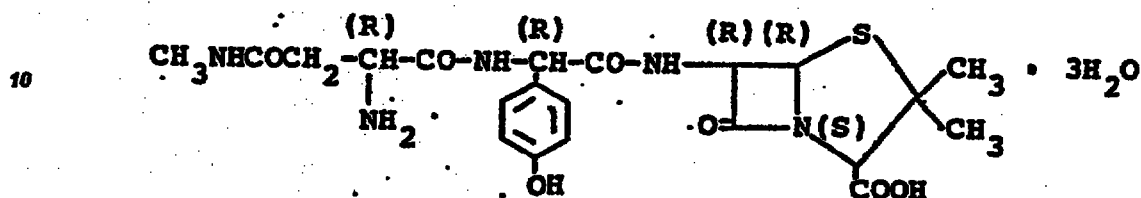


Description

This invention relates to a stable hydrate of a penicillin derivative and a process for preparing same. More particularly, it relates to (2S, 5R, 6R)-6-[(2R)-2-[(2R)-2-amino-3-(N-methylcarbamoyl)propionamido]-2-(p-hydroxyphenyl)-acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid trihydrate (hereinafter referred to as "N⁴-methyl-D-asparaginylamoxicillin") of the formula :



N⁴-Methyl-D-asparaginylamoxicillin is useful as chemotherapeutic agents because it shows potent antimicrobial activity against both of gram-positive and gram-negative microorganisms. It has been known that N⁴-methyl-D-asparaginylamoxicillin can be prepared either (i) by condensing 6-aminopenicillanic acid with D-2-(D-2-amino-3-N-methylcarbamoyl-propionamido)-2-p-hydroxyphenylacetic acid, or (ii) by condensing amoxicillin with D-2-amino-3-N-methylcarbamoylpropionic acid (U.S. Patent No. 4,053,609). It has also been known that pure N⁴-methyl-D-asparaginylamoxicillin can be obtained as amorphous anhydrate by contacting an aqueous solution of crude N⁴-methyl-D-asparaginylamoxicillin with a non-polar macroporous adsorption resin, eluting the adsorbed product and liophilizing the eluate containing the desired product (U.S. Patent No. 4,313,875). However, the amorphous N⁴-methyl-D-asparaginylamoxicillin anhydrate obtained by the known method gradually moistens in an ambient atmosphere and it is not stable unless it is stored by protecting from moisture and light.

Also it is known from GB-A-1,216,714 and GB-A-1,241,844 that crystalline trihydrates are stable phases for ampicillin amoxycillin.

It has now been found that N⁴-methyl-D-asparaginylamoxicillin can be obtained in the form of a crystalline trihydrate. The new trihydrate of N⁴-methyl-D-asparaginylamoxicillin is of superior quality in terms of its crystallinity and stability. In particular, the new trihydrate has been found to have a well-defined crystalline structure and it has been found to be remarkably stable in storage. These properties render the crystalline trihydrate of N⁴-methyl-D-asparaginylamoxicillin of value in pharmaceutical use.

Accordingly the present invention includes the crystalline hydrate of N⁴-methyl-D-asparaginylamoxicillin of value in pharmaceutical use.

According to a further aspect of the present invention, N⁴-methyl-D-asparaginylamoxicillin trihydrate is prepared by adjusting the pH of a solution of N⁴-methyl-D-asparaginylamoxicillin or a salt thereof in water to 3 to 6, preferably 3.5 to 5.5, and crystallizing the desired crystalline trihydrate.

N⁴-Methyl-D-asparaginylamoxicillin to be used in the present invention may be either a crude product prepared by the method described in U.S. Patent No. 4,053,609 or an amorphous anhydrate prepared by the method described in U.S. Patent No. 4,313,875. Examples of the salt of N⁴-methyl-D-asparaginylamoxicillin include alkali metal salts (e.g., sodium or potassium salt), alkaline earth metal salts (e.g., calcium or magnesium salt), amino acid salts (e.g., L-lysine, L-arginine, L-histidine or L-ornithine salt) or the salts with organic bases (e.g., triethylamine, tributylamine or N-methylmorpholine). Water, aqueous lower alkanols (e.g., aqueous methanol, aqueous ethanol or aqueous propanol) and aqueous lower alkanones (e.g., aqueous acetone or aqueous methylethylketone) are preferably used as the aqueous medium. From an industrial view-point, water is the most preferable as the aqueous medium.

In carrying out the method of the present invention, the solution of N⁴-methyl-D-asparaginylamoxicillin or a salt thereof in the aqueous medium is firstly adjusted to a pH of 3 to 6, preferably 3.5 to 5.5. For example, when N⁴-methyl-D-asparaginylamoxicillin is employed in the form of its free form, the adjustment of pH is carried out by adding an acid or an alkali to the solution of said compound. When N⁴-methyl-D-asparaginylamoxicillin is employed in the form of its salt, the adjustment of pH is carried out by adding an acid to the solution of said salt. Examples of the acid to be used for the pH adjustment include a mineral acid such as hydrochloric acid or sulfuric acid or an organic acid such as citric acid, and examples of the alkali include an alkali metal hydroxide such as sodium hydroxide or potassium hydroxide. After the solution of N⁴-methyl-D-asparaginylamoxicillin or its salts is adjusted to the prescribed pH, the desired trihydrate is crystallized from said solution. The crystallization of the desired trihydrate may be preferably conducted by cooling or condensation of the solution, or by addition of a water-miscible organic solvent, or by a combination

thereof. For example, when the crystallization is carried out by cooling the solution, it is preferred that the solution which has previously been warmed to about 30 to 70°C is cooled to 0 to 10°C. When the crystallization is carried out by condensation, it is preferred that the solution of N⁴-methyl-D-asparaginyloxycillin or its salt is condensed until the concentration of said compound is 10 to 45 w/w%, especially about 30 to 40 w/w%. Further, when the crystallization is carried out by the addition of a water-miscible organic solvent, said water-miscible organic solvent may be added intermittently or continuously to the solution of N⁴-methyl-D-asparaginyloxycillin or its salt. Examples of such water-miscible organic solvent include a lower alkanol such as methanol, ethanol or propanol, and a lower alkanone such as acetone or methyl ethyl ketone. The precipitated trihydrate can be readily separated by a conventional solid-liquid separation technique such as filtration or centrifugation.

As mentioned hereinbefore, N⁴-methyl-D-asparaginyloxycillin trihydrate of the present invention is difficult to moisten in an ambient atmosphere and is stable in storage. Therefore, said trihydrate is superior to the known amorphous anhydrate as bulk substance for medicine. Moreover, the trihydrate of the present invention is easy to handle because the bulk density and electrification thereof is less than those of the known amorphous anhydrate. Further, the trihydrate of the present invention is characterized in that it can be readily prepared in an industrial scale because of its good crystallinity.

Experiment

(Stability test)

Each of crystalline N⁴-methyl-D-asparaginyloxycillin trihydrate of the present invention and amorphous N⁴-methyl-D-asparaginyloxycillin anhydrate (prepared according to the method described in U.S. Patent No. 4,313,875) was allowed to stand at 40°C in a sealed tube for 5, 10, 15 or 30 days, and the stability of each compound was estimated by measuring the content of N⁴-methyl-D-asparaginyloxycillin. The results are shown in the following Table 1.

The content of N⁴-methyl-D-asparaginyloxycillin was measured by high performance liquid chromatography (HPLC) under the conditions mentioned below.

[Conditions]

Column : Octadecylsilane (4.6 mmØX 150 mm)
Mobile phase : Phosphate buffer-acetonitril (87 : 13)(pH : 3.0)
Flow speed : 1.0 ml/minute
Temperature of column : 40°C

Remaining ratio (%)

of N⁴-methyl-D-asparaginyloxycillin

$$= \frac{\left[\begin{array}{l} \text{Content of N}^4\text{-methyl-D-aspara-} \\ \text{ginyloxycillin after it was} \\ \text{allowed to stand} \end{array} \right]}{\left[\begin{array}{l} \text{Content of N}^4\text{-methyl-D-aspara-} \\ \text{ginyloxycillin before it was} \\ \text{allowed to stand} \end{array} \right]} \times 100$$

Table 1

Test compound Nos.	Remaining ratio of N ⁴ -methyl-D-asparaginy- lamoxicillin (%)				
	A period of time during which the test compound was allowed to stand (days)				
	initial	5	10	15	30
1.	100.0	100.0	100.0	100.0	99.5
2.	100.0	91.0	90.0	88.5	85.5

Test Compound

1. Crystallin N⁴-methyl-D-asparaginyloxycillin trihydrate (The compound of the present invention)
2. Amorphous anhydrate of N⁴-methyl-D-asparaginyloxycillin (prepared according to U.S. Patent No. 4,313,875)

Example 1

Amorphous N⁴-methyl-D-asparaginyloxycillin anhydrate (10 g) is dissolved at 40°C in water (50 ml). The solution is adjusted to pH 4.0 with diluted hydrochloric acid and stirred at 30°C for one hour and then cooled to 5°C. The resultant precipitates are collected by filtration, washed with water and dried, whereby N⁴-methyl-D-asparaginyloxycillin trihydrate (8 g) is obtained as a white crystalline solid.

Water content (Karl Fisher Method) : 10.05% (corresponds to 3 moles)

Content of product (HPLC) : 99.7%

[α] 0255 + 179.5° (c= 1.0, water)

Infrared spectrum : shown in Figure 1

X-ray powder diffraction pattern : shown in the following (Power source ; Cu :Ni, 40KV, Table 2 35mA, λ =1.5405)

Just for reference, the X-ray powder diffraction pattern of amorphous N⁴-methyl-D-asparaginyloxycillin anhydrate is also shown in Table 2.

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Figure 1

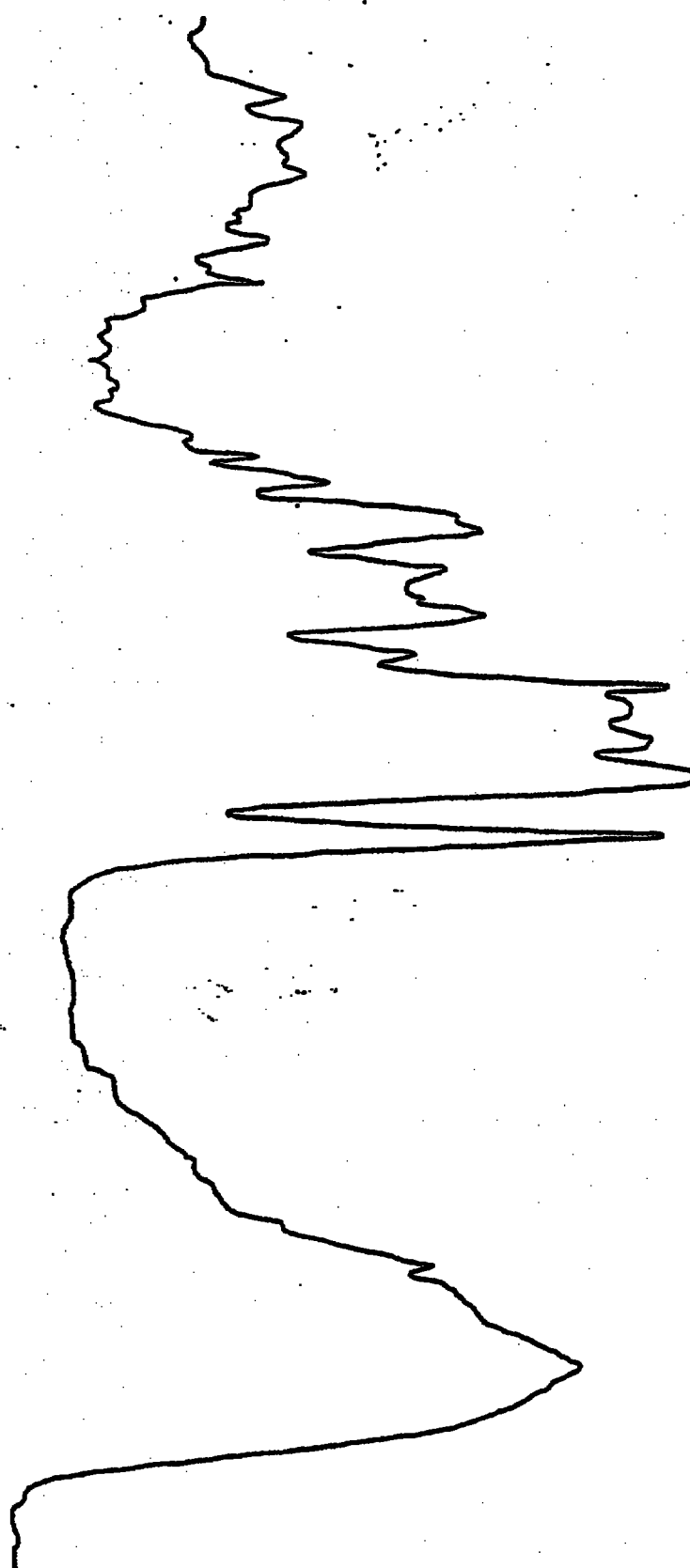


Table 2

Crystalline N ⁴ -methyl-D-asparaginylamoxicillin trihydrate		Amorphous N ⁴ -methyl-D-asparaginylamoxicillin anhydrate	
'd' value	Relative intensity*	'd' value	Relative intensity*
15.77	w	5.94	vw
10.77	w	5.50	vw
9.21	vw	4.57	vw
7.89	w		
5.98	s		
5.57	vs		
5.34	w		
4.98	vw		
4.62	vs		
4.39	w		
4.23	m		
3.97	m		
3.77	vw		
3.64	vw		
3.54	w		
3.48	w		
3.37	w		
3.25	m		
3.13	vw		
3.01	w		
2.86	vw		
2.81	vw		
2.65	vw		
2.60	vw		
2.40	w		

*: The relative intensities were estimated by comparing the line intensities by comparing the line intensities against a set of standards : vs= very strong, s= strong, m= medium, w= weak, vw= very weak

Example 2

Amorphous N⁴-methyl-D-asparaginylamoxicillin anhydrate (10 g) is dissolved in water (500 ml) [The pH of the solution is 4.3]. The solution is concentrated under reduced pressure to 250 g, and ethanol (250 g) is added thereto. The resultant precipitates are collected by filtration and dried, whereby N⁴-methyl-D-asparaginylamoxicillin trihydrate (5 g) is obtained as a white crystalline solid.

Water content (Karl Fisher Method) : 10.74% (corresponds to 3 moles)

Content of product (HPLC) : 99.3%

$[\alpha]_{\text{D}}^{20} + 179.3^{\circ}$ (c= 1.0, water)

Example 3

Water (70 ml) is added to amorphous N⁴-methyl-D-asparaginylamoxicillin anhydrate (22.2 g), and an

aqueous 20% sodium hydroxide solution (5.4 ml) is added dropwise thereto to dissolve said anhydrate therein. Activated charcoal is added to the solution, and the mixture is filtered. The filtrate is adjusted to pH 3.5 with 20% hydrochloric acid (about 5.5 ml). The resultant precipitates are collected by filtration and dried, whereby N⁴-methyl-D-asparaginyloxycillin trihydrate (17 g) is obtained as a white crystalline solid.

Water content (Karl Fisher Method) : 10.21% (corresponds to 3 moles)

Content of product (HPLC) : 99.7%

$[\alpha]_D^{20} + 179.9^\circ$ (c= 1.0, water)

Example 4

Thiobenzamide (20.5 g) is dissolved in ethanol (290 ml), and (2S, 5R, 6R)-6-[(2R)-2-[(2R)-2-(o-nitrophenylsulfonyl)-amino-3-(N-methylcarbamoyl)propionamido]-2-(p-hydroxyphenyl)-acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid trihydrate (32.21 g) is added thereto. The mixture is stirred at 15 to 20°C for 17 hours to remove the amino-protecting group (i.e., o-nitrophenylsulfonyl group). The precipitates (i.e., N⁴-methyl-D-asparaginyloxycillin) are collected by filtration, dried and then added to a suspension of activated charcoal (3 g) in water (50 ml). The suspension is adjusted to pH 5.5 with 1% hydrochloric acid (about 3 ml) and stirred at room temperature for one hour. Insoluble materials are filtered off, and the filtrate is concentrated under reduced pressure until the concentration of N⁴-methyl-D-asparaginyloxycillin is 40 w/w%. The concentrated solution is stirred at 30°C for one hour and then cooled to 5°C. The resultant precipitates are collected by filtration and dried, whereby N⁴-methyl-D-asparaginyloxycillin trihydrate (12.5 g) is obtained as a white crystalline solid.

Water content (Karl Fisher Method) : 10.1% (corresponds to 3 moles)

Content of product (HPLC) : 99.7%

$[\alpha]_D^{20} + 180.0^\circ$ (c= 1.0, water)

Claims

CLAIMS FOR THE CONTRACTING STATES : BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. Crystalline trihydrate of (2S, 5R, 6R)-6-[(2R)-2-[(2R)-2-amino-3-(N-methylcarbamoyl)propionamido]-2-(p-hydroxyphenyl)acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

2. A process for preparing crystalline trihydrate of (2S, 5R, 6R)-6-[(2R)-2-[(2R)-2-amino-3-(N-methylcarbamoyl)propionamido]-2-(p-hydroxyphenyl)-acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, which comprises adjusting the pH of a solution of (2S, R, 6R)-6-[(2R)-2-[(2R)-2-amino-3-(N-methylcarbamoyl)propionamido]-2-(p-hydroxyphenyl)acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid or a salt thereof in water to 3 to 6 and crystallizing the desired crystalline trihydrate.

3. A process according to claim 2, wherein the pH is adjusted to 3.5 to 5.5.

4. A process according to claim 2, wherein the crystallization is carried out by cooling or condensation of the solution, or by addition of a water-miscible organic solvent.

5. A process according to claim 4, wherein the organic solvent is methanol, ethanol, propanol, acetone or methyl ethyl ketone.

CLAIMS FOR THE CONTRACTING STATES : AT, GR, ES

1. A process for preparing crystalline trihydrate of (2S, 5R, 6R)-6-[(2R)-2-[(2R)-2-amino-3-(N-methylcarbamoyl)propionamido]-2-(p-hydroxyphenyl)-acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, which comprises adjusting the pH of a solution of (2S, R, 6R)-6-[(2R)-2-[(2R)-2-amino-3-(N-methylcarbamoyl)propionamido]-2-(p-hydroxyphenyl)acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid or a salt thereof in water to 3 to 6 and crystallizing the desired crystalline trihydrate.

2. A process according to claim 2, wherein the pH is adjusted to 3.5 to 5.5.

3. A process according to claim 2, wherein the crystallization is carried out by cooling or condensation of the solution, or by addition of a water-miscible organic solvent.

4. A process according to claim 4, wherein the organic solvent is methanol, ethanol, propanol, acetone



aqueous 20% sodium hydroxide solution (5.4 ml) is added dropwise thereto to dissolve said anhydrate therein. Activated charcoal is added to the solution, and the mixture is filtered. The filtrate is adjusted to pH 3.5 with 20% hydrochloric acid (about 5.5 ml). The resultant precipitates are collected by filtration and dried, whereby N⁴-methyl-D-asparaginyloxycillin trihydrate (17 g) is obtained as a white crystalline solid.

Water content (Karl Fisher Method) : 10.21% (corresponds to 3 moles)
Content of product (HPLC) : 99.7%

$[\alpha]_{\text{D}}^{20} + 179.9^{\circ}$ (c= 1.0, water)

10 Example 4

Thiobenzamide (20.5 g) is dissolved in ethanol (290 ml), and (2S, 5R, 6R)-6-[(2R)-2-[(2R)-2-(o-nitrophenylsulfonyl)-amino-3-(N-methylcarbamoyl)propionamido]-2-(p-hydroxyphenyl)-acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid trihydrate (32.21 g) is added thereto. The mixture is stirred at 15 to 20°C for 17 hours to remove the amino-protecting group (i.e., o-nitrophenylsulfonyl group). The precipitates (i.e., N⁴-methyl-D-asparaginyloxycillin) are collected by filtration, dried and then added to a suspension of activated charcoal (3 g) in water (50 ml). The suspension is adjusted to pH 5.5 with 1% hydrochloric acid (about 3 ml) and stirred at room temperature for one hour. Insoluble materials are filtered off, and the filtrate is concentrated under reduced pressure until the concentration of N⁴-methyl-D-asparaginyloxycillin is 40 w/w%. The concentrated solution is stirred at 30°C for one hour and then cooled to 5°C. The resultant precipitates are collected by filtration and dried, whereby N⁴-methyl-D-asparaginyloxycillin trihydrate (12.5 g) is obtained as a white crystalline solid.

Water content (Karl Fisher Method) : 10.1% (corresponds to 3 moles)
Content of product (HPLC) : 99.7%

$[\alpha]_{\text{D}}^{20} + 180.0^{\circ}$ (c= 1.0, water)

Claims

CLAIMS FOR THE CONTRACTING STATES : BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. Crystalline trihydrate of (2S, 5R, 6R)-6-[(2R)-2-[(2R)-2-amino-3-(N-methylcarbamoyl)propionamido]-2-(p-hydroxyphenyl)acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid.

2. A process for preparing crystalline trihydrate of (2S, 5R, 6R)-6-[(2R)-2-[(2R)-2-amino-3-(N-methylcarbamoyl)propionamido]-2-(p-hydroxyphenyl)-acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, which comprises adjusting the pH of a solution of (2S, R, 6R)-6-[(2R)-2-[(2R)-2-amino-3-(N-methylcarbamoyl)propionamido]-2-(p-hydroxyphenyl)acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid or a salt thereof in water to 3 to 6 and crystallizing the desired crystalline trihydrate.

3. A process according to claim 2, wherein the pH is adjusted to 3.5 to 5.5.

4. A process according to claim 2, wherein the crystallization is carried out by cooling or condensation of the solution, or by addition of a water-miscible organic solvent.

5. A process according to claim 4, wherein the organic solvent is methanol, ethanol, propanol, acetone or methyl ethyl ketone.

CLAIMS FOR THE CONTRACTING STATES : AT, GR, ES

1. A process for preparing crystalline trihydrate of (2S, 5R, 6R)-6-[(2R)-2-[(2R)-2-amino-3-(N-methylcarbamoyl)propionamido]-2-(p-hydroxyphenyl)-acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, which comprises adjusting the pH of a solution of (2S, R, 6R)-6-[(2R)-2-[(2R)-2-amino-3-(N-methylcarbamoyl)propionamido]-2-(p-hydroxyphenyl)acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid or a salt thereof in water to 3 to 6 and crystallizing the desired crystalline trihydrate.

2. A process according to claim 2, wherein the pH is adjusted to 3.5 to 5.5.

3. A process according to claim 2, wherein the crystallization is carried out by cooling or condensation of the solution, or by addition of a water-miscible organic solvent.

4. A process according to claim 4, wherein the organic solvent is methanol, ethanol, propanol, acetone



or methylethylketone.

Ansprüche

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PATENTANSPRUCHE für die Vertragsstaaten : BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. Kristallines Trihydrat von (2S,5R,6R)-6-((2R)-2-((2R)-2-Amino-3-(N-methylcarbamoyl)propionamido)-2-(p-hydroxyphenyl)acetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptan-2-karbonsäure.
2. Verfahren zum Herstellen des kristallinen Trihydrates von (2S,5R,6R)-6-((2R)-2-((2R)-2-Amino-3-(N-methylcarbamoyl)propionamido)-2-(p-hydroxyphenyl)acetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptan-2-karbonsäure, welches das Einstellen des pH-Wertes einer Lösung von (2S,5R,6R)-6-((2R)-2-((2R)-2-Amino-3-(N-methylcarbamoyl)propionamido)-2-(p-hydroxyphenyl)acetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptan-2-karbonsäure oder eines Salzes davon in Wasser auf 3 bis 6 und das Kristallisieren des gewünschten kristallinen Trihydrates umfasst.
3. Verfahren gemäss Anspruch 2, worin der pH-Wert auf 3,5 bis 5,5 eingestellt ist.
4. Verfahren gemäss Anspruch 2, worin die Kristallisation durch Kühlen oder Kondensieren der Lösung, oder durch Zugabe eines mit Wasser mischbaren organischen Lösungsmittels ausgeführt wird.
5. Verfahren gemäss Anspruch 4, worin das organische Lösungsmittel Methanol, Ethanol, propanol, Aceton oder Methylethylketon ist.

PATENTANSPRUCHE für die Vertragsstaaten : AT, GR, ES

1. Verfahren zum Herstellen des kristallinen Trihydrates von (2S,5R,6R)-6-((2R)-2-((2R)-2-Amino-3-(N-methylcarbamoyl)propionamido)-2-(p-hydroxyphenyl)acetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptan-2-karbonsäure, welches das Einstellen des pH-Wertes einer Lösung von (2S,5R,6R)-6-((2R)-2-((2R)-2-Amino-3-(N-methylcarbamoyl)propionamido)-2-(p-hydroxyphenyl)acetamido)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptan-2-karbonsäure oder eines Salzes davon in Wasser auf 3 bis 6 und das Kristallisieren des gewünschten kristallinen Trihydrates umfasst.
2. Verfahren gemäss Anspruch 1, worin der pH-Wert auf 3,5 bis 5,5 eingestellt ist.
3. Verfahren gemäss Anspruch 1, worin die Kristallisation durch Kühlen oder Kondensieren der Lösung, oder durch Zugabe eines mit Wasser mischbaren organischen Lösungsmittels ausgeführt wird.
4. Verfahren gemäss Anspruch 3, worin das organische Lösungsmittel Methanol, Ethanol, propanol, Aceton oder Methylethylketon ist.

Revendications

Revendications pour les Etats Contractants : BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. Trihydrate cristallin de l'acide (2S, 5R, 6R)-6-((2R)-2-((2R)-2-amino-3-(N-méthylcarbamoyl)propionamido)-2-(p-hydroxyphényl)-acétamido)-3,3-diméthyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylique.
2. Procédé pour préparer du trihydrate cristallin de l'acide (2S, 5R, 6R)-6-((2R)-2-((2R)-2-amino-3-(N-méthylcarbamoyl)propionamido)-2-(p-hydroxyphényl)-acétamido)-3,3-diméthyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylique, qui comprend l'ajustement du pH d'une solution d'acide (2S, R, 6R)-6-((2R)-2-((2R)-2-amino-3-(N-méthylcarbamoyl)propionamido)-2-(p-hydroxyphényl)-acétamido)-3,3-diméthyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylique ou d'un de ses sels dans de l'eau à une valeur de 3 à 6 et la cristallisation du trihydrate cristallin voulu.
3. Procédé selon la revendication 2, dans lequel on ajuste le pH à une valeur comprise entre 3,5 et 5,5.
4. Procédé selon la revendication 2, dans lequel on effectue la cristallisation par refroidissement ou condensation (concentration) de la solution, ou par addition d'un solvant organique miscible à l'eau.
5. Procédé selon la revendication 4, dans lequel le solvant organique est le méthanol, l'éthanol, le propanol, l'acétone ou la méthyléthylcétone.

**Revendications pour les Etats Contractants : AT, GR, ES**

5 1. Procédé pour préparer du trihydrate cristallin de l'acide (2S, 5R, 6R)-6-[(2R)-2-[(2R)-2-amino-3-(N-méthylcarbamoyl)-propionamido]-2-(p-hydroxyphényl)-acétamido]-3,3-diméthyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylique, qui comprend l'ajustement du pH d'une solution d'acide (2S, R, 6R)-6-[(2R)-2-[(2R)-2-amino-3-(N-méthylcarbamoyl)propionamido]-2-(p-hydroxyphényl)acétamido]-3,3-diméthyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylique ou d'un de ses sels dans de l'eau à une valeur de 3 à 6 et la cristallisation du trihydrate cristallin voulu.

10 2. Procédé selon la revendication 1, dans lequel on ajuste le pH à une valeur comprise entre 3,5 et 5,5.

3. Procédé selon la revendication 1, dans lequel on effectue la cristallisation par refroidissement ou condensation (concentration) de la solution, ou par addition d'un solvant organique miscible à l'eau.

15 4. Procédé selon la revendication 3, dans lequel le solvant organique est le méthanol, l'éthanol, le propanol, l'acétone ou la méthyléthylcétone.

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