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(54) Title: PROCESS FOR PREPARING PHARMACEUTICAL FORMULATION IN FORM OF ANTIOXIDANT-FREE SOLUTION FOR INJECTION CONTAINING PEMETREXED OR ITS SALT

(57) Abstract: The present invention provides a method for preparing a pharmaceutical formulation in the form of an antioxidant-free solution for injection, the method of which comprises: (a) controlling a dissolved oxygen concentration in a solution for injection comprising pemetrexed or its salt to 1 ppm or less; and (b) filling a container for injection with the solution obtained from the step (a), in a closed system having an oxygen partial pressure of 0.2 %v/v or less.



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Description

Title of Invention: PROCESS FOR PREPARING PHARMACEUTICAL FORMULATION IN FORM OF ANTIOXIDANT-FREE SOLUTION FOR INJECTION CONTAINING PEMETREXED OR ITS SALT

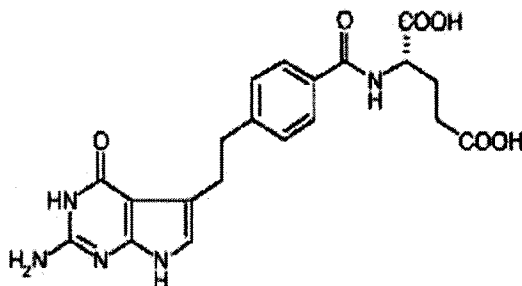
Technical Field

- [1] The present invention relates to a method for preparing a pharmaceutical formulation in the form of an antioxidant-free solution for injection containing pemetrexed or its salt as an active ingredient.

Background Art

- [2] Pemetrexed or its salt (e.g., disodium salt) is an antifolate antineoplastic agent and has the chemical name of N-[4-[2-(2-amino-4,7-dihydro-4-oxo-1H-pyrrolo[2,3-d]pyrimidin-5-yl)ethyl]benzoyl]-L-glutamic acid, which is represented by the following formula (US Patent No.: US5,344,932).

[3]



- [4] Pemetrexed or its salt is currently being used in the form of a lyophilized powder formulation for injection. The lyophilized powder formulation is administered to patients after it is reconstituted with e.g., physiological saline. However, such a lyophilized powder formulation is prepared through complicated lyophilizing processes, which results in increasing manufacturing costs thereof. In addition, in order to administer a lyophilized powder formulation to patients in clinics or hospitals, there is required a reconstitution step using physiological saline, thereby causing inconveniences and risks of contamination by microorganisms in the reconstitution step.
- [5] Therefore, it would be desirable that pemetrexed or its salt is formulated in the form of injectable formulations, e.g., solutions for injection, so as to allow direct use in clinics or hospitals. However, pemetrexed has very low stability in a medium for injection (e.g., water for injection) and thus causes the occurrence of various degradation products. To address this problem, International Patent Publication No.: WO2001/56575 discloses a liquid formulation comprising pemetrexed and an an-

tioxidant such as monothioglycerol, L-cysteine, or thioglycolic acid. According to the disclosures of WO2001/56575, while common antioxidants such as sodium metabisulfite, ascorbic acid, sodium EDTA, monoethanolamine gentisate, sodium formaldehyde sulfoxylate, and sodium bisulfite do not provide the desired formulation characteristics, the three antioxidants (i.e., monothioglycerol, L-cysteine, and thio-glycolic acid) may be used to prepare a stable liquid formulation for injection.

- [6] In addition, International Patent Publication No.: WO2010/30598 discloses a solid pharmaceutical formulation comprising amorphous pemetrexed or its salt and a method for preparing the same. International Patent Publication No.: WO2001/62760 discloses a heptahydrate form of pemetrexed and a process for preparing the same. According to the disclosures of WO2001/62760, the heptahydrate form has higher stability in the moisture content than the hemipentahydrate form.

Disclosure of Invention

Technical Problem

- [7] The present inventors have conducted various researches in order to develop a pharmaceutical formulation in the form of a solution for injection, having an improved stability. We surprisingly found that, when dissolved oxygen in the solution for injection was substantially removed (i.e., approximately 1 ppm or less) by e.g., degassing, a stable liquid formulation containing pemetrexed or its salt could be prepared, even without using a stabilizing agent such as an antioxidant.
- [8] Therefore, the present invention provides a method for preparing a pharmaceutical formulation in the form of an antioxidant-free solution for injection containing pemetrexed or its salt as an active ingredient.

Solution to Problem

- [9] In accordance with an aspect of the present invention, there is provided a method for preparing a pharmaceutical formulation in the form of an antioxidant-free solution for injection, the method of which comprises: (a) controlling a dissolved oxygen concentration in a solution for injection comprising pemetrexed or its salt to 1 ppm or less; and (b) filling a container for injection with the solution obtained from the step (a), in a closed system having an oxygen partial pressure of 0.2 %v/v or less.
- [10] In an embodiment of the present invention, the step (a) may be performed by dissolving pemetrexed or its salt and at least one excipient selected from the group consisting of sodium chloride, mannitol, and a pH-control agent, in water for injection having a dissolved oxygen concentration of 1 ppm or less. And also, in this embodiment, both the step (a) and the step (b) may be performed in a closed system having an oxygen partial pressure of 0.2 %v/v or less.
- [11] In another embodiment of the present invention, the step (a) may be performed by

dissolving pemetrexed or its salt and at least one excipient selected from the group consisting of sodium chloride, mannitol, and a pH-control agent in water for injection; and then bubbling an inert gas in the obtained solution to adjust a dissolved oxygen concentration to 1 ppm or less. In this embodiment, the bubbling of the inert gas may be performed under reduced pressure or in vacuum.

Advantageous Effects of Invention

- [12] It is newly found by the present invention that, when dissolved oxygen in the solution for injection is substantially removed (i.e., approximately 1 ppm or less) by e.g., degassing, a stable liquid formulation containing pemetrexed or its salt may be prepared, even without using a stabilizing agent such as an antioxidant. Therefore, the method according to the present invention can minimize the use of excipients required for preparing medicinal products, thereby giving desirable characteristics in terms of safety. Especially, the method of the present invention can avoid generating both any unknown degradation product(s) according to the use of an antioxidant and any oxidative degradation product(s) derived from the antioxidant per se.

Best Mode for Carrying out the Invention

- [13] A pharmaceutical formulation in the form of a solution for injection is typically prepared by filling contents in an ampoule or a vial and then replacing the ambient air in the headspace thereof with an inert gas such as nitrogen gas, for avoiding any stability problems which may be caused during the storage. Since pemetrexed has very low stability in a medium for injection (e.g., water for injection) and thus causes the occurrence of various degradation products, it is required to use an antioxidant such as monothioglycerol, L-cysteine, or thioglycolic acid (see WO2001/56575). In other words, a stable pemetrexed-containing injectable formulation cannot be obtained only by replacing the headspace thereof with an inert gas such as nitrogen gas; and thus a specific antioxidant needs to be used to stabilize the pemetrexed-containing pharmaceutical formulation. However, it is desirable in terms of safety to minimize the use of excipients required for preparing medicinal products. The present invention provides a method for preparing a pharmaceutical formulation in the form of a stable solution for injection without using a stabilizing agent, such as an antioxidant.
- [14] The present invention provides a method for preparing a pharmaceutical formulation in the form of an antioxidant-free solution for injection, the method of which comprises: (a) controlling a dissolved oxygen concentration in a solution for injection comprising pemetrexed or its salt to 1 ppm or less; and (b) filling a container for injection with the solution obtained from the step (a), in a closed system having an oxygen partial pressure of 0.2 %v/v or less.
- [15] It is newly found by the present invention that, when dissolved oxygen in the

solution for injection is substantially removed (i.e., approximately 1 ppm or less) by e.g., degassing, a stable liquid formulation containing pemetrexed or its salt may be prepared, even without using a stabilizing agent such as an antioxidant. Therefore, the method according to the present invention can minimize the use of excipients required for preparing medicinal products, thereby giving desirable characteristics in terms of safety. Especially, the method of the present invention can avoid generating both any unknown degradation product(s) according to the use of an antioxidant and any oxidative degradation product(s) derived from the antioxidant per se.

[16] In the method according to the present invention, any degassing method known in the art may be used (refer to Table 1 below).

[17] Table 1

[Table 1]

Degassing method	Mechanism
Vacuum degassing	Reducing gas partial pressure, thereby removing dissolved oxygen
Distillation degassing	Degassing by distilling and cooling under inert gas atmosphere
N ₂ bubbling degassing	Degassing by bubbling N ₂ in water
Membrane degassing	Vacuating the permeated water side of a hydrophobic polymer membrane to remove dissolved oxygen
Catalyst-resin degassing	Immobilizing a reducing catalyst, e.g., palladium, on a resin, followed by reacting with a reducing agent, e.g., hydrogen to remove dissolved oxygen

[18] Two or more degassing methods may be used in combination. Examples of the degassing methods used in combination include a combination of vacuum degassing and N₂ bubbling degassing, a combination of vacuum degassing and membrane degassing, and a combination of vacuum degassing and catalyst-resin degassing. In addition, the degassing method(s) may be performed more than once.

[19] In the method according to the present invention, the controlling a dissolved oxygen concentration may be performed with respect to water for injection or with respect to a solution obtained by dissolving pemetrexed or its salt (along with an excipient) in water for injection. The degassing method(s) that may be used in the respective controlling method may be appropriately selected by one of ordinary skill in the art. For example, distillation degassing and/or catalyst-resin degassing may be performed with respect to water for injection. And also, membrane degassing, vacuum degassing, and/or N₂ bubbling degassing may be performed with respect to both water for injection

and a solution.

- [20] In an embodiment of the present invention, the step (a) may be performed by dissolving pemetrexed or its salt and at least one excipient selected from the group consisting of sodium chloride, mannitol, and a pH-control agent, in water for injection having a dissolved oxygen concentration of 1 ppm or less. In this case, the step (a) and the step (b) may be performed in separate systems or in the same system. That is, if the steps (a) and (b) are performed in the same system, both of the steps (a) and (b) may be performed in a closed system having an oxygen partial pressure of 0.2 %v/v or less.
- [21] In another embodiment of the present invention, the step (a) may be performed by dissolving pemetrexed or its salt and at least one excipient selected from the group consisting of sodium chloride, mannitol, and a pH-control agent in water for injection; and then bubbling an inert gas (e.g., nitrogen or argon) in the obtained solution to adjust a dissolved oxygen concentration to 1 ppm or less. In this embodiment, the bubbling of the inert gas may be performed under reduced pressure or in vacuum.
- [22] The salt of pemetrexed may be, for example, pemetrexed disodium, but are not limited thereto. Sodium chloride may be added as an isotonic agent; and mannitol may be added as an additive. In addition, if needed, a pH-control agent such as sodium hydroxide or hydrochloric acid may be used.
- [23] The method according to the present invention includes filling a container for injection, for example, an ampoule or a vial, with the injectable solution obtained by degassing. That is, the method of the present invention includes filling a container for injection with the solution obtained from the step (a), in a closed system having an oxygen partial pressure of 0.2 %v/v or less.
- [24] The closed system having an oxygen partial pressure of 0.2 %v/v or less may be provided by injecting an inert gas (e.g., nitrogen or argon) thereto so as to adjust the oxygen partial pressure. For example, conventional systems such as a glove bag may be used as the closed system. When the filling process is performed in the closed system as described above, the headspace of the container for injection may be automatically replaced with the inert gas.
- [25] The pharmaceutical formulation in the form of a solution for injection prepared according to the present invention may be sterilized using conventional methods, for example, sterilization using membrane filters and/or heat sterilization.
- [26] The present invention will be described in further detail with reference to the following examples and experimental examples. These examples and experimental examples are for illustrative purposes only and are not intended to limit the scope of the present invention.
- [27] In the following Examples, the dissolved oxygen concentration in water for injection was measured via a Winkler-Azide titration method (the sodium azide modification to

the Winkler method) and a method using diaphragm electrodes (instrumental analysis). That is, the method using diaphragm electrodes was first used and the measurement results were then verified via the Winkler-Azide titration method. The dissolved oxygen concentration in a pemetrexed-containing solution was measured only using the method using diaphragm electrodes. The method using diaphragm electrodes was performed by following steps: (i) adding a sample to a BOD bottle after calibration prior to analysis, (ii) immersing the oxygen sensor equipped with an agitator that was connected to the dissolved oxygen measuring device into the BOD bottle containing the sample, (iii) switching on the operating switch of the dissolved oxygen measuring device, and then, (iv) measuring a dissolved oxygen concentration after waiting for approximately 5 minutes until the values of dissolved oxygen concentration in the sample included in the BOD bottle are stabilized.

[28]

[29] **Example 1: Preparation of solution for injection by distillation degassing**

[30] 700 ml of water for injection was distilled by heating in a heating mantle under nitrogen atmosphere and then cooled under nitrogen atmosphere to remove the dissolved oxygen therein. The dissolved oxygen concentration in the obtained water for injection (degassed water) was 0.5 ppm. Pemetrexed disodium (1.25 g as pemetrexed) and 0.45 g of sodium chloride were put into a 100 ml glass container, which was then placed in a glove bag. A vial, a rubber closure, and a sterile filter were also put into the glove bag. An electrode of a pH meter was calibrated, washed, and then put into the glove bag. Nitrogen gas was injected into the glove bag under reduced pressure so as to adjust an oxygen partial pressure to 0.1 %v/v. 50 ml of the degassed water was taken with a syringe and then added to the 100 ml glass container in the glove bag to dissolve the components. The pH measured in the glove bag was 7.66. The resulting solution was taken again using a syringe and then filtered through a 0.2 μm sterile filter equipped in the syringe. The 5 ml vial was filled with 4 ml of the obtained filtrate and then sealed with the rubber closure. The glove bag was opened up and the vial was capped with an aluminum cap. The obtained vial was sterilized in a high pressure steam sterilizer for 15 minutes.

[31]

[32] **Example 2: Preparation of solution for injection by vacuum degassing**

[33] Pemetrexed disodium (2.5 g as pemetrexed) and 0.9 g of sodium chloride were added to a 250 ml glass bottle equipped with a gas inlet and a vacuum outlet formed at its cap, and then 100 ml of water for injection was added thereto to dissolve the components. The dissolved oxygen concentration in the water for injection was 6.5 ppm. Nitrogen was connected to the gas inlet and a diaphragm vacuum pump was connected to the vacuum outlet. A vacuum degassing operation and nitrogen purging

were repeatedly performed. An aliquot of the resulting solution was taken for measuring the dissolved oxygen concentration therein and the pH thereof. As the result of the measurement, the dissolved oxygen concentration was 0.8 ppm and the pH was 7.68.

- [34] The resulting solution was taken with a syringe and then put in a glove bag. A vial, a rubber closure, and a sterile filter were also put in the glove bag. Nitrogen gas was injected into the glove bag under reduced pressure so as to adjust an oxygen partial pressure to 0.1 %v/v. The solution was taken using a syringe and then filtered through a 0.2 μm sterile filter equipped in the syringe. The 5 ml vial was filled with 4 ml of the obtained filtrate and then sealed with the rubber closure. The glove bag was opened up and the vial was capped with an aluminum cap. The obtained vial was sterilized in a high pressure steam sterilizer for 15 minutes.

[35]

[36] **Comparative Example 1**

- [37] Pemetrexed disodium (1.25 g as pemetrexed) and 0.45 g of sodium chloride were dissolved in 50 ml of water for injection that was not subjected to a degassing process. The dissolved oxygen concentration therein and the pH thereof were measured, respectively. As the result of the measurement, the pH was 7.68 and the dissolved oxygen concentration was 6.4 mg/L (6.4 ppm). The resultant solution was filtered with a 0.2 μm sterile filter. A 5 ml vial was filled with 4 ml of the obtained filtrate and then sealed with the rubber closure.

[38]

[39] **Comparative Example 2**

- [40] Pemetrexed disodium (1.25 g as pemetrexed) and 0.45 g of sodium chloride were dissolved in 50 ml of water for injection that was not subjected to a degassing process. The dissolved oxygen concentration therein and the pH thereof were measured, respectively. As the result of the measurement, the pH of the solution was 7.67 and the dissolved oxygen concentration in the solution was 6.4 mg/L (6.4 ppm). The resulting solution was taken with a syringe and then put in a glove bag. A vial, a rubber closure, and a sterile filter were also put in the glove bag. Nitrogen gas was injected into the glove bag under reduced pressure so as to adjust an oxygen partial pressure to 0.1 %v/v. The solution was taken using a syringe and then filtered through a 0.2 μm sterile filter equipped in the syringe. The 5 ml vial was filled with 4 ml of the obtained filtrate and then sealed with the rubber closure. The glove bag was opened up and the vial was capped with an aluminum cap.

[41]

[42] **Experimental Example: Stability test**

- [43] Stabilities of the solutions for injection prepared according to the Examples and the

Comparative Examples were evaluated under stress conditions (75 ± 2 °C, a relative humidity of 85 ± 5 %). The amounts of pemetrexed and degradation products were analyzed by high performance liquid chromatography (HPLC) according to a known method disclosed in *Chromatographia* 2007, 66, pp. 431-434. The stability test results under the stress condition for 7 days are shown in Table 2 below.

[44] Table 2

[Table 2]

		Comparative Example 1	Comparative Example 2	Example 1	Example 2
Initial	Appearance	Transparent pale yellow	Transparent pale yellow	Transparent pale yellow	Transparent pale yellow
	pH	7.68	7.67	7.66	7.68
	Amounts	99.8%	100.2%	100.1%	99.9%
	Degradation Products	Not detected	Not detected	Not detected	Not detected
1 day	Amounts	96.5%	99.7%	99.9%	99.8%
	Degradation Products	- Total: 3.73%- Individual compounds: more than 0.1%	- Total: 0.19%- Individual compound more than 0.1% was not detected	Not detected	Not detected
3 days	Amounts	93.7%	99.9%	100.2%	100.0%
	Degradation Products	- Total: 8.23%- Individual compounds: more than 0.1%	- Total: 0.34%- Individual compounds: more than 0.1%	Not detected	- Total: 0.06%- Individual compound more than 0.1% was not detected
7 days	Appearance	Dark yellow	Yellow	Transparent pale yellow	Transparent pale yellow
	pH	6.81	7.49	7.67	7.64
	Amounts	91.9%	99.7%	100.1%	99.9%
	Degradation Products	- Total: 8.67%- Individual compounds: more than 0.1%	- Total: 0.36%- Individual compounds: more than 0.1%	Not detected	- Total: 0.06%- Individual compound more than 0.1% was not detected

- [46] As seen in Table 2, in case of the solutions for injection according to Comparative Examples 1 and 2 (prepared by using water for injection in which dissolved oxygen was not adjusted), changes in the appearance and increases in degradation products were notably observed when the solutions for injection were stored under the stress conditions for 1 week, even though not being sterilized in a high pressure steam sterilizer. By contrast, in case of the solutions for injection prepared in Examples 1 and 2 according to the present invention, degradation products were generated at less than 0.1% or were not detected, even after high pressure steam sterilization. These results indicate that the solutions for injection according to the present invention have very excellent stability.

Claims

- [Claim 1] A method for preparing a pharmaceutical formulation in the form of an antioxidant-free solution for injection, the method of which comprises:
(a) controlling a dissolved oxygen concentration in a solution for injection comprising pemetrexed or its salt to 1 ppm or less; and
(b) filling a container for injection with the solution obtained from the step (a), in a closed system having an oxygen partial pressure of 0.2 %v/v or less.
- [Claim 2] The method of claim 1, wherein the step (a) is performed by dissolving pemetrexed or its salt and at least one excipient selected from the group consisting of sodium chloride, mannitol, and a pH-control agent, in water for injection having a dissolved oxygen concentration of 1 ppm or less.
- [Claim 3] The method of claim 2, wherein both the step (a) and the step (b) are performed in a closed system having an oxygen partial pressure of 0.2 %v/v or less.
- [Claim 4] The method of claim 1, wherein the step (a) is performed by dissolving pemetrexed or its salt and at least one excipient selected from the group consisting of sodium chloride, mannitol, and a pH-control agent in water for injection; and then bubbling an inert gas in the obtained solution to adjust a dissolved oxygen concentration to 1 ppm or less.
- [Claim 5] The method of claim 4, wherein the bubbling of the inert gas is performed under reduced pressure or in vacuum.