PATENT SPECIFICATION (11) 1407342

1407342

(21) Application No. 16358/73 (22) Filed 5 April 1973

(44) Complete Specification published 24 Sept. 1975

(51) INT CL² C07D 501/32 A61K 31/545

(52) Index at acceptance

C2C 1314 214 220 226 22Y 256 25Y 29X 29Y 303 321 32Y 342 34Y 351 352 366 367 628 650 KE

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(19)



(10)

(54) PROCESS FOR THE PREPARATION OF ANTIBIOTIC DERIVATIVES

(71) We, BRISTOL-MYERS COMPANY, a Corporation organised and existing under the laws of the State of Delaware, United States of America, having Offices located at 345 Park Avenue, New York, New York, United States of America, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to the preparation of a stable form of crystalline cephalexin monohydrate.

Cephalexin is an antibacterial agent used in human therapy and marketed as the monohydrate of the free acid (i.e., the zwitterion). It is described, for example, in J. Med. Chem. *12*, 310—313 (1969), J. Org. Chem. *36(9)*, 1259—1267 (1971) and J. Org. Chem. *37 (17)*, 2765—2767 (1972) [See Belgium 765,596; Farmdoc 67,511S] and in U.S. 3,507,861, U.K. 1,174,335 and Canada 856,786.

There are numerous disclosures of alternative methods for the production and purification of cephalexin (as the free acid). Examples include U.S. 3,634,416; U.S. 3,668,201; U.S. 3,668,202; U.S. 3,671,449 (Example 3); U.S. 3,676,434; U.S. 3,676,437 and U.S. 3,689,483 and U.S. 3,694,437.

Various disclosures with regard to crystalline forms and hydrates of cephalexin are given, for example, by Pfeiffer et al., in Journal of Pharmaceutical Sciences, *59(12)*, 1809—1814 (1970), by Bond et al. in Pharmaceutical Journal, 210—214 (August 22, 1970), in U.S. Patents 3,502,663; 3,531,481; 3,655,656 and 3,692,781, in Belgium 753,910 (Farmdoc 8214S) and Belgium 777,789 (Farmdoc 48556T) and in France 2,096,117.

This invention provides a process of producing a specific crystalline form of cephalexin monohydrate having a low water solubility and exhibiting essentially the following X-ray diffraction data:

Interplanar Spacing d Å	Relative Intensities I/I_1
16.01	.19
12.07	1.00
10.82	.13
9.64	.03
8.83	.07
8.53	.13
8.10	.26
7.07	.08
6.10	.14
5.60	.22
5.43	.64
4.98	.17
4.76	.09
4.57	.17
4.39	.18
4.23	.18
4.02	.30
3.94	.13
3.86	.19
3.79	.04
3.70	.05
3.61	.18
3.44	.08
3.24	.10
3.20	.05
3.11	.13
2.99	.08
2.91	.09
2.80	.07
2.73	.07
2.67	.09

which comprises preparing a concentrated, acidic aqueous solution of cephalexin (preferably having a pH less than 2.4, more preferably in the range of 1.7—2.3, and especially about 2.3), adding sufficient n - butanol to form two liquid phases (preferably being at least 0.5 volumes n - butanol per each

[*Price 33p*]

volume of aqueous solution and preferably also adding seed crystals of said crystalline form of cephalexin monohydrate) and then slowly raising the pH of the said biphasic system whilst agitating said system (preferably at a temperature no higher than 45°C and preferably in the range of 20°C to 25°C, preferably up to pH 4.0—5.0, suitably about pH 4.5, and preferably with triethylamine, sodium hydroxide, potassium hydroxide or ammonium hydroxide) to precipitate said crystalline form of cephalexin monohydrate.

The use of lower temperatures (in the range of 20°—25°C) is particularly valuable because it reduces the decomposition observed when higher temperatures are used.

The pH values are determined by a glass electrode inserted in the stirred two-phase system during the addition of the base and in the aqueous phase which separates when agitation is stopped at the end of the base addition.

The preferred pH elevating agents are sodium hydroxide, potassium hydroxide and ammonium hydroxide.

The specific crystalline form of cephalexin monohydrate referred to here has an approximate water-solubility of 10 mg/ml. This was determined in a somewhat crude manner. 30 mg was suspended in 1.0 ml of deionized water and shaken for 5 minutes; some solids remained in suspension. Another 1.0 ml addition of water was made and not all of the solids had gone into solution after 5 minutes of shaking. Another 1.0 ml addition of water was made (total 3.0 ml H_2O) and at this point there was obtained a clear solution. The solubility therefore is between 10 and 15 mg per ml.

The present invention provides a process for preparing a specific crystalline form of cephalexin monohydrate having a low water-solubility and characteristic X-ray diffraction data. The utility of this crystalline form is identical to that of the cephalexin products extensively reported in the previous scientific and medical literature. Thus this crystalline form of cephalexin monohydrate is a useful antibacterial agent in animal therapy, including man, possesses good stability and provides effective blood levels on oral administration. Its precise and reproducible chemical and physical nature makes it highly suitable for efficient commercial production and subsequent formulation. A further aspect of the invention is concerned with a pharmaceutical composition which comprises the specific crystalline form of cephalexin monohydrate, when prepared by the process of this invention, together with a biologically-inert physiologically-acceptable carrier.

The following examples are given in illustration of the present invention.

Example 1

Preparation of the present form of cephalexin monohydrate by BuOH-H_2O system at 20—25°C

25.0 g cephalexin primary grade was added slowly to 60 ml H_2O. Then 6*N* HCl (9.8 ml) was added dropwise, giving a final pH of 1.7. The solution was slightly hazy. There was added 2.5 g decolorizing charcoal. The mixture was stirred for 15 minutes and then filtered. Meanwhile 20 g NaOH was dissolved in enough water to make 100 ml of 20% NaOH solution.

The charcoal was washed with water to give a final filtrate volume of 110 ml. With stirring at 20—25°C, there was added 3.5 ml 20% NaOH over about 2 minutes giving a final pH of 2.3. There was then added to the solution as seed crystals 1.0 g of a previous batch of the present form of cephalexin monohydrate. Then 50 ml n-BuOH was added and the mixture was stirred at 23°C while a total of 8.65 ml additional 20% NaOH was added in increments of about 0.5 ml over a period of 4-1/2 hours.

The pH was thus slowly raised to a final pH of 4.5 while the temperature was maintained at 20—25°C. Analyses gave Karl Fischer H_2O=3.6%; isopropyl alcohol=0.089%; BuOH=0.72%; Chem. Potency=898 mcg/mg; Bio Potency=920 mcg/mg.

The precipitated desired form of cephalexin monohydrate was collected by filtration, washed successively with n-BuOH (50 ml), water (40 ml), n-BuOH (50 ml) and isopropyl alcohol (100 ml) and then dried 22 hours at 53—55°C (atmospheric pressure oven) to yield 20.0 g of the desired form of dry, crystalline cephalexin monohydrate of a pharmaceutical grade.

Example 2

Preparation of the present form of cephalexin monohydrate via Butanol-water recrystallization

The following procedure has been found to yield a very white product having high purity as well as the desired crystal form. The butanol layer removes residual productive impurities such as dimethylaniline (DMA) and colored impurities while the aqueous layer purges water-soluble impurities.

1. 1000 g of primary-grade cephalexin was added slowly to 2400 ml of deionized water containing 40 ml of 6*N* HCl. The addition was made slowly over a period of about 15 minutes while maintaining the pH at 1.6—1.8 by adding 6*N* HCl. The pH was checked constantly and a total of 390—400 ml of 6*N* HCl was necessary for complete solution. The temperature was maintained at about 20°C.

2. 100 g of decolorizing charcoal was added to the solution and the slurry was stirred for 30 minutes. The slurry was filtered through a precoated filter.

3. The cake was washed with enough deionized water to bring the filtrate volume to 4.0 liters. Higher volumes would have lead to undesirably high losses in the final mother liquor.

4. The filtrate was adjusted to pH 2.1—2.2 with about 30—35 ml triethylamine (TEA). The pH was monitored (a slight turbidity may result at this stage).

5. 2000 ml of n - butanol was added to the aqueous solution and the temperature was brought to 40°C. About 100 ml of TEA was added and the batch was seeded with crystals of the present form of cephalexin monohydrate to promote complete crystallization.

6. TEA was slowly added in a constant manner over about a five-minute period while holding the temperature at 40°C to give a final pH 4.4—4.6. The total amount of TEA required was about 345—350* ml. The temperature was then lowered to 20°C over a 30-minute period, and then the slurry was stirred at 15—20°C for 3 hours.

7. The slurry was filtered on a Buchner funnel and the cake was washed with 2 liters of butanol or until the washing butanol started to come through colorless.

8. The cake was then washed with 1500 ml of deionized water at 0—5°C.

9. The cake was then washed with 1500 ml of butanol. These washes and the original mother liquor were saved and allowed to stand. The aqueous phase which separated was used for recycle in the inventive process.

10. The cake was finally washed with 2000 ml of isopropanol. It was then dried at 53—55°C overnight, (about 16—24 hours): Yields of 800—820 g of the desired form of cephalexin monohydrate per 1000 g input were obtained.

Example 3

Preparation of the present form of cephalexin Monohydrate by direct crystallization by BuOH-H_2O system from pilot plant rich aqueous cephalexin

1,000 ml of pilot plant partly concentrated aqueous acidic solution of cephalexin was concentrated under vacuum to about 200 ml. The pH of the concentrate was 1.1, and its temperature was 22°C. The pH was adjusted to 2.1 with 10.0 ml of TEA. The solution was seeded with 1.0 g of the present form of cephalexin monohydrate (insoluble form) and 150 ml of n - butanol was added. A heavy crystal mass formed very readily. The slurry was stirred and TEA (8.0 ml) was added dropwise in small increments of about 1.0 ml over a period of 3 hours to give a final pH of 4.5.

The slurry was stirred for 1 hour at 20°C and then the solid product was collected by filtration and the filter cake was washed with 200 ml of wet butanol, followed by 100 ml of cold (0—5°C) deionized water. The cake was then washed with 100 ml of dry butanol followed by 100 ml of isopropanol. The cake was dried at 50°C. A yield of 30.0 g of the desired form of cephalexin monohydrate was obtained which represented a yield of 29.0 g cephalexin monohydrate when allowing for the seed addition of 1.0 g

Assays:

Karl Fischer H_2O=3.9%
Isopropyl alcohol=0.15%
BuOH=0.57%
Chem. Potency=892 mcg/mg

WHAT WE CLAIM IS:—

1. The process of producing a specific crystalline form of cephalexin monohydrate having a low water-solubility and exhibiting essentially the following X-ray diffraction data:

Interplanar Spacing d Å	Relative Intensities I/I_1
16.01	.19
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5.60	.22
5.43	.64
4.98	.17
4.76	.09
4.57	.17
4.39	.18
4.23	.18
4.02	.30
3.94	.13
3.86	.19
3.79	.04

*As a check on its concentration, an aliquot of *6N* HCl should be titrated with TEA beforehand to see how much TEA is required to attain pH 4.5. The HCl should be diluted with 8 volumes of deionized water before the titration is carried out. In this way over-addition of TEA during the crystallization can be prevented.

2. 100 g of decolorizing charcoal was added to the solution and the slurry was stirred for 30 minutes. The slurry was filtered through a precoated filter.

3. The cake was washed with enough deionized water to bring the filtrate volume to 4.0 liters. Higher volumes would have lead to undesirably high losses in the final mother liquor.

4. The filtrate was adjusted to pH 2.1—2.2 with about 30—35 ml triethylamine (TEA). The pH was monitored (a slight turbidity may result at this stage).

5. 2000 ml of n - butanol was added to the aqueous solution and the temperature was brought to 40°C. About 100 ml of TEA was added and the batch was seeded with crystals of the present form of cephalexin monohydrate to promote complete crystallization.

6. TEA was slowly added in a constant manner over about a five-minute period while holding the temperature at 40°C to give a final pH 4.4—4.6. The total amount of TEA required was about 345—350* ml. The temperature was then lowered to 20°C over a 30-minute period, and then the slurry was stirred at 15—20°C for 3 hours.

7. The slurry was filtered on a Buchner funnel and the cake was washed with 2 liters of butanol or until the washing butanol started to come through colorless.

8. The cake was then washed with 1500 ml of deionized water at 0—5°C.

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10. The cake was finally washed with 2000 ml of isopropanol. It was then dried at 53—55°C overnight, (about 16—24 hours): Yields of 800—820 g of the desired form of cephalexin monohydrate per 1000 g input were obtained.

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The slurry was stirred for 1 hour at 20°C and then the solid product was collected by filtration and the filter cake was washed with 200 ml of wet butanol, followed by 100 ml of cold (0—5°C) deionized water. The cake was then washed with 100 ml of dry butanol followed by 100 ml of isopropanol. The cake was dried at 50°C. A yield of 30.0 g of the desired form of cephalexin monohydrate was obtained which represented a yield of 29.0 g cephalexin monohydrate when allowing for the seed addition of 1.0 g

Assays:

Karl Fischer H_2O=3.9%
Isopropyl alcohol=0.15%
BuOH=0.57%
Chem. Potency=892 mcg/mg

WHAT WE CLAIM IS:—

1. The process of producing a specific crystalline form of cephalexin monohydrate having a low water-solubility and exhibiting essentially the following X-ray diffraction data:

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5.60	.22
5.43	.64
4.98	.17
4.76	.09
4.57	.17
4.39	.18
4.23	.18
4.02	.30
3.94	.13
3.86	.19
3.79	.04

*As a check on its concentration, an aliquot of 6*N* HCl should be titrated with TEA beforehand to see how much TEA is required to attain pH 4.5. The HCl should be diluted with 8 volumes of deionized water before the titration is carried out. In this way over-addition of TEA during the crystallization can be prevented.

Interplanar Spacing d Å	Relative Intensities I/I_1
3.70	.05
3.61	.18
3.44	.08
3.24	.10
3.20	.05
3.11	.13
2.99	.08
2.91	.09
2.80	.07
2.73	.07
2.67	.09

which comprises preparing a concentrated, acidic aqueous solution of cephalexin, adding sufficient n - butanol to form two liquid phases, and then slowly raising the pH of said biphasic system whilst agitating said system to precipitate said crystalline form of cephalexin monohydrate.

2. A process according to claim 1 which comprises preparing the said concentrated, acidic aqueous solution of cephalexin such that it has a pH of less than 2.4.

3. A process according to calim 1 or 2 wherein at least 0.5 volumes n - butanol are added per each volume of said aqueous solution.

4. A process according to any of claims 1 to 3 wherein seed crystals of said specific crystalline form of cephalexin monohydrate are added to said aqueous solution or biphasic system.

5. A process according to any of claims 1 to 4 which comprises slowly adding base to said biphasic system to raise the pH to from 4.0 to 5.0.

6. A process according to claim 5 wherein the pH is raised to a pH of about 4.5.

7. A process according to any of claims 1 to 6 wherein the procedure of raising the pH is carried out at a temperature no higher than 45°C.

8. A process according to claim 7 wherein the said temperature is from 20 to 25°C.

9. A process according to any of claims 1 to 8 wherein the pH is raised by adding triethylamine, sodium hydroxide, potassium hydroxide, or ammonium hydroxide.

10. A process for producing the crystalline form of cephalexin monohydrate which is specified in claim 1, substantially as herein described in any of the Examples.

11. The specific crystalline form of cephalexin monohydrate when prepared by the process of any of claims 1 to 10.

12. A pharmaceutical composition which comprises the specific crystalline form of cephalexin monohydrate as claimed in claim 11 together with a biologically-inert physiologically-acceptable carrier.

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Printed for Her Majesty's Stationery Office, by the Courier Press, Leamington Spa, 1975.
Published by The Patent Office, 25 Southampton Buildings, London, WC2A 1AY, from which copies may be obtained.