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(72) Inventors; and


(54) Title: AZTREONAM L-LYSINE AND METHODS FOR THE PREPARATION THEREOF

(57) Abstract: The present invention relates to the L-lysine salt of aztreonam and methods for making the L-lysine salt of aztreonam.
AZTREONAM L-LYSINE AND METHODS FOR
THE PREPARATION THEREOF

CROSS-REFERENCED TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application Serial Nos. 60/484,861 filed July 2, 2003 and 60/550,098 filed March 4, 2004, the disclosures of which are incorporated by reference in their entirety herein.

FIELD OF THE INVENTION

The present invention relates to the L-lysine salt of aztreonam and methods for making the L-lysine salt of aztreonam.

BACKGROUND OF THE INVENTION

Aztreonam is a monobactam antibiotic disclosed in U.S. patent 4,775,670, which is incorporated by reference herein in its entirety. Aztreonam has the chemical name (Z)-2-[[[(2-amino-4-thiazolyl)[(2S,-3S)-2-methyl-4-oxo-1-sulfo-3-azetidinyl]carbamoyl[methylene]amino]oxy]-2-methylpropionic acid. Aztreonam is also known as [3S-[3a(Z),4β]]-3-[[2-amino-4-thiazolyl][(1-carboxy-l-methylethoxy)imino]acetyl]amino]-4-methyl-2-oxo-l-azetidinesulfonic acid and (2S, 3S)-3-[[ 2-[2-amino-4-thiazolyl]-(Z)-2-[(1-carboxy-l-methylethoxy)imino]acetyl]amino]-4-methyl-2-oxo-l-azetidine-1-sulfonic acid.

Aztreonam has the structure:
U.S. patent 4,775,670 discloses a process for making Aztreonam and pharmaceutically acceptable salts thereof. However, U.S. patent 4,775,670 does not teach how to prepare salts of Aztreonam with amines or amino acids.

Applicants encountered unexpected difficulties when trying to prepare salts of Aztreonam with amines and amino acids by dissolution of the acid and base in a solvent and precipitation of the salt. In the majority of experiments an oil, which was impossible to crystallize and which decomposed very rapidly, was obtained.

Applicants have discovered methods that enable the preparation of a solid, stable Aztreonam L-lysine salt.

SUMMARY OF THE INVENTION

The invention relates to an amorphous, solid Aztreonam L-lysine salt. The invention also relates to methods for making the amorphous L-lysine salt. The first method comprises freeze-drying an aqueous solution of Aztreonam L-lysine. The second method comprises spray-drying an aqueous solution of Aztreonam L-lysine. The third method comprises precipitating Aztreonam L-lysine from an aqueous solution.

DETAILED DESCRIPTION OF THE INVENTION

Aztreonam is converted into its L-lysine salt in aqueous solution. The pH plays an important role in the stability of the Aztreonam L-lysine aqueous solution and it should not be more than 5.5. The salt may be isolated from the aqueous solution as an amorphous solid by three different techniques. The three techniques include freeze-drying, spray-drying and precipitation in an organic solvent. All three techniques provide an amorphous product.

Aztreonam L-lysine salt may be obtained by freeze drying an aqueous solution of aztreonam L-lysine. The ratio of Aztreonam and L-lysine used to form the aqueous solution is preferably between 1:1 and 1:2:1. The product obtained by this method appears as white to yellowish flakes and contains about 3 to about 6 % water.

Aztreonam L-lysine may also be obtained by spray drying an aqueous solution
of Aztreonam L-lysine. The Aztreonam L-lysine salt obtained by spraying is a white to off-white powder. The water content of the product obtained by this method is between about 4 to about 7%. The preferred spray drying parameters are listed in the following table. The parameters apply to a Büchi laboratory spray-drier B-191 (Aspirator rate: 31.5 m³/h).

<table>
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<tr>
<th>Pump speed (m³/h)</th>
<th>Inlet temperature (°C)</th>
<th>Spray flow (l/h)</th>
<th>Concentration of solution (m/m %)</th>
<th>Outlet temperature (°C)</th>
</tr>
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<tbody>
<tr>
<td>240...750</td>
<td>115...195</td>
<td>400...800</td>
<td>7...29</td>
<td>45...127</td>
</tr>
</tbody>
</table>

* The outlet temperature depends mainly on the pump speed, inlet temperature, spray flow and concentration of solution.

Using optimal drying parameters, the product exhibited good handling properties, i.e., it was free-flowing. The particle size can also be influenced by regulating the specific drying parameters.

The L-lysine salt of aztreonam can also be isolated by precipitating Aztreonam L-lysine from an aqueous solution. The aqueous solution of aztreonam L-lysine is preferably dropped into an aqueous or anhydrous organic solvent, e.g., ethanol, acetone, etc. The water content of the alcohol used for the precipitation is preferably between about 0 and 9% (m/m).

The Aztreonam L-lysine obtained using these methods was stable in the sense that during 3 months at 2-8°C:

1. the assay of Aztreonam was maintained constant in the limit 60-66%; and
2. no impurity exceed 0.3 area %.

EXAMPLES

The impurity content of Aztreonam lysine salt using the HPLC method is determined as follows:
a. Aztreonam Lysine salt sample is dissolved in 0.02 M KH₂PO₄ buffer solution (pH adjusted 2.0 with 25 w/w % phosphoric acid) diluent,

b. The sample solution (ca. 10 µl) is injected into a 100.0 mm x 4.0 mm, 3 µm RP-18 HPLC column,

c. Gradient eluting with a mixture of 0.02 M KH₂PO₄ buffer solution (pH adjusted 3.0 with 25 w/w % phosphoric acid) (A) and acetonitrile (B) according to the following profile:

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<tr>
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<tbody>
<tr>
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<tr>
<td>1.2</td>
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<tr>
<td>1.2</td>
<td>30.0</td>
<td>100.0</td>
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</table>

d. The amounts of each impurity was measured at 230 nm wavelength with a UV detector and appropriate recording device.

e. The amount of each impurity was calculated referring to an Aztreonam working standard at a concentration of 2.5 g/ml.

In the above method, Aztreonam has a retention time of about 10.2 minutes.

The assay of Aztreonam Lysine salt using the HPLC method was determined as follows:

a. Dissolving Aztreonam Lysine salt sample in a mixture of 0.02 M KH₂PO₄ buffer solution (pH adjusted 3.0 with 25 w/w % phosphoric acid) and methanol (80:20) diluent,

b. Injecting the sample solution (ca. 10 µl) into a 50.0 mm x 4.6 mm, 3 µm RP-18 HPLC column,

c. Isocratic eluting at 1.5 ml/min with a mixture of 0.02 M KH₂PO₄ buffer solution (pH adjusted 3.0 with 25 w/w % phosphoric acid) and methanol in a 83:17 v/v % ratio.
d. Measuring of the amounts of each impurity at 270 nm wavelength with a UV detector and appropriate recording device.

e. Calculating of the assay referring to the Aztreonam working standard at a concentration of 100μg/ml.

In the above method, Aztreonam has a retention time of about 2.3 minutes

EXAMPLE 1

Aztreonam (5.00 g, water content: 12.2 %) was suspended in 25 ml distilled water at 0-5 °C. A solution of 2.70 g L-lysine in 13.5 ml distilled water was added dropwise to the above suspension with cooling (ice-water bath). The solution of Aztreonam L-lysine salt obtained by this method was filtered on a glass filter and freeze dried.

Product: white flakes.

Yield: 6.8 g (quant.)

EXAMPLE 2

Aztreonam (35.0 g, water content: 12.6 %) was suspended in 230 ml distilled water at 0-5 °C. A solution of 17.5 g L-lysine in 45 ml distilled water was added dropwise to the above suspension with cooling (ice-water bath). The solution of Aztreonam L-lysine salt obtained by this method was decolorized with charcoal, filtered on a glass filter and spray dried using laboratory spray dryer Büchi B-191.

Product: white powder.

Yield: 31 g (62 %)

The Aztreonam L-lysine salt produced according to this example, does not contain any impurity exceeding 0.3 area %, and/or maintains at least about 63 weight % of the Aztreonam, after storage for three months at about 2-8 °C.

EXAMPLE 3

Aztreonam (3.50 g, water content: 11.3 %) was suspended in 8 ml distilled water at 0-5 °C. A solution of 1.80 g L-lysine in 3.5 ml distilled water was added dropwise to the above suspension with cooling (ice-water bath). The solution of Aztreonam L-
lysine salt obtained by this method was diluted with 23 ml ethanol and added dropwise to the stirred mixture of 60 ml ethanol and 4.75 ml water at 0-5 °C in 15 min. 120 ml pure ethanol was added dropwise together with the Aztreonam L-lysine salt solution but from another dropping funnel in the same time period. The precipitation was filtered off and dried in air-circulated oven at 38 °C.

Product: white powder.

Yield: 3.86 g (77 %)

Having thus described the invention with reference to particular preferred embodiments and illustrated it with examples, those of skill in the art may appreciate modifications to the invention as described and illustrated that do not depart from the spirit and scope of the invention as disclosed in the specification.
What is claimed is:

1. An amorphous Aztreonam L-lysine salt.

2. The amorphous L-lysine of claim 1, wherein the water content is 3-7% water by weight.

3. A method for making the L-lysine salt of Aztreonam comprising freeze-drying an aqueous solution of Aztreonam L-lysine, wherein the aqueous solution has a pH of not more than 5.5.

4. The method of claim 3, wherein the ratio of Aztreonam to L-lysine used to form the aqueous solution is between about 1:1 and 1:2.1.

5. A method for making the L-lysine salt of Aztreonam comprising spray-drying an aqueous solution of Aztreonam L-lysine, wherein the aqueous solution has a pH of not more than 5.5.

6. A method for making the L-lysine salt of Aztreonam comprising precipitating Aztreonam L-lysine from an aqueous solution of Aztreonam L-lysine, wherein the aqueous solution has a pH of not more than 5.5.

7. The method of claim 6, wherein the aqueous solution of Aztreonam L-lysine is dropped into an aqueous or anhydrous organic solvent.

8. The method of claim 7, wherein the solvents are selected from a group consisting of acetone and ethanol.

9. The product obtained by the method of claim 3.

10. The product obtained by the method of claim 5.
11. The product obtained by the method of claim 6.

12. The product of claim 9, wherein the water content of the Aztreonam L-lysine is about 3% to about 6% by weight.

13. The product of claim 10, wherein the water content of the Aztreonam L-lysine is about 4% to about 7% by weight.

14. The product of any of claims 1-13, which is stable for at least three months when stored at about 2-8 degrees Celsius.

15. The product of claim 1, which after storage for three months at about 2-8°C, does not contain any impurity exceeding 0.3 area %.

16. The product of claim 1, which after storage for three months at about 2-8°C, maintains at least about 60-66 weight % of the Aztreonam L-lysine.

17. The product of claim 16, which after storage for three months at about 2-8°C, maintains at least about 63 weight % of the Aztreonam.
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07D41/12 A61K31/427 A61P11/00 A61P31/04

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)
IPC 7 C07D A61K A61P

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 practical, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS, INSPEC, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<td>EP 0 297 580 A (SQUIBB &amp; SONS INC) 4 January 1989 (1989-01-04) the whole document</td>
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Further documents are listed in the continuation of box C. Patent family members are listed in annex.

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  * "E" earlier document but published on or after the international filing data
  * "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
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  * "S" document member of the same patent family

Date of the actual completion of the international search
23 November 2004

Date of mailing of the international search report
09/12/2004

Name and mailing address of the ISA
European Patent Office, P.B. 5816 Patentbulletin 2 NL – 2280 HV Rijswijk Tel. (31-70) 340-3200, Tx. 31 651 eipo nl, Fax (31-70) 340-3016

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**INTERNATIONAL SEARCH REPORT**

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<td>WO 2004/052333 A (PARI GMBH ; KELLER MANFRED (DE); LINTZ FRANK-CHRISTOPHE (DE); OPOLSKI) 24 June 2004 (2004-06-24) page 7, line 10 - line 25 page 10 - page 12, line 10 page 13 page 15, line 21 - page 16, line 2 page 17, line 13 - line 16 page 18, line 27 - page 19, line 2 claims 1-27; examples 1-6</td>
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