyzed product was deposited on a silicon single-crystal to form a thin layer. Incident CuK radiation ($\lambda=1.5406$ Å) was supplied by a copper anticathode tube (40 kV/40
15 mA). Also, the analysis was performed under the following conditions: step width: 0.02°; measuring range: 3-45°; and scan speed: 10°/min.

Fourier transform infrared (FT-IR) spectrometry

This analysis was performed using a JASCO FT/IR-4100 type A spectrometer.

20 Hereinafter, the present invention will be described in further with reference to examples. It is to be understood, however, that these examples are for illustrative purposes and are not intended to limit the scope of the present invention in any way.

Example 1: Preparation of crystalline form of bortezomib

96 g of bortezomib (purity: 99.68%) was dissolved completely in 200 mL of methanol, and then concentrated in a vacuum at 40~45°C. 4 L of acetonitrile was added to the oily material and stirred at 20~25°C. After 1 hour, a crystal started to precipitate, and the solution was stirred at an internal temperature of 10~15°C for 10 hours. The crystal was filtered, washed with 300 mL of acetonitrile, and then dried in a vacuum at 50°C for 2 hours, thereby obtaining 77 g of the desired compound as a white solid.

Yield: 80%; HPLC purity: 99.97%; K/F moisture content: 6.9%.

Residual solvents: 173 ppm of acetonitrile, and 109 ppm of methanol.

FIG. 1 shows the powder X-ray diffraction peaks of a crystalline form of bortezomib prepared in Example 1.

FIG. 2 shows a differential scanning calorimetry (DSC) thermogram of a crystalline form of bortezomib prepared in Example 1.

FIG. 3 shows the Fourier transform infrared (FT-IR) spectrum of a crystalline form of bortezomib prepared in Example 1.

Example 2: Stability test (temperature: 25 ± 2°C, and relative humidity: 60 ± 5%)

The crystalline form of bortezomib prepared in Example 1 was subjected to a stability test in a sealed state at a temperature of 25 ± 2°C and a relative humidity of 60 ± 5% for 12 months. As a result, it was shown that the crystalline form of bortezomib was maintained in a stable state without any change in the HPLC purity, content, moisture content and crystalline form (PXRD) thereof.

Table 2.

	Initial	1 month	2 months	3 months	4 months	5 months	6 months	8 months	12 months
HPLC purity (%)	99.97	99.97	99.98	99.95	99.98	99.96	99.97	99.96	99.95
HPLC content (%)	102.1	101.7	101.7	101.8	102.0	101.9	101.8	101.5	101.6
Moisture content	6.9	6.8	6.7	7.0	6.6	6.9	6.8	6.9	7.0

Example 3: Stability test (temperature: 25 ± 2°C, and relative humidity: 60 ± 5%)

Crystalline form II of bortezomib as disclosed in International Patent Publication No. WO 08/075376, and crystalline form A of bortezomib as disclosed in International

Patent Publication No. WO 09/036281, were prepared according to the methods described in the patent documents. For comparison, the prepared crystalline forms together with the novel crystalline form of bortezomib according to the present invention were subjected to a moisture content test in a stability chamber at a temperature of $25 \pm 2^\circ\text{C}$ and a relative humidity of $60 \pm 5\%$ for 20 days in a state in which the cap was open.

Table 3.

		0 day	3 days	6 days	10 days	20 days	Crystalline form
Form II	Purity	99.92%	99.87%	99.47%	98.64%	97.23%	Maintained
	Moisture content	5.50%	5.41%	5.49%	5.92%	5.98%	
Form A	Purity	99.90%	99.89%	99.79%	99.21%	98.71%	Maintained
	Moisture content	2.51%	6.29%	8.75%	9.11%	9.42%	
Novel crystalline form	Purity	99.97%	99.95%	99.94%	99.89%	99.89%	Maintained
	Moisture content	6.90%	6.97%	6.99%	7.05%	7.12%	

As can be seen in Table 3 above, in the case of crystalline form II, there was a slight change in the moisture content, but the HPLC purity was reduced while impurities were produced.

In the case of crystalline form A, it was shown that the moisture content increased rapidly, and the HPLC purity also decreased with the passage of time. In the case of the novel crystalline form of the present invention, it was shown that there was little or no change in the moisture content and the HPLC purity.

Example 4: Stability test ($60 \pm 2^\circ\text{C}$)

Crystalline form II of bortezomib as disclosed in International Patent Publication No. WO 08/075376, and crystalline form A of bortezomib as disclosed in International Patent Publication No. WO 09/036281, were prepared according to the methods described in the patent documents. For comparison, the prepared crystalline forms together with the novel crystalline form of bortezomib according to the present invention were subjected to a moisture content test in a stability chamber at a temperature of $60 \pm 2^\circ\text{C}$ for 20 days in a sealed state.

Table 4.

		0 day	3 days	6 days	10 days	20 days
Form II	Purity	99.92%	99.85%	91.56%	89.03%	76.64%
Form A	Purity	99.90%	99.87%	99.26%	98.54%	96.78%
Novel crystalline form	Purity	99.97%	99.90%	99.90%	99.88%	99.71%

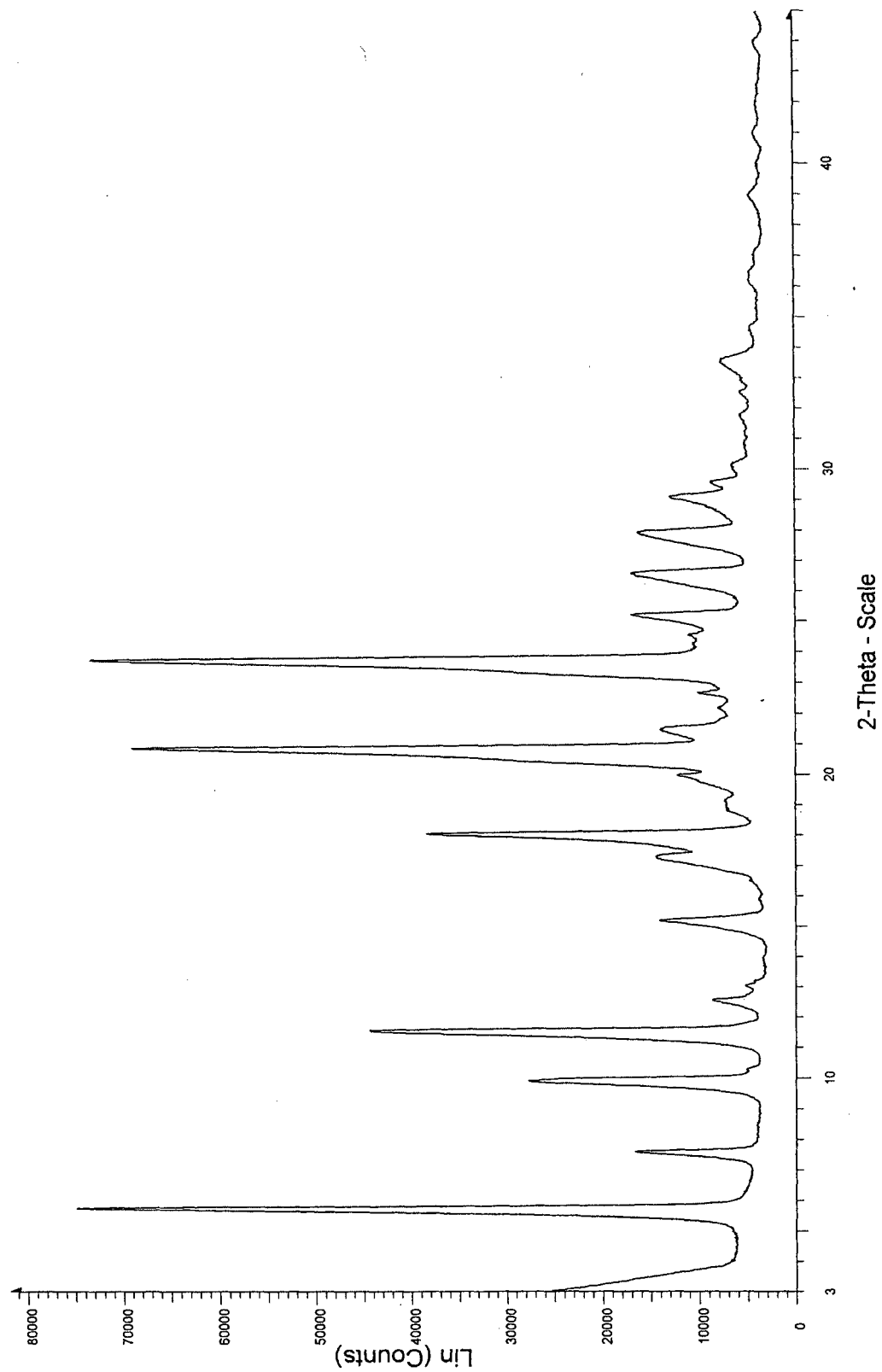
As can be seen in Table 4 above, in the case of the novel crystalline form of the present invention, there was a slight change in the purity at high temperature, but the content of related compounds was 0.1% or less. However, it was shown that crystalline form II and crystalline form A according to the prior art were decomposed at high temperature, and thus their purity decreased. Therefore, it can be seen that the novel crystalline form of bortezomib according to the present invention has substantially improved stability compared to the crystalline forms of the prior arts.

CLAIMS

1. A crystalline form of bortezomib, characterized by X-ray powder diffraction with peaks at 2θ of 5.7, 7.5, 9.9, 11.5, 18.0, 20.8 and $23.7 \pm 0.2^\circ$.
- 5 2. A crystalline form of bortezomib, characterized by X-ray powder diffraction with peaks at 2θ of 5.7, 7.5, 9.9, 11.5, 15.2, 17.2, 18.0, 20.4, 20.8, 23.7, 25.2, 26.6 and $27.9 \pm 0.2^\circ$.
- 10 3. The crystalline form of bortezomib of claim 1, which characterized by X-ray powder diffraction with peaks at 2θ of 5.7, 7.5, 9.9, 11.5, 18.0, 20.8 and $23.7 \pm 0.2^\circ$, and shows endothermic peaks at 69°C and 180°C in differential scanning calorimetry (DSC; heating from 30°C to 200°C at a rate of $10^\circ\text{C}/\text{min}$).
- 15 4. The crystalline form of bortezomib of claim 2, which characterized by X-ray powder diffraction with peaks at 2θ of 5.7, 7.5, 9.9, 11.5, 15.2, 17.2, 18.0, 20.4, 20.8, 23.7, 25.2, 26.6 and $27.9 \pm 0.2^\circ$, and shows endothermic peaks at 69°C and 180°C in differential scanning calorimetry (DSC; heating from 30°C to 200°C at a rate of $10^\circ\text{C}/\text{min}$).
- 20 5. The crystalline form of bortezomib of claim 4, which has a moisture content of 6-9%.

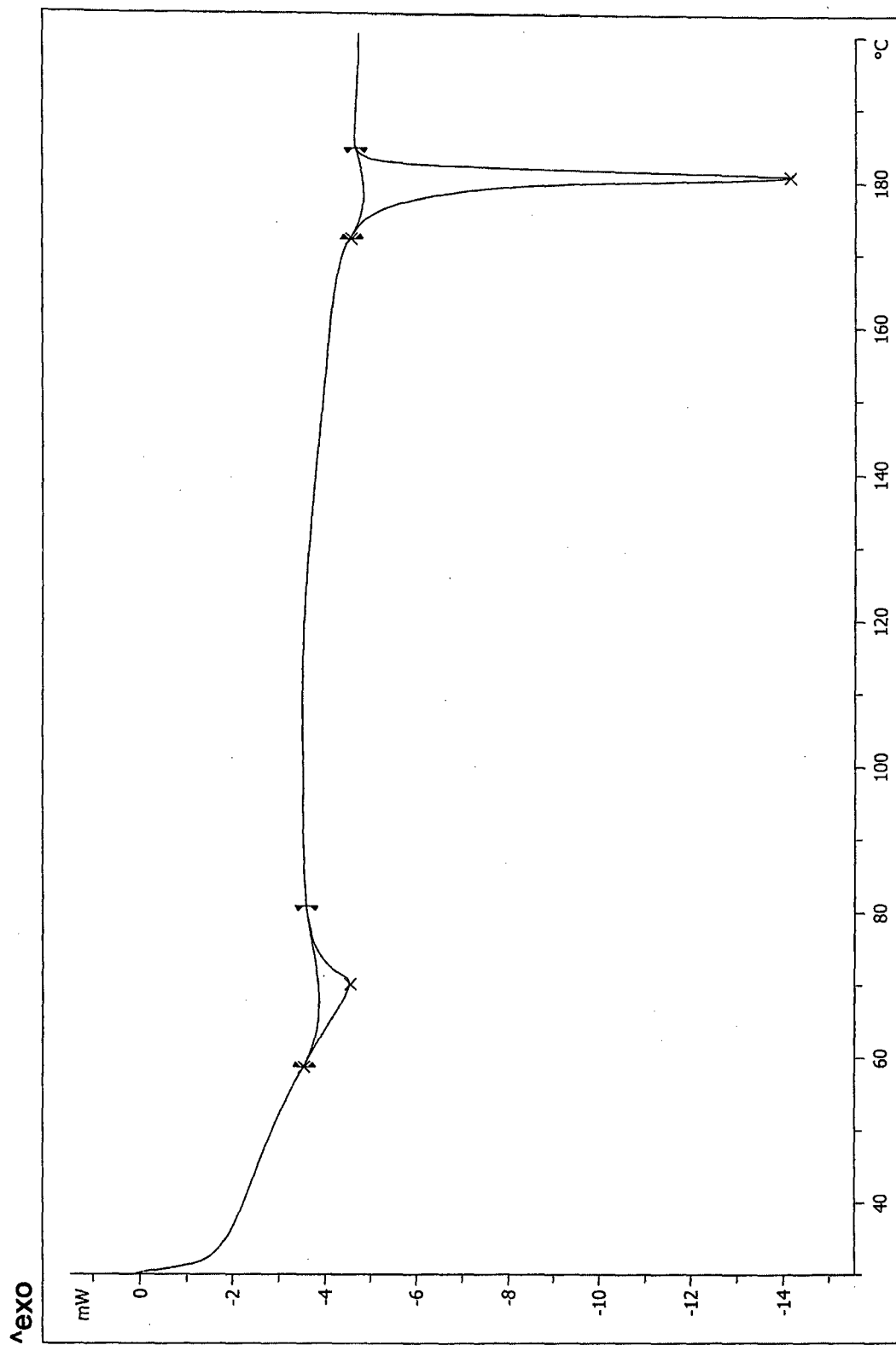
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Fig. 1



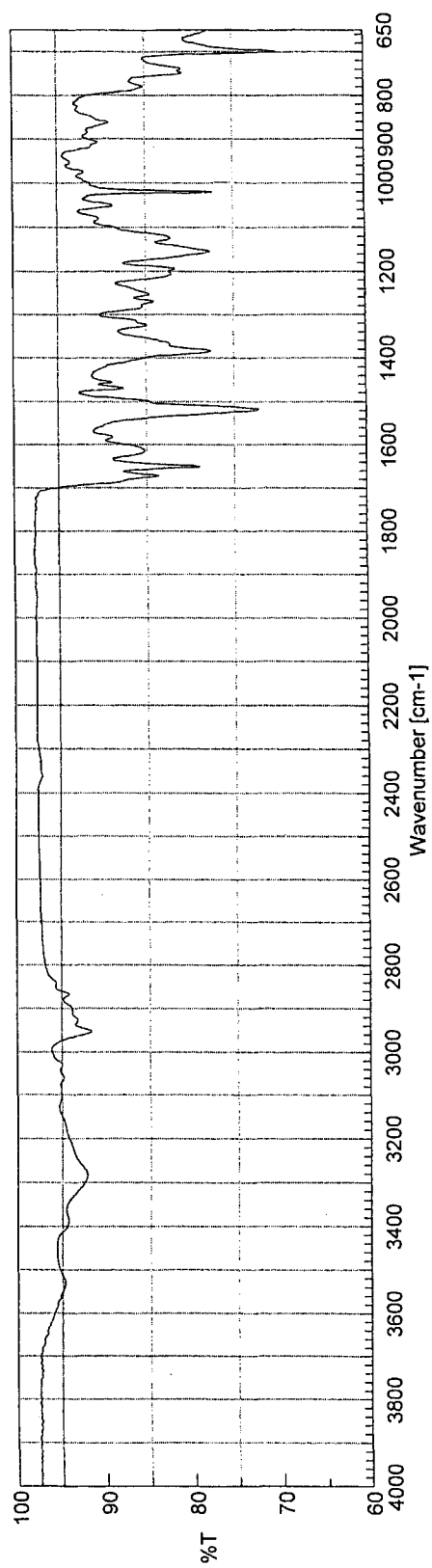
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Fig. 2



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Fig. 3



INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2015/001424**A. CLASSIFICATION OF SUBJECT MATTER****C07F 5/02(2006.01)i, A61K 31/69(2006.01)i**

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)

C07F 5/02; A61K 31/517; A61K 31/4965; C07F 5/04; A61K 31/69

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

Korean utility models and applications for utility models

Japanese utility models and applications for utility models

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

eKOMPASS(KIPO internal) & Keywords:bortezomib, crystalline

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2008-075376 A1 (NATCO PHARMA LIMITED et al.) 26 June 2008 See claims 1-2 and figures 1-6.	1-5
X	KR 10-2010-0051828 A (DR. REDDY'S LABORATORIES LIMITED, DR. REDDY'S LABORATORIES, INC.) 18 May 2010 See examples 1-9 and figures 1-10.	1-5
X	WO 2012-131707 A2 (LAURUS LABS PRIVATE LIMITED et al.) 04 October 2012 See claims 1-6 and figures 1-4.	1-5
X	WO 2011-099018 A1 (HETERO RESEARCH FOUNDATION et al.) 18 August 2011 See claims 1-2 and figures 1-2.	1-5
X	US 2012-0289699 A1 (JULIAN PAUL HENSCHKE et al.) 15 November 2012 See claims 20-21 and figures 1-5.	1-5



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

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"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

28 April 2015 (28.04.2015)

Date of mailing of the international search report

29 April 2015 (29.04.2015)

Name and mailing address of the ISA/KR

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/KR2015/001424

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2008-075376 A1	26/06/2008	WO 2008-075376 A8	15/04/2010
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WO 2011-099018 A1	18/08/2011	None	
US 2012-0289699 A1	15/11/2012	US 8497374 B2	30/07/2013